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Research Article

Simultaneous Determination of Six Components in Beagle Dog Plasma by UPLC-MS/MS and Its Application to a Comparative Pharmacokinetic Study of Three Different Yuanhu Zhitong Preparations

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This study has developed a sensitive, simple, and reliable ultrahigh performance liquid chromatography tandem mass spectrometry method for the simultaneous determination of corydaline, tetrahydropalmatine, tetrahydroberberine, tetrahydrocoptisine, byakangelicin, and byakangelicol in beagle dog plasma after oral administration of Yuanhu Zhitong (YHZT) oral liquid, dispersible tablet, and dropping pills. A one-step protein precipitation method was used with methanol-acetonitrile (50 : 50, v/v) for plasma sample pretreatment. Six analytes and one internal standard were separated on a Waters Acquity UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) by gradient elution with 0.1% formic acid-water and 0.1% formic acid-acetonitrile at a flow rate of 0.4 mL/min. Detection was conducted by multiple reaction monitoring mode in the positive ionization. The calibration curves showed good linearity ($r > 0.9920$). The lower limit of quantization ranged from 0.1 to 1 ng/mL. Moreover, the other results of the method validation were within the acceptable range. After oral administration, the pharmacokinetic characteristics of the three YHZT preparations were different. Compared with the two solid preparations, the absorption of the six analytes in oral liquid was more rapid. Moreover, the area under the plasma drug concentration-time curve and the maximum plasma concentration of tetrahydropalmatine (the main analgesic component of YHZT prescription) in the oral liquid group were higher than those in the other two groups. The results showed oral liquid can exert its efficacy quickly, while dispersible tablet and dropping pills exhibit relatively slow release pattern. The comparative pharmacokinetic study would be helpful to the clinical rational selection and application of YHZT preparations.

1. Introduction

Traditional Chinese medicine (TCM) has been used in Asia (China, Japan, and South Korea, etc.) for thousands of years. Lots of clinical applications of TCM have shown satisfactory efficacy and safety, especially for chronic, difficult, and complicated diseases, which is attracting more and more attention all over the world [1, 2]. In TCM, the etiology, pathogenesis, location, and characteristics of pain were first recorded in Inner Canon of Huangdi. Furthermore, different types of pains should be treated according to syndrome differentiation, which was initially recorded in Shanghan Zabing Lun (a classical work of TCM written by the famous Chinese physician Zhang Zhongjing).

Yuanhu Zhitong (YHZT) prescription is a typical TCM formula for relieving pain, which has been officially recorded in all the editions of Chinese Pharmacopoeia since 1985. It is composed of *Corydalis rhizome* processed with vinegar (CR)

and *Radix Angelicae dahurica* (RAD) in the radio of 2 : 1 and is widely used for treating stomach pain, hypochondriac pain, headache, and dysmenorrhea due to qi stagnation and blood stasis [3]. There are many tapes of YHZT preparations recorded in Chinese Pharmacopoeia, including tablets, oral liquids, granules, and capsules.

Natural pharmaceutical chemistry research finds that the main chemical compositions in CR and RAD are alkaloids [4] and coumarins [5], respectively. Modern pharmacological studies have shown that these active components exhibit many biological activities, in which the most important role of alkaloids and coumarins has been found to be effective on alleviating pain [4–9].

Nevertheless, from the perspective of *in vivo*pharmacokinetics (PK), not all the constituents in TCM prescription used can exert efficacy. Only the ones that can be absorbed by blood that is possible to play pharmacological role. The PK study of TCM prescription can effectively characterize the interaction between different components and reveal theirs *in vivo* process [10–12]. At present, the reports on YHZT prescription are often focusing on the identification of effective components and evaluation of the pharmacological mechanism of pain treatment. Occasionally, there are PK studies on individual components or an oral preparation [13–21]. However, so far, there is no comparative PK study of different YHZT preparations based on multiple components related to analgesic effect. Consequently, it is essential to develop an appropriate analysis method to reflect the *in vivo* process of these preparations.

In the present study, we developed a rapid, simple, sensitive, and specific ultrahigh performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for simultaneous determination of six components including corydaline (Cde), tetrahydropalmatine (Thp), tetrahydroberberine (Thb), tetrahydrocoptisine (Thc), byakangelicin (Bcin), and byakangelicol (Bcol) in beagle dog plasma. Then, the method was applied to the comparative PK study of these components in dogs after oral administration of YHZT oral liquid (OL), dispersible tablet (DT) and dropping pills (DP), respectively. It is the first study on PK profiles of these six active ingredients in different YHZT preparations. The result of the investigation would be helpful to better explain the effect of dosage forms on the *in vivo* process of YHZT prescription and better guide its clinical rational application.

2. Materials and Methods

2.1. Reagents and Materials. Three types of YHZT preparations produced by different manufacturers were purchased from local drug stores (Table 1). Cde, Thp, Thb, Thc, Bcin, Bcol, and promethazine hydrochloride (internal standard, IS) purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The purities of all standards were higher than 98% and suitable for LC-MS/MS analysis. Their chemical structures are shown in Figure 1.

HPLC grade acetonitrile, methanol, and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Pure water was obtained from a Milli-Q system (Millipore, Bedford, USA). All the other reagents were of analytical grade.

2.2. Experimental Animals. Male beagle dogs (8–12 kg, aged about 1, certificate no. SCXK 2016-0001) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). All animals were raised in Tianjin International Joint Academy of Biomedicine (Breeding license no 2017-0003), given standard feed, regular ventilation, light, and free drinking water. The animal experiment was approved by the Animal Ethics Committee of Tianjin International Joint Academy of Biomedicine.

2.3. UPLC-MS/MS Conditions. A UPLC I-Class system (Waters Corp., Milford, MA, USA) interfaced with a Waters Xevo™ triple-quadrupole mass spectrometer (Waters, USA) equipped with an electrospray ionization (ESI) source.

LC separation was performed on an Acquity UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 μm), and the column temperature was maintained at 35°C during the analysis. The flow rate was 0.4 mL/min and the mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution conditions were as follows: 0-1 min, 90% A; 1–5 min, 90%–60% A; 5–7.5 min, 60%–0% A; 7.5–8.5 min, 0% A; 8.5–9 min, 0%–90% A; 9–11 min, 90% A. The autosampler temperature was set at 4°C, and the injection volume was 8 μL.

MS/MS spectra were acquired in positive ion mode with multiple reactions monitoring (MRM). The conditions of MS analysis were designed as follows: source voltages 3.00 kV; source temperature 400°C; cone gas flow 50 L/h, and desolvation gas flow 700 L/h. The MS/MS transitions (m/z), cone, and collision energy (CE) of the analytes and IS are listed in Table 2.

2.4. Content Determination of the Three Types of YHZT Preparations. The pulverized sample (1 g) of the two solid preparations was precisely weighed, and the OL (10 ml) was accurately measured, putting them into 50 mL volumetric flask and adding methanol to make volume, respectively. All samples were ultrasonically extracted at room temperature for 30 min and then filtered through 0.22 μm organic filter membrane. The contents of these six analytes in the three preparations are shown in Table 3.

2.5. Preparation of Standard and Quality Control Samples. Standard stock solution of Cde, Thp, Thb, Thc, Bcin, Bcol, and IS was prepared in methanol (1 mg/mL). We take appropriate amount of stock solution of the six analytes and mix well, which contained 200 ng/mL of Thp, Thb, and Thc; 400 ng/mL of Cde and Bcol; and 2000 ng/mL of Bcin. The mixed working standard solutions were prepared by sequential dilution (at the ratios of 1, 2, 4, 10, 20, 40, 100, and 200) with methanol, respectively. The stock solution of IS was diluted with methanol to 100 ng/mL as the working solution. The abovementioned working

Table 1: Sample information.

Sample no.	Manufacturer	Batch no.	Production date
YHZT OL	Henan Xichuan County Fusen Pharmaceutical Co., Ltd.	19070221	Jul 15, 2019
YHZT DT	Chengdu Yongkang Pharmaceutical Co., Ltd.	200502	Jun 9, 2020
YHZT DP	Gansu Longshenrongfa Pharmaceutical Industry Co., Ltd.	20200603	Jun 2, 2020

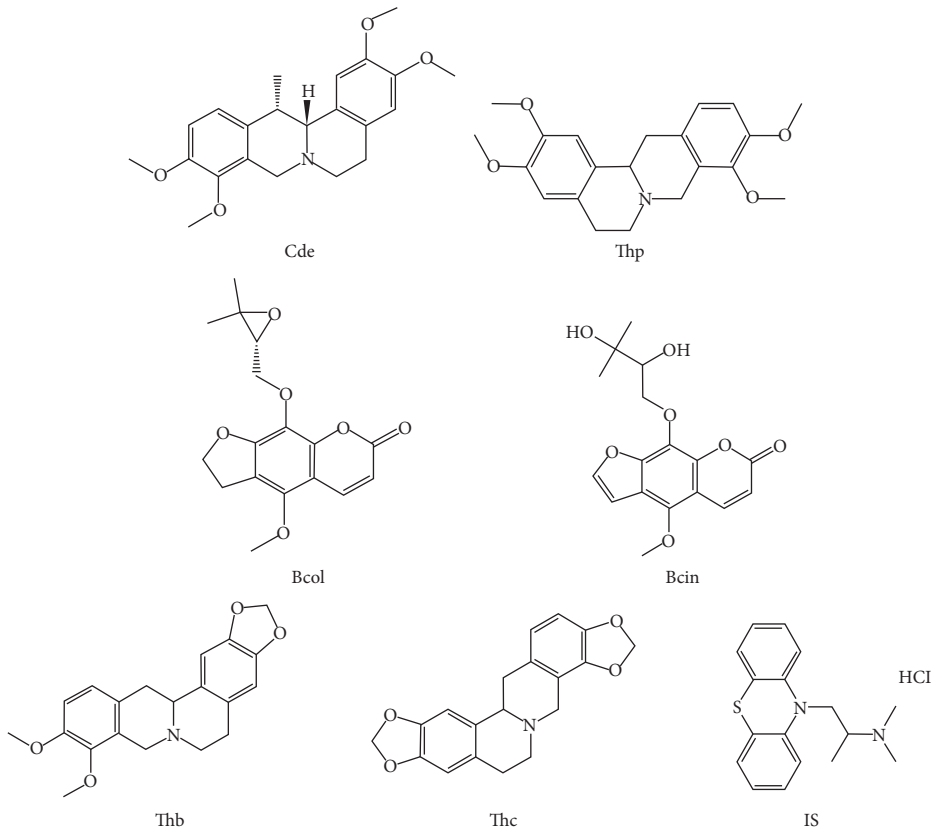

Figure 1: Chemical structures of the analytes and IS. Corydaline, Cde; tetrahydropalmatine, Thp; tetrahydroberberine, Thb; tetrahydrocoptisine, Thc; byakangelicin, Bcin; byakangelicol, Bcol; promethazine hydrochloride, IS.

Table 2: Optimized MS/MS transitions, cone and collision energy of the analytes and internal standards.

Compound	Parent ion (m/z)	Daughter ion (m/z)	Cone (V)	CE (V)
Cde	370.2	192.0	50	28
Thp	356.2	192.1	48	26
Thb	340.1	176.0	44	24
Bcin	335.0	317.0	14	6
Thc	324.0	176.0	44	26
Bcol	317.0	232.9	32	12
Is	285.1	86.0	26	16

Table 3: The quantitative results of the six analytes in the three different YHZT preparations.

Analyte	*Concentration (ng/g)*		
	YHZT OL	YHZT DT	YHZT DP
Cde	276.81	348.80	230.77
Thp	295.51	200.60	213.46
Thb	32.42	43.41	40.38
Bcin	351.62	221.56	250.00
Thc	23.69	79.34	59.62
Bcol	110.97	7.49	100.00

Calculated by the amount of crude material.

solutions were stored in a refrigerator (4°C) until they were required for use.

Calibration standards solutions were prepared by adding 5 μL of the corresponding standard solutions, 5 μL of the IS working solution and 150 μL protein precipitant (methanol-acetonitrile, 50 : 50, v/v) to 50 μL of blank plasma. Consequently, the final concentration of calibration standards solutions were 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 ng/mL for Thp, Thb, and Thc; 0.2, 0.4, 1, 2, 4, 10, 20, and 40 ng/mL for Cde and Bcol; and 1, 2, 5, 10, 20, 50, 100, and 200 ng/mL for Bcin. Quality control (QC) samples at low, medium, and high concentrations (0.1, 2, and 16 ng/mL for Thp, Thb, and Thc; 0.2, 4, and 32 ng/mL for Cde and Bcol; and 1, 20, and 160 ng/mL for Bcin.) were obtained in the same way. All of the above calibration standards solutions and QC samples were freshly prepared each analyzing day.

2.6. Sample Pretreatment. 50 μL of plasma samples were spiked with 5 μL of methanol, 5 μL of IS working solution, and 150 μL methanol-acetonitrile (50 : 50, v/v) to a Eppendorf tube, vortexed for 2 min, and centrifuged at 13,000 rpm for 10 min at 4°C. The supernatant was transferred to internal intubation and injected into the UPLC-MS/MS system for analysis.

2.7. Method Validation. The quantitative analysis method was validated in terms of specificity, linearity, lower limit of quantification (LLOQ), precision, accuracy, recovery, matrix effect, and stability, which was performed in accordance with the "Guideline on bioanalytical method validation" of the European Medicines Agency (EMA) [22].

2.7.1. Specificity. Specificity was assessed by the comparison of the MRM chromatograms of drug-free blank plasma, drug-free blank plasma spiked with six analytes and IS, and plasma samples acquired after oral administration of the three YHZT preparations.

2.7.2. Linearity and LLOQ. The linearity of the assay was assessed by analyzing the calibration curves in plasma using a weighted ($1/\chi^2$) linear regression of the peak area ratios of these analytes to the IS versus the nominal concentrations of the calibration standards. The LLOQ was determined as at least 10 times of the signal/noise ratio, which was defined as the lowest concentration on the calibration curve that can be quantitated with accuracy and precision less than 20%.

2.7.3. Precision and Accuracy. To assess the intraday accuracy and precision of the method, with QC samples at three concentrations (low, medium, and high), and six replicates independently prepared at each concentration. Similarly, the interday accuracy and precision was evaluated the QC samples on three consecutive days. The precision was calculated from the relative standard deviation (RSD) of the replicates, and the accuracy was calculated by comparison of measured value of QC samples with the theoretical value (relative error, RE).

2.7.4. Extraction Recovery and Matrix Effect. The recovery efficiency of the six analytes from plasma samples was estimated by comparing the peak areas of QC samples at three concentrations with that of post-extracted QC samples spiked with the analytes and IS in corresponding concentrations. The matrix effects were determined by comparing the peak areas of post-extracted spiked QC samples at three concentrations with the standard working solutions in corresponding concentrations. Meanwhile, the extraction recoveries and matrix effects of IS (at 10 ng/mL) were evaluated by the same method.

2.7.5. Stability. The QC samples at three concentrations in different conditions, including room temperature storage (25°C for 4 h); place in the autosampler (4°C for 24 h); three freeze-thaw cycles, and long term storage (−80°C frozen for 30 days) were evaluated the stability of these analytes in dog plasma.

2.8. PK Study. Six male beagle dogs were randomly divided into groups and self-crossover controlled trial design was used. Each dog was given a single clinical equivalent dose of the three YHZT preparations, fasted for 12 h before administration, drank freely, and crossed after a cleaning period of 1 week. Blood samples were collected into heparinized Eppendorf tubes from forelimb vein at 0.083, 0.167, 0.333, 0.667, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 48 h after oral administration and then immediately centrifuged at 3500 r/min for 10 min. The supernatant were separated and stored at −80°C until analysis.

Phoenix WinNonlin 6.1 was applied to fit the data; the PK parameters calculations were performed by a non-compartment analysis. The PK characteristics of different YHZT preparations were compared with maximum concentration time (T_{max}), maximum plasma concentration (C_{max}), area under the plasma drug concentration–time curve (AUC), mean residence time (MRT) terminal half-life value ($T_{1/2}$), clearance (CL), and apparent volume of distribution (Vd). The results were expressed as mean ± standard deviation. SPSS 22 was applied to statistical analysis, and one-way ANOVA was used to compare the differences between multiple groups. Before statistics, the data were tested for normal distribution and variance homogeneity, and the skew distribution data were analyzed by Mann–Whitney U test. $P < 0.05$ indicates that the difference was statistically significant.

3. Results and Discussion

3.1. UPLC-MS/MS Condition Optimization. Acetonitrile-water (containing 0.1% formic acid) was selected as the mobile phase system for simultaneous quantitative analysis of the six analytes. In order to achieve better peak shapes and a suitable run time, a gradient elution was employed. The addition of formic acid can effectively improve the peak shape of the analytes. As Figure 2 shows under the developed chromatographic condition, all analytes and IS were eluted out within 9 min.

The full scanning results of precursor ion have shown that all analytes showed higher sensitivity in positive ion mode than in negative ion mode. The most abundant quasimolecular ion for the analytes and IS $[M+H]^+$. The selection of IS is also a very important part for the stability and accuracy of quantitative results. It is reported [23] that promethazine hydrochloride showed appropriate chromatographic behaviors and good response in positive mode when it as IS for the *in vivo* quantitative analyses of YHZT preparations. Therefore, it was selected as the IS for the determination of the six analytes. Through optimize mass spectrometry conditions (cone and CE), the quantitative ion pairs with the best abundance were obtained. The MS/MS conditions are shown in Table 2. In MS/MS mode, the main fragment ions were meaning including $[M+H\text{-}C_{11}H_{14}O_2]^+$,

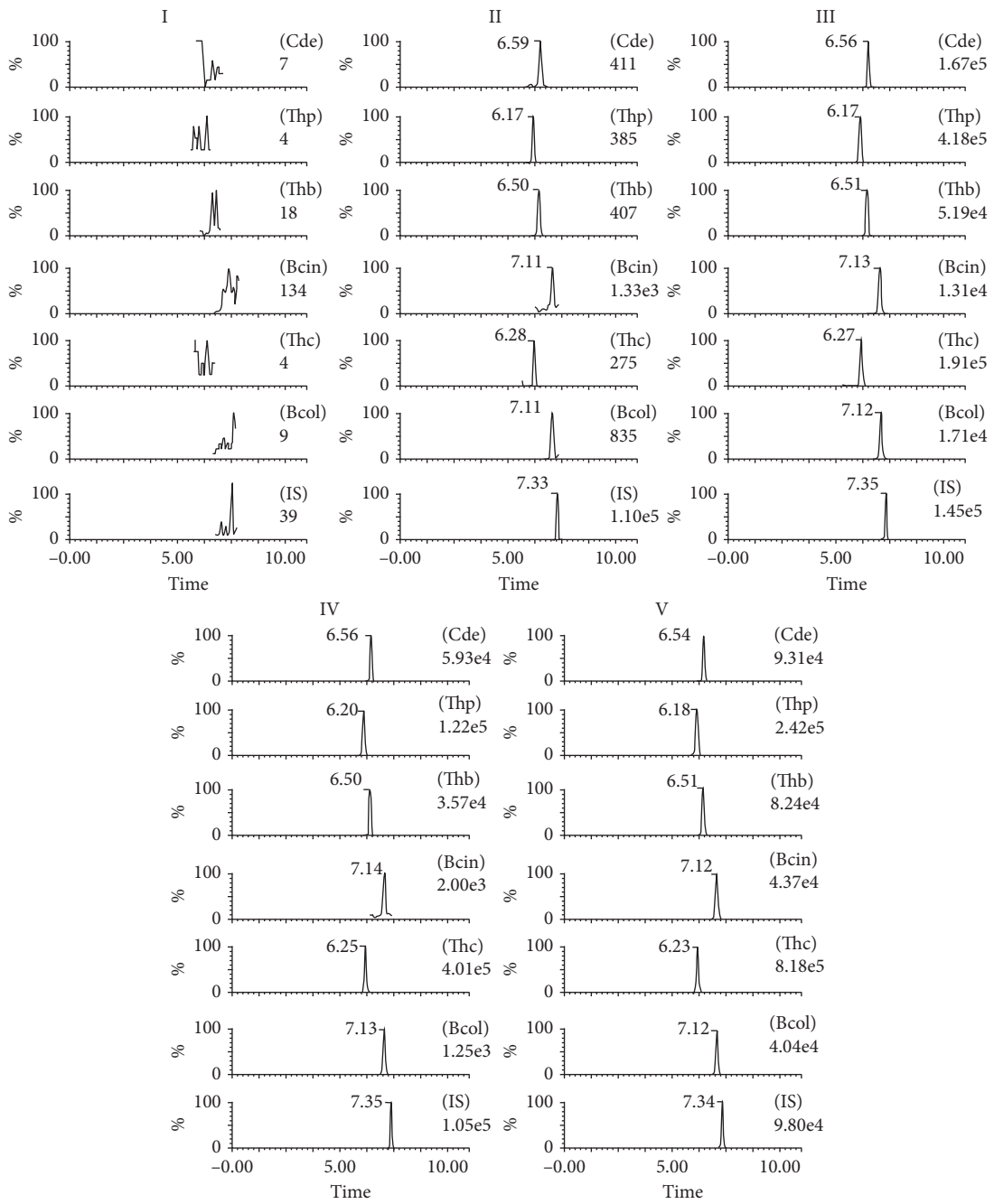

Figure 2: MRM chromatograms of the analytes and IS: (I) blank dog plasma; (II) blank dog plasma spiked with the analytes at LLOQ and IS; (III) plasma sample after oral administration of YHZT OL at 0.667 h; (IV) plasma sample after oral administration of YHZT DT at 0.667 h; (V) plasma sample after oral administration of YHZT DP at 0.667 h.

$[M + H\text{-}C_{10}H_{13}O_2]^+$, $[M + H\text{-}C_{11}H_{13}O_2N\text{-}CH_3]^+$, $[M + H\text{-}C_9H_8O_2]^+$, $[M + H\text{-}H_2O]^+$, $[M + H\text{-}C_5H_8O]^+$, and $[M + H\text{-}C_{12}H_9NS\text{-}HCl]^+$, which correspond to Cde, Thp, Thb, Thc, Bcin, Bcol, and IS, respectively. The MS/MS spectra of the analytes and IS are shown in Figure 3.

3.2. Optimization of Sample Preparation. Appropriate sample preparation methods play a very important role in the study of PK. The extraction efficiency of sample pretreatment method was investigated by liquid-liquid extraction (ethyl acetate and chloroform) and protein precipitation (methanol, acetonitrile, and their equal volume mixture). The results have shown that the extraction recovery of liquid-liquid extraction for the analytes and IS was very low, and the operation process was very complex. However, protein precipitation with methanol-acetonitrile (50 : 50, v/v) is easier to operate and the resulting precipitation is more uniform and thorough, which also has shown good response, high recovery, and lower matrix effect for all the analytes and IS in plasma samples. By comparison, a one-step protein precipitation with 3 fold of

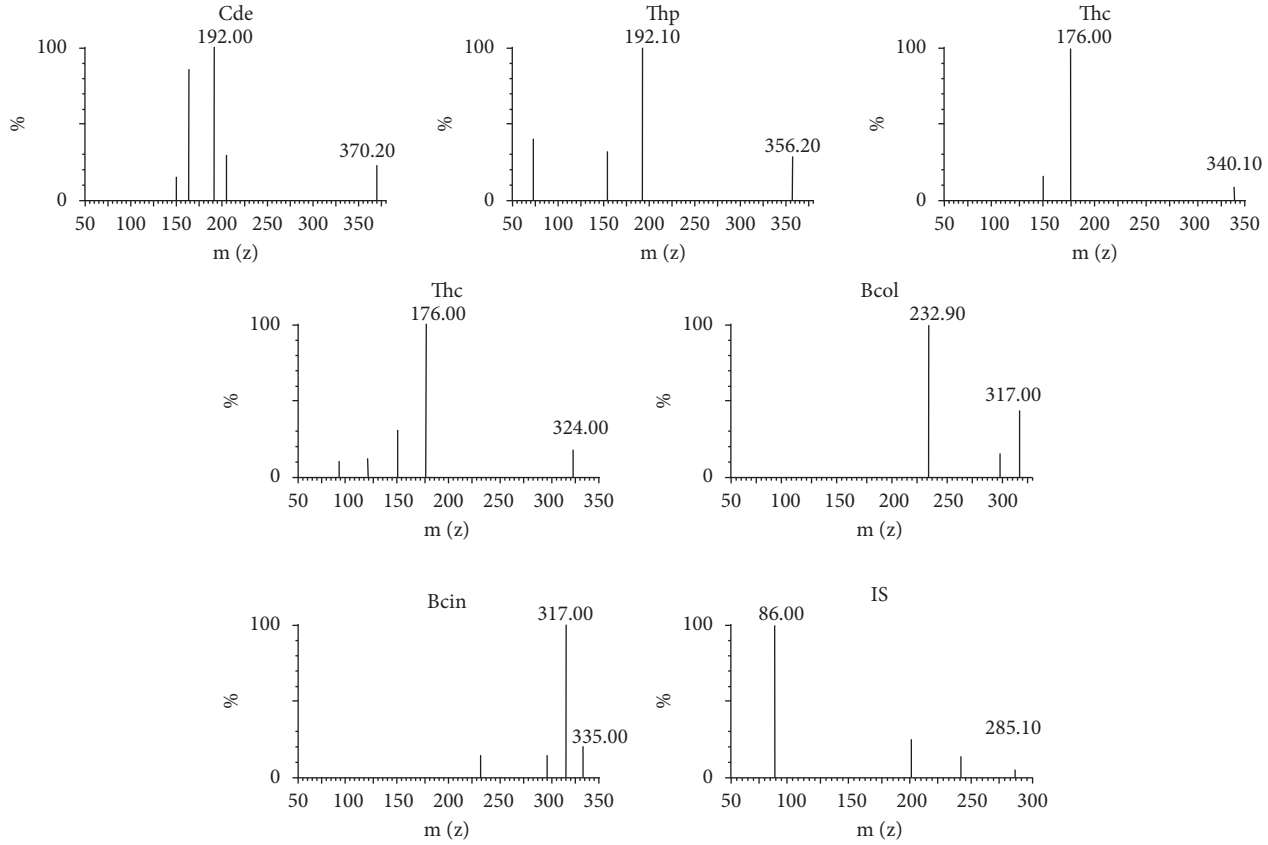

Figure 3: The MS/MS spectra of the analytes and IS.

methanol-acetonitrile (50 : 50, v/v) was selected for the sample preparation.

3.3. Method Validation

3.3.1. Specificity. Typical MRM chromatograms of blank plasma, blank plasma spiked with the six analytes and IS, and plasma samples at 0.667 h after oral administration of the three YHZT preparations are shown in Figure 2. There were no significant metabolites and endogenous components in plasma interfere with the chromatographic peaks of the analytes and IS.

3.3.2. Linearity and LLOQ. The regression equation, retention time, linear ranges, and LLOQs of Cde, Thp, Thb, Thc, Bcin, and Bcol are listed in Table 4. All correlation coefficients exceeded 0.9920, which indicate that the linear relationship of these analytes was good within the set concentration range. The LLOQ of Cde, Thp, Thb, Bcin, Thc, and Bcol were Cde 0.2, 0.1, 0.1, 1, 0.1, and 0.2 ng/mL, respectively.

3.3.3. Precision and Accuracy. The intra- and interday precision and accuracy of the analytes in QC samples at three concentrations are shown in Table 5. The precision was less than 10% and the accuracies were in the range of −13.13% to 10%, which means the developed method was accurate, reliable, and reproducible for quantitative analysis of these analytes in plasma samples.

3.3.4. Extraction Recovery and Matrix Effect. Table 6 shows the extraction recoveries and matrix effects of the six analytes (at three concentrations) and IS (at 10 ng/mL). The extraction recoveries of these analytes were in the range of 99.50% to 125.63%, and the matrix effects were within the range of 82.85 to 118.44%. Moreover, for IS, the matrix effect was 112.06% and the extraction recovery was 125.50%. These results have indicated that the pretreatment method was effective and stable, and there was no obvious ion suppression or enhancement in the analytical process.

3.3.5. Stability. The stability results of the six analytes in plasma samples under different storage conditions are summarized in Table 7. The data indicated that these analytes were relatively stable in dog plasma after short-term storage, post-preparation in autosample, three freeze-thaw cycles, and long term storage.

3.4. PK Study. The validated UPLC-MS/MS method was well applied to the comparative PK study of Cde, Thp, Thb, Thc, Bcin, and Bcol in dog plasma after oral administration of YHZT OL, DT, and DP. The quantitative result was used to generate PK parameters. The data are listed in Table 8, and

Table 4: The regression equation, retention time, linear ranges, and LLOQs of the six analytes in dog plasma.

Compound name	Regression equation ($W = 1/\chi^2$)	r	Retention time (min)	Linear range (ng/mL)	LLOQ (ng/mL)
Cde	$y = 0.27x - 0.02$	0.9972	6.58	0.2–40 ng/mL	0.2
Thp	$y = 0.45x - 0.02$	0.9962	6.17	0.1–20 ng/mL	0.1
Thb	$y = 0.65x - 0.001$	0.9928	6.49	0.1–20 ng/mL	0.1
Bcin	$y = 0.05x + 0.03$	0.9976	7.11	1–200 ng/mL	1
Thc	$y = 0.41x - 0.01$	0.9926	6.28	0.1–20 ng/mL	0.1
Bcol	$y = 0.18x - 0.02$	0.9926	7.11	0.2–40 ng/mL	0.2

Table 5: Accuracy and precision of the six analytes in dog plasma (mean, $n = 3$ days, 6 replicates per day).

Analyte	Nominal concentration (ng/mL)	*Intraday*			*Interday*		
		Measured concentration (ng/mL)	Precision (% RSD)	Accuracy (% bias)	Measured concentration (ng/mL)	Precision (% RSD)	Accuracy (% bias)
Cde	0.2	0.20	0.00	0.00	0.20	0.00	0.00
	4	4.20	3.30	5.00	4.00	6.30	0.00
	32	31.80	3.50	−0.62	31.40	5.50	−1.88
Thp	0.1	0.10	0.00	0.00	0.10	0.00	0.00
	2	2.20	1.80	10.00	2.20	2.70	10.00
	16	14.30	2.70	−10.63	14.90	5.80	-6.88
Thb	0.1	0.10	0.00	0.00	0.10	0.00	0.00
	2	2.00	4.50	0.00	2.00	6.30	0.00
	16	16.00	1.80	0.00	15.70	4.50	−1.88
Bcin	1	1.00	10.00	0.00	1.00	9.20	0.00
	20	21.10	3.40	5.50	21.20	3.80	6.00
	160	139.00	1.90	−13.13	143.70	5.30	−10.19
Thc	0.1	0.10	0.00	0.00	0.10	0.00	0.00
	2	2.10	3.60	5.00	2.10	5.50	5.00
	16	15.80	1.90	−1.25	15.80	3.70	−1.25
Bcol	0.2	0.20	0.00	0.00	0.20	0.00	0.00
	4	4.20	3.20	5.00	4.20	4.60	5.00
	32	28.30	2.30	−11.56	29.20	4.50	−8.75

Table 6: Extraction recovery and matrix effect of the six analytes and internal standard in dog plasma (mean, $n = 6$).

Analyte	Spiked concentration (ng/mL)	*Extraction recovery*		*Matrix effect*	
		Mean	RSD%	Mean	RSD%
Cde	0.2	107.32	12.50	104.47	16.90
	4	109.38	4.64	107.92	4.61
	32	105.69	1.39	103.38	2.56
Thp	0.1	99.50	11.06	105.68	8.47
	2	114.02	3.57	111.99	4.50
	16	106.07	2.35	107.07	2.36
Thb	0.1	109.11	16.12	84.11	13.05
	2	111.57	2.80	85.50	6.10
	16	105.43	1.24	86.89	1.94
Bcin	1	119.52	6.53	118.44	9.96
	20	109.55	4.82	112.60	6.38
	160	109.19	2.95	106.22	4.91
Thc	0.1	108.91	9.58	87.03	19.15
	2	109.92	3.42	90.21	5.29
	16	107.02	2.40	85.43	2.49
Bcol	0.2	125.63	10.29	87.59	7.84
	4	120.47	5.06	82.85	5.08
	32	107.05	0.99	85.43	5.19
Is	10	125.50	6.43	112.06	2.75

Table 7: Stability of the six analytes in dog plasma under different conditions (mean, $n = 6$).

Analyte	Spiked concentration (ng/mL)	*25°C for 4 h*		*4°C for 24 h*		*Three freeze-thaw cycles*		*−80°C frozen for 30 days*	
		Accuracy (%bias)	Precision (%RSD)	Accuracy (%bias)	Precision (%RSD)	Accuracy (%bias)	Precision (%RSD)	Accuracy (%bias)	Precision (%RSD)
Cde	0.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	2.08	2.90	7.22	5.30	1.67	5.10	−7.92	3.20
	32	9.43	6.50	5.50	5.20	−4.06	2.80	−3.28	2.40
Thp	0.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	−1.67	5.30	0.28	6.70	5.83	4.60	5.00	4.30
	16	4.06	9.30	1.08	5.10	−12.19	3.10	−9.17	3.50
Thb	0.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	−4.17	7.70	−5.56	4.40	−2.50	5.40	−9.17	6.40
	16	3.33	9.30	−1.08	8.00	−0.63	1.40	−5.42	4.90
Bcin	1	8.33	9.10	1.11	7.50	5.00	5.20	−3.33	8.40
	20	−0.83	3.00	1.00	6.50	11.92	2.40	6.92	6.30
	160	0.91	8.70	−2.34	4.60	−7.22	2.70	−11.73	1.10
Thc	0.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.83	7.30	−0.56	6.90	4.17	4.70	−7.50	6.60
	16	1.35	8.90	−4.62	4.50	−4.27	3.50	−7.81	3.10
Bcol	0.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	5.00	2.10	6.25	3.70	8.75	2.40	6.67	5.70
	32	−1.15	7.60	−3.65	4.50	−9.53	2.50	−10.36	1.30

Table 8: PK parameters of the six analytes after oral administration of YHZT OL, YHZT DT, and YHZT DP (mean ± SD, $n = 6$).

Analyte	Group	T_{max} (h)	C_{max} (NG/ML)	$T_{1/2}$ (h)	$AUC_{0\text{-}t}$ (NG H/ML)	$MRT_{0\text{-}t}$ (h)	Vd (ML/KG)	CL (ML/H/KG)
Cde	YHZT OL	0.44 ± 0.17	36.75 ± 12.67	15.96 ± 6.24	74.96 ± 20.43	7.05 ± 1.54	67.93 ± 39.84	2.83 ± 0.67
	YHZT DT	0.83 ± 0.62	18.78 ± 10.85*	22.47 ± 6.57	65.94 ± 17.18	11.06 ± 1.25*	123.43 ± 44.49*	3.84 ± 1.13
	YHZT DP	0.56 ± 0.17	30.77 ± 10.80	26.86 ± 11.17*	92.14 ± 24.83	12.00 ± 2.45*	59.50 ± 20.14	1.64 ± 0.58*
Thp	YHZT OL	0.42 ± 0.20	80.83 ± 31.40	7.81 ± 0.50	254.31 ± 82.50	7.30 ± 1.37	11.42 ± 3.98	1.01 ± 0.34
	YHZT DT	1.33 ± 1.30	45.70 ± 20.34*	7.94 ± 0.67	179.81 ± 51.98	7.69 ± 1.67	11.15 ± 4.24	0.96 ± 0.31
	YHZT DP	0.61 ± 0.25	52.50 ± 15.23*	7.55 ± 2.63	172.35 ± 62.79*	5.23 ± 2.64	12.01 ± 6.07	1.07 ± 0.40
Thb	YHZT OL	0.31 ± 0.19	7.42 ± 3.67	2.58 ± 1.36	6.79 ± 3.14	1.57 ± 0.42	15.05 ± 7.72	5.01 ± 4.16
	YHZT DT	0.72 ± 0.65	4.53 ± 2.62	4.52 ± 2.05	8.60 ± 4.01	3.40 ± 1.37*	30.09 ± 22.46	4.58 ± 2.45
	YHZT DP	0.53 ± 0.22	9.87 ± 4.64	4.60 ± 4.53	14.24 ± 6.99	2.63 ± 1.24	14.99 ± 11.72	2.52 ± 1.03
Bcin	YHZT OL	0.31 ± 0.19	21.57 ± 10.39	0.27 ± 0.05	10.56 ± 3.63	0.43 ± 0.08	9.99 ± 4.03	25.76 ± 8.49
	YHZT DT	ND	ND	ND	ND	ND	ND	ND
	YHZT DP	0.50 ± 0.18	44.48 ± 19.09	0.45 ± 0.40	28.86 ± 13.06*	0.59 ± 0.18	5.13 ± 6.78	6.54 ± 2.28*
Thc	YHZT OL	0.31 ± 0.19	49.17 ± 17.76	1.94 ± 0.38	58.28 ± 22.30	1.72 ± 0.15	1.08 ± 0.66	0.38 ± 0.20
	YHZT DT	0.72 ± 0.65	130.67 ± 66.39*	1.68 ± 0.39	222.98 ± 96.04*	2.30 ± 0.93	0.82 ± 0.43	0.34 ± 0.18
	YHZT DP	0.56 ± 0.17	201.33 ± 114.79*	2.49 ± 1.29	329.31 ± 188.70	2.08 ± 0.38	0.68 ± 0.48	0.18 ± 0.09*
Bcol	YHZT OL	0.31 ± 0.19	4.30 ± 2.10	0.27 ± 0.05	2.16 ± 0.73	0.44 ± 0.08	15.68 ± 6.72	39.44 ± 11.44
	YHZT DT	ND	ND	ND	ND	ND	ND	ND
	YHZT DP	0.50 ± 0.18	8.58 ± 3.98	0.49 ± 0.49	5.74 ± 2.62	0.63 ± 0.14	11.99 ± 17.04	13.69 ± 4.75*

*$P < 0.05$, compared with oral liquid. ND, nondetected value.

the average plasma concentration-time curve is illustrated in Figure 4.

As shown in Table 8, compared with the other two groups, Cde, Thp, Thb, Bcin, Thc, and Bcol were more rapidly absorbed in YHZT OL group, and all of them reached the C_{max} within 1 hour. The result was consistent with some previous studies [24, 25]. Because the alkaloids in YHZT prescription have the characteristic of large fat solubility and easy permeability to cell membrane, it has been previously reported which are easy to dissolve in blood, and the absorption begins in the stomach [26].

The contents of Cde and Thp in the three preparations were similar (Table 3), while the value of C_{max} of the two analytes after oral administration YHZT OL was higher than that of in DT ($P < 0.05$) and DP. However, the rest four components were just the opposite (OL *versus* DP). Bcin and Bcol were not detected in the YHZT DT group, and the C_{max} of the two in OL and DP was also very low, which may be related to its structural characteristics that are not easy to be absorbed *in vivo*. [27] In addition, as shown in Table 3, due to the production process, the content of Bcol in DT was very low. Although the *in vitro* content of Cde and Thp is similar; on the contrary, one can observe that the bioavailability

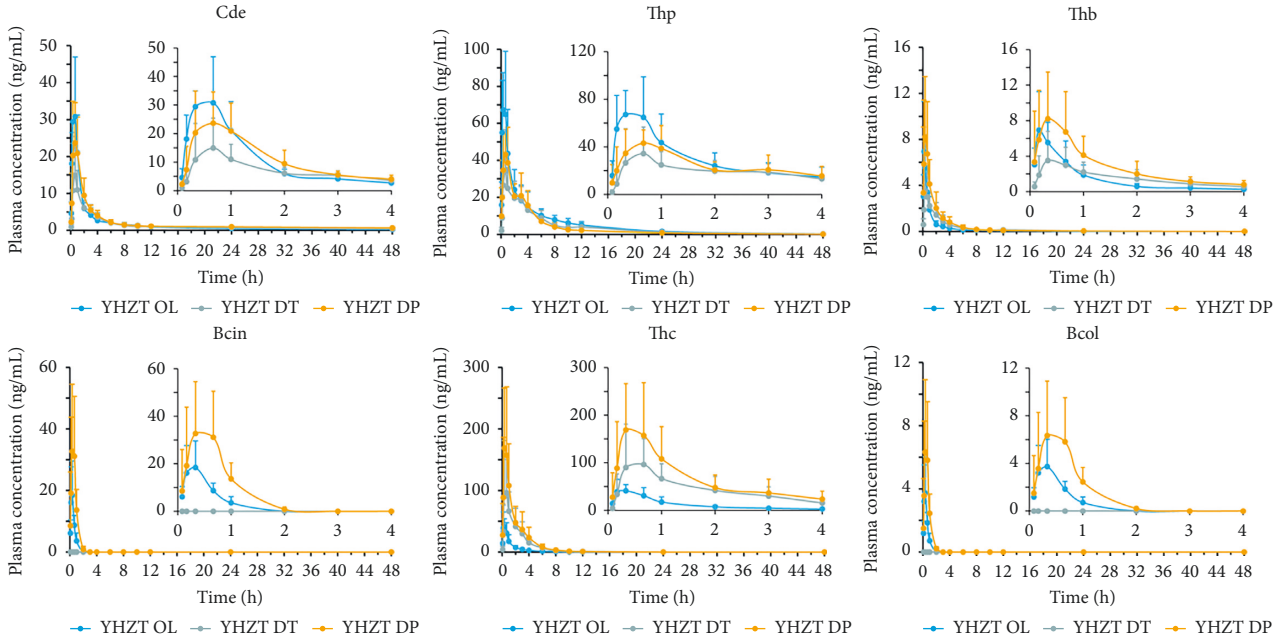

Figure 4: Mean plasma concentration-time curve of the analytes in dog plasma after oral administration of YHZT OL, DT, and DP.

(AUC and C_{max}) of Thp are significantly higher than those of Cde *in vivo*. This phenomenon is consistent with previous reports, which may explain that Thp is the main analgesic component of CR. [28] Although the Thc was not high in the three prescriptions, but it's *in vivo* exposure level did increase significantly, and the absorption intensity in solid preparations was significantly higher ($P < 0.05$) than that of in YHZT OL. These results may be related to the differences of the processing of the three preparations and the changes of PK behavior caused by the physicochemical properties of various components or theirs *in vivo* interactions. [29–31]

The high Vd values of Cde in all groups means that it was easily absorbed and distributed into tissues after oral administration, which was in conformity with the previous reference [32, 33]. It can be seen from Table 8, the $T_{1/2}$ and MRT of active ingredients in OL is shorter than that of solid preparation, indicates that the drug components in OL were easily released and absorbed. The CL of these six compounds in OL group was higher than the other two groups, suggesting that the elimination of drug in OL was fast. It is widely known that OL does not need to disintegrate, and can direct release drug components *in vivo*. Moreover, it is rich in high polar chemicals but poor in low polar compounds. Compared with solid preparations, OL was absorbed more rapidly, distributed more widely and eliminated faster, which can exert its efficacy quickly. On the contrary, solid preparations have a relatively slow release pattern.

In summary, the mentioned results have indicated that the statistically significant differences of PK parameters of different preparations were mainly reflected in the C_{max}, AUC, $T_{1/2}$, MRT, CL, and Vd.

4. Conclusions

In this paper, a rapid, reliable, and sensitive UPLC-MS/MS method was developed and validated for simultaneous determination of six analytes including Cde, Thp, Thb, Thc, Bcin, and Bcol in beagle dog plasma, and the method was successfully applied to the comparative study on PK after oral administration of YHZT OL, DT and DP. The results showed that there are significant differences in the PK parameters among the three groups. Our findings demonstrated the pharmaceutical dosage form is of great significance for the exertion of drug efficacy. In clinical application, appropriate preparations should be selected according to the different state of an illness, so as to give full play to its maximum therapeutic effect and avoid adverse reactions. This study will play an important role in more reasonable selection of the YHZT preparations and better ensure their efficacy in clinics.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Yang Wang and Zhaorui Yin contributed equally to this work.

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