

# Study on Integrated Pharmacokinetics of the Component-Based Chinese Medicine of *Ginkgo biloba* Leaves Based on Nanocrystalline Solid Dispersion Technology

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**Background:** To improve the dissolution and bioavailability of the component-based Chinese medicine of *Ginkgo biloba* leaves (GBCCM), a novel nanocrystalline solid dispersion of GBCCM (GBCCM NC-SD) was first prepared.

**Methods:** GBCCM mainly containing high pure flavonoid aglycones (FAs) and terpenoid lactones (TLs) was used as the model drug. PVP K30 and SDS were used as solubilizers, combined stabilizers and carriers, and GBCCM NC-SD was prepared by high-pressure homogenization combined with freeze-dryer. Morphology and crystal characteristic of GBCCM NC-SD were analyzed. The dissolution and bioavailability evaluation were performed to investigate the feasibility of GBCCM NC-SD by in vitro dissolution and in vivo integrated pharmacokinetic models.

**Results:** After homogenizing for 30 cycles under the pressure of 650 bar and freeze-drying, GBCCM NC-SD with uniform quality would be obtained. The particle size, PDI and zeta potential were found to be  $335.9 \pm 32.8$  nm,  $0.29 \pm 0.02$  and  $-28.4 \pm 0.7$  mV respectively. Based on charged aerosol detector (CAD) technology, a new chromatographic method for simultaneous detection of eight components in GBCCM was developed. In vitro drug release study showed that the cumulative dissolution of FAs and TLs in GBCCM NC-SD increased from 12.77% to 52.92% ( $P < 0.01$ ) and 90.91% to 99.21% ( $P < 0.05$ ) respectively. In comparison with physical mixture of GBCCM and stabilizer (PM), the integrated pharmacokinetics  $AUC_{0-t}$  of FAs and TLs in GBCCM NC-SD were significantly increased ( $P < 0.05$ ), and the  $T_{1/2}$  of TLs was also significantly prolonged ( $P < 0.05$ ).

**Conclusion:** This study demonstrated that novel GBCCM NC-SD was prepared using Polyvinylpyrrolidone K30 (PVP K30) and Sodium dodecyl sulfate (SDS) as a synergetic stabilizer and also provided a feasible way to improve the dissolution and oral bioavailability of poorly soluble candidate antihypertensive drugs.

**Keywords:** *Ginkgo biloba*, flavonoid aglycones, terpenoid lactones, nanocrystalline solid dispersion, integrated pharmacokinetics, charged aerosol detector

## Introduction

The component-based Chinese medicine of *Ginkgo biloba* leaves (GBCCM) mainly contained high pure flavonoid aglycones (FAs, >90%) and terpenoid lactones (TLs, >95%), as prepared in the previous study. Compared with *Ginkgo biloba* extract (GBE), GBCCM had more clear chemical structures, better antihypertensive effect, and could improve the

effect of relieving left ventricular hypertrophy caused by long-term hypertension.<sup>1</sup> It had the potential to become a new drug for the treatment of hypertension complicated with ventricular hypertrophy. FAs were mainly composed of quercetin (QCT), kaempferol (KMF) and isorhamnetin (ISR); TLs were composed of ginkgolide A (GA), ginkgolide B (GB), ginkgolide C (GC), ginkgolide J (GJ) and bilobalide (BB). Preliminary study found that FAs were practically insoluble and TLs were slightly soluble, and there was almost no change in the solubility of these two components in the range of pH 1.2–7.4. Just like GBE, the low water solubility of the main components in GBCCM might lead to poor oral absorption and low bioavailability.<sup>2</sup>

Nanocrystals (NCs) had become one of the internationally recognized effective strategies to improve the oral bioavailability of insoluble drugs. It could solve the problem of oral delivery of insoluble drugs by reducing particle size, increasing specific surface area, significantly increasing the apparent solubility and dissolution of insoluble drugs, and improving bioavailability and efficacy.<sup>3–5</sup> Solid dispersion (SD) was a new technology for dispersing insoluble drugs in water-soluble carriers.<sup>6–8</sup> Because drugs were highly dispersed in molecular, amorphous or microcrystalline state, it increased the wettability of the drug, which could increase the dissolution of the drug and improve the bioavailability. If the two solubilization technologies were used together, unexpected results might be obtained.

In order to further improve the oral bioavailability of GBCCM, this study intended to use hydrophilic polymer and ionic surfactant as solubilizer, composite stabilizer and carrier, prepare nanocrystalline dispersion by high-pressure homogenization combined with freeze-drying, and investigate its dissolution characteristics *in vitro*.

Multicomponent overall pharmacokinetics was to introduce the idea of “Holistic View” into the study of pharmacokinetics.<sup>9,10</sup> According to the weight contribution of each component to the overall drug metabolism, appropriate modeling method was selected to integrate the pharmacokinetic parameters of each component, so as to obtain the maximum characterization of the overall disposal kinetic characteristics of traditional Chinese medicine *in vivo*. This study intends to use self-defined weight coefficient method according to  $AUC_{0-t}$  to investigate the integrated pharmacokinetics of GBCCM, which will provide data support for the development of new antihypertensive drugs.

## Materials and Methods

### Materials

Hydroxypropyl cellulose (HPC)-SL was obtained from Japan Caoda Co. Ltd (Hong Kong, China). Hydroxypropyl methylcellulose E5 (HPMC E5) was purchased from Dow Chemical Company (Michigan, USA). Kolliphor HS 15, Poloxamer 188 and Polyvinylpyrrolidone K30 (PVP K30) were generously provided by BASF (China) Ltd. (Shanghai, China). Sodium dodecyl sulfate (SDS) was purchased from Hunan Erkang Pharmaceutical Co. Ltd. (Changsha, China). Ginkgolide A (GA  $\geq 95\%$ ), ginkgolide B (GB  $\geq 95\%$ ), ginkgolide C (GC  $\geq 95\%$ ), ginkgolide J (GJ  $\geq 95\%$ ), bilobalide (BB  $\geq 98\%$ ), quercetin (QCT  $\geq 98\%$ ), isorhamnetin (ISR  $\geq 98\%$ ), kaempferol (KMF  $\geq 98\%$ ) were purchased from National Institutes for Food and Drug Control (Beijing, China). Acetonitrile (HPLC grade), formic acid (MS grade), trifluoroacetic acid (HPLC grade), tetrahydrofuran (HPLC grade) and methanol (HPLC grade) were acquired from Merck (Darmstadt, Germany).

### High-Performance Liquid Chromatography (HPLC) Analysis of GBCCM

Based on charged aerosol detector (CAD) technology, this study intended to develop a new analytical method for the simultaneous detection of FAs and TLs in GBCCM. The HPLC system (Ultimate 3000, Thermo Fisher Scientific, USA) equipped with an autosampler. A reversed-phase column (150  $\times$  4.6 mm, 5 $\mu$ m; Eclipse XDB-C18) was used. The injection volume was 5  $\mu$ L. The flow rate was 1.0 mL/min. The column temperature was 40°C. The data acquisition frequency of CAD was 5 Hz, the filter was 10.0 S, and the evaporation tube temperature was set to Low. The mobile phases were listed in Table 1. System suitability, precision, specificity, linearity and accuracy were validated to ensure the accuracy of the method.

**Table 1** Gradient Elution with the Mobile Phase

T/min	0	17	18	38	39	45
A (Tetrahydrofuran: Water: Methanol =10: 65: 25)	82	82	0	0	82	82
B (0.01% Formic acid)	18	18	52	52	18	18
D (Methanol)	0	0	48	48	0	0

## Effects of Different Stabilizers on the Solubility of FAs and TLs in GBCCM

The apparent solubility of FAs and TLs were measured in phosphate buffer (pH 6.8) with PVP K30, SDS, HPMC E5, Poloxamer 188 and HPC-SL. Weighed 20mg of analyte and stabilizer respectively and added them to the centrifuge tube containing 1mL of phosphate buffer (pH 6.8). These centrifugal tubes were sonicated for 30 minutes in  $37 \pm 0.5^\circ\text{C}$  to ensure adequate dissolution. The sample was centrifuged at 12,000 r/min for 10 min to remove the undissolved substance. The supernatant was used to determine the content of FAs and TLs according to the method described in “2.1”. Each sample was measured in triplicate.

## Preparation of GBCCM Nanocrystalline Solid Dispersion (NC-SD)

GBCCM nanosuspension was first prepared by a high-pressure homogenizer (SCIENTZ-150, Ningbo Scientz Biotechnology CO., LTD, Zhejiang, China). It was found that when the ratio of the primary stabilizer to FAs was 1:1, nanocrystals with small particle size and high drug loading could be obtained in the preliminary experiment. The secondary stabilizer could help improve the stability of the suspension, and the addition quantity was generally about 0.05%.<sup>11</sup>

GBCCM (FAs (0.54%, w/v) and TLs (0.34%, w/v)), primary stabilizer (0.54%, w/v) and secondary stabilizer (0.05%, w/v) were dispersed in aqueous solution (50mL) by ultrasonic dispersion method. The GBCCM nanosuspension with appropriate particle size could be obtained by homogenizing 30 times under the pressure of 650 bar. Then, the nanosuspension was pre-frozen for 8h in the  $-20^\circ\text{C}$  refrigerator and dried using a freeze dryer (FDU-1100, Tokyo Rikakikai CO., LTD., Tokyo, Japan). The lyophilization process was performed for 48 h at  $-60^\circ\text{C}$  under a vacuum pressure of 100 mTorr. Finally, the GBCCM nanocrystalline solid dispersion (NC-SD) was obtained by crushing through a 100-mesh sieve.

Accurately weigh an appropriate amount of GBCCM NC-SD ( $W_1$ ). Methanol was added to make it completely dissolved, and then filtered through 0.22  $\mu\text{m}$  membrane. The concentration of each component was detected according to the method of “2.1”, and converted into the weight ( $W_2$ ) of the total effective component in GBCCM NC-SD. The drug loading was calculated according to the formula, drug loading =  $W_2 / W_1 * 100\%$ . Each sample was analyzed in triplicate.

## Characterization of GBCCM NC-SD

### Particle Size, Polydispersity Index and Zeta Potential

The mean particle size and polydispersity index (PDI) of GBCCM NC-SD were measured using a Zetasizer Nano ZS system (Nano-ZS, Malvern, UK). Zeta potential value was measured by laser doppler micro-electrophoresis technique using the same instrument. GBCCM NC-SD was re-dispersed with deionized water to form nanosuspension and further diluted to achieve a suitable concentration for analysis. Each sample was analyzed in triplicate.

### Powder X-Ray Diffraction Analysis (PXRD)

The crystal types of FAs, TLs, physical mixture of GBCCM and stabilizers, GBCCM NC-SD, stabilizers and freeze-dried product of stabilizers were determined by powder X-ray diffractometry (PXRD) (Empyrean, Panalytical, Netherlands) with Cu-K $\alpha$  radiation operated at 40 kV and 25 mA. Data were obtained in the range of  $3-50^\circ$  ( $2\theta$ ) at  $2^\circ$  /min with a step of  $0.05^\circ$ .

## Scanning Electron Microscopy (SEM)

The micro-morphology structures of FAs, TLs, blank excipient, physical mixture of GBCCM and excipients, lyophilized physical mixture of GBCCM and excipients, GBCCM NC-SD and lyophilized centrifugal precipitation of GBCCM nanosuspension were observed using a scanning electron microscope (SEM) (EM-30Plus, COXEM, Korea). The samples were fixed on the stubs using double-side adhesive tape, and sputter coated with gold-palladium to make a thickness of 10 nm before observing.

## In vitro Dissolution Study

Dissolution studies of GBCCM, GBCCM NC-SD, physical mixture of GBCCM and stabilizers (PM) were performed in automated dissolution test apparatus (UDT 812A-12, Logan Instrument. Corp., Somerset, New Jersey, USA). The temperature and paddle speed were set at  $37 \pm 0.5^\circ\text{C}$  and 100 rpm, respectively. The dissolution media was 0.5% SDS phosphate buffer (pH 6.8). The samples (equivalent to 27.0 mg flavone aglycones and 17.0 mg terpene lactones) were added into the vessels containing 500 mL dissolution medium.<sup>12</sup> 3 mL of dissolution medium were withdrawn at the predetermined time intervals of 5, 10, 20, 30, 45, and 60 min, and immediately add equal volume of fresh dissolution medium to the vessels. The collected sample was centrifuged at 12,000 g for 10 min to remove the undissolved drug. The supernatant was used to determine the concentration of each component according to the method of “2.1”. Each sample was measured in triplicate.

## In vivo Bioavailability

### Administration Method and Sample Collection

Male Sprague-Dawley rats (Weight:  $300 \pm 20$  g) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (Beijing, China; animal license number: SCXK [Zhe] 2019–0001). All animal experimental procedures were carried out according to the Guide and Use of Laboratory Animals and approved by the Ethics Committee for Experimental Animals at State Key Laboratory of Generic Manufacture Technology of Chinese Traditional Medicine (Approved on November 18th, 2021; No. NH-IACUC-2021-54) for minimizing animal suffering.

Fifteen rats were randomly separated into three groups, GBCCM group (GBCCM was dissolved with 5% HS-15, iv), PM group (physical mixture of GBCCM and stabilizer was dissolved with 0.5% CMC-Na, ig), GBCCM NC-SD group (GBCCM NC-SD was dissolved with purified water, ig). The rats had free access to water in 12 h before the experiment. Injection administration of GBCCM at dose of 14.65 mg/kg and oral administration of PM and GBCCM NC-SD at doses of 88.00 mg/kg. 0.3 mL of blood samples were collected in heparinized tube from the subclavian vein at the predetermined time intervals of 0.033, 0.167, 0.5, 1, 2, 4, 8, 24 h (Injection group) and 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24 h (Oral group), respectively. The plasma was separated by centrifuging the blood sample at 4000 r/min for 10 min and stored at  $-20^\circ\text{C}$  until further analysis.

### Plasma Sample Pretreatment

150  $\mu\text{L}$  of extracting solvent of acetonitrile was added to each 50  $\mu\text{L}$  plasma sample. The mixture was vortexed for 10 min and centrifuged at 9000 r/min for 10 min. The supernatant was used to determine the content of each component.

### Analysis Method

A UPLC-QTRAP-MS/MS (SCIEX QTRAP5500+, SCIEX, USA) method was developed for the studies of pharmacokinetics (PK) of BB, GA, GB, GJ, GC, QCT, KMF and ISR concentrations in rat plasma. Specificity, linearity, quantification limits, precision and accuracy had been validated to ensure the reliability of the UPLC-ESI-MS/MS method.

The liquid chromatographic separation of all analytes was carried out on a Thermo Hypersil Gold analytical column ( $2.1 \times 100$  mm,  $1.9 \mu\text{m}$ ) with 0.1% formic acid-water as phase A and methanol as phase B. The flow rate was 0.2 mL/min. The column temperature was  $40^\circ\text{C}$ , and the injection volume was 2  $\mu\text{L}$ . The gradient elution is listed in Table 2.

The active ingredients in GBCCM were analyzed by electron spray ionization mass spectrometry (ESI-MS) and the scan range was  $m/z$  100–1500. Curtain gas: 35 psi; Ionspray voltage:  $-4500$  V; Collision gas: 10 psi; Temperature:  $450^\circ\text{C}$ ; Ion source gas 1 (spray gas): 45 psi; Ion source gas 2: 45 psi. Data acquisition was performed using multiple



**Table 2** Gradient Elution Program of 0.1% Formic Acid-Water as Phase A and Methanol as Phase B

T/min	Phase A /%	Phase B /%
0	80	20
0.5	80	20
5	10	90
9	10	90
9.1	80	20
10	80	20

reaction monitoring (MRM) of the active ingredients under negative ionization mode. Optimized mass parameters MRM (m/z), Fragmentor voltage, Collision energy and the structures of each analyte are shown in Table 3 and Figure 1.

### Analysis of Pharmacokinetic (PK) Parameters

The plasma concentrations of BB, GA, GB, GC, GJ, QCT, KMF and ISR were calculated according to the method described in “2.7.4” and the concentration–time curves were drawn. Pharmacokinetics parameters such as half-life ( $T_{1/2}$ ), areas under the curve (AUC) and mean residence time (MRT) were estimated using DAS 2.1.1 software package edited by the Mathematics Pharmacological Committee of the Chinese Pharmacological Society.

### Establishment of GBCCM Multi-Component Integrated PK Model Based on Self-Defined Weight Coefficient According to Area Under Curve ( $AUC_{0-t}$ )

Self-define the weight coefficient of each compound in the comprehensive concentration according to the ratio of each compound in the total  $AUC_{0-t}$  of all components. The blood concentration of each compound at each time point was assigned with their respective weight coefficients to calculate the comprehensive concentration of FAs and TLs, and then the integrated pharmacokinetic parameters were further estimated. The calculation formula of self-defined weight coefficient and comprehensive concentration of each component in GBCCM was as follows:

$$\omega_i = \frac{AUC_{0-t_i}}{\sum_{i=1}^n AUC_{0-t_i}} (n = 3) \quad (1)$$

$$\omega_j = \frac{AUC_{0-t_j}}{\sum_{j=1}^m m AUC_{0-t_j}} (m = 5) \quad (2)$$

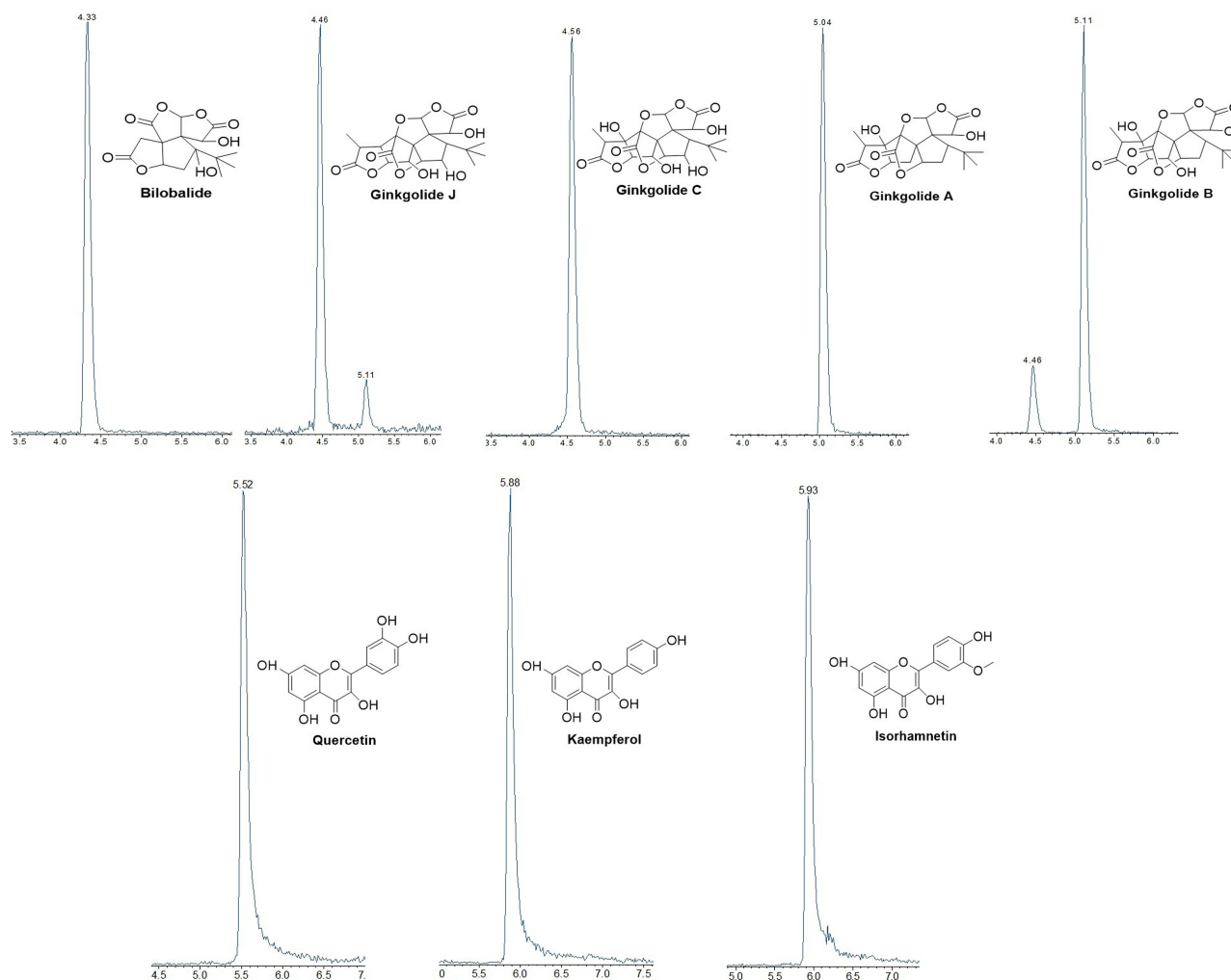
$$C_{TFAs} = \omega_1 \times C_1 + \omega_2 \times C_2 + \dots + \omega_n \times C_n (n = 3) \quad (3)$$

$$C_{TTLs} = \omega_1 \times C_1 + \omega_2 \times C_2 + \dots + \omega_m \times C_m (m = 5) \quad (4)$$

**Table 3** MRM Quantitative Parameters of the Active Ingredients in GBCCM

Components	M m/z	Ion Pair m/z	DP (V)	CE (V)
Ginkgolide A	408.3	351.3	-114	-20
Ginkgolide B	424.3	367.3	-105	-23
Ginkgolide C	440.1	383.2	-115	-22
Ginkgolide J	424.1	349.2	-112	-31
Bilobalide	326.2	163.1	-90	-27
Quercetin	302.0	151.0	-115	-29
Isorhamnetin	316.1	300.0	-119	-29
Kaempferol	286.0	185.0	-132	-36

**Abbreviations:** DP, declustering potential; CE, collision energy.



**Figure 1** The structures and MRM chromatograms of a blank sample spiked analytes.

*i* represented three flavonoid aglycones (1. QCT, 2. KMF, 3. ISR), and *j* represented five terpene lactones (1. BB, 2. GA, 3. GB, 4. GC, 5. GJ).  $\omega_i$  and  $\omega_j$  represented the proportions of corresponding  $AUC_{0-t}$  of each compound in the total  $AUC_{0-t}$  of three flavonoid aglycones and five terpene lactones compounds, respectively.  $C_{TFAS}$  and  $C_{TTLs}$  were the comprehensive concentrations of FAs and TLs in GBCCM after self-defined weight coefficient correction, respectively.

## Statistical Analysis

Statistical analysis was performed using SPSS software (Version 23.0; IBM SPSS Statistics Inc., Chicago, IL, USA). The results were expressed as mean  $\pm$  SD, and three repeats were performed for each experiment. One-way analysis of variance (ANOVA) was performed to evaluate the differences in mean values. Significant differences were verified by the Tukey-Kramer honestly significant difference test ( $P < 0.05$ ).

## Results and Discussion

### Validation of HPLC Methods for Detection of GBCCM

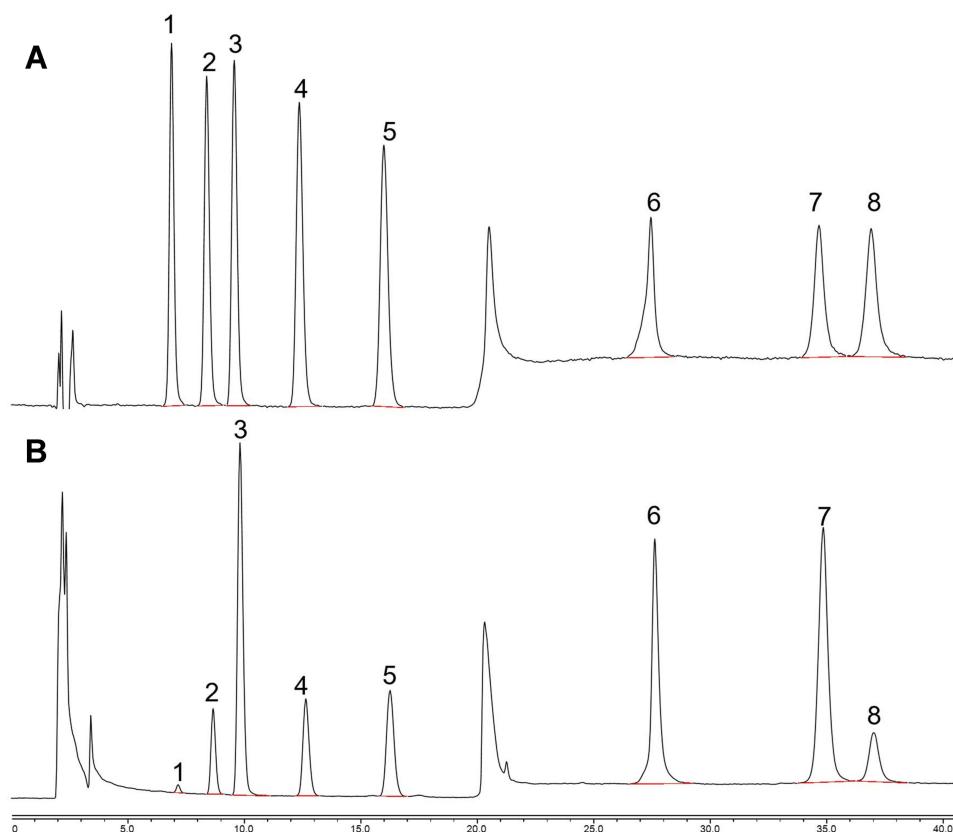
Because FAs have strong UV absorption, they were usually detected by HPLC-UV, while TLs were usually detected by HPLC-ELSD.<sup>13,14</sup> Using the two methods to detect GBCCM would bring a lot of inconvenience. Although it had been reported that HPLC-MS could simultaneously determine FAs and TLs, the test cost was high.<sup>15</sup> So, it was necessary to

develop a new method for simultaneous detection of FAs and TLs, which could not only overcome the complexity of traditional detection methods, but also avoid the high cost of MS methods.

Based on CAD detector, an analysis method for simultaneous detection of eight components in GBCCM was established in this experiment (Figure 2). The LODs of eight components, including GJ, GC, BB, GA, GB, QCT, KMF and ISR were 1.82, 2.15, 2.17, 2.00, 3.16, 1.57, 2.13 and 1.92  $\mu\text{g}\cdot\text{mL}^{-1}$ , respectively. The linear concentration ranges were 5.75~30.00  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9993$ ), 21.60~152.00  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9998$ ), 104.50~557.40  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9996$ ), 31.30~167.00  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9997$ ), 28.60~152.80  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9993$ ), 146.4~780.80  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9998$ ), 154.6~824.80  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9994$ ) and 35.40~188.60  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $r = 0.9999$ ); The average recoveries of eight components were 96.55%, 98.72%, 99.47%, 99.57%, 98.20%, 104.67%, 100.80% and 100.88%; RSDs of system suitability, stability and intra-day precision were all less than 2.0%.

## Effects of Different Stabilizers on the Solubility of FAs and TLs in GBCCM

The effects of different stabilizers on the solubility of TLs and FAs are shown in Table 4. As could be seen from the table, the saturated solubility of total flavonoid aglycones and terpenoids was the lowest in PBS (pH 7.4), about 2.15 mg/L and 3.32 mg/mL, respectively. The effects of stabilizers on the solubility of FAs were as follows: PVP K30 > SDS > HPC-SL > HPMC E5 > poloxamer 188. In the solution added with PVP K30, the solubility was 625 times higher than that in PBS (pH 6.8). The reason might be that the phenolic hydroxyl groups contained in flavonoids were easy to form hydrogen bonds with the carbonyl groups in PVP K30 molecules, and the insoluble FAs were dispersed in PVP K30 macromolecules through hydrogen bonds, making them easy to dissolve.<sup>16</sup> All of the stabilizers had little effect on the solubility of TLs. In the solution containing SDS, the solubility of TLs reached 7.26 mg/mL. It could be seen that PVP K30 and SDS had better solubilization effects on FAs and



**Figure 2** HPLC chromatogram of GBCCM. Chromatogram of mixed reference substance (A); Chromatogram of test preparation (B). 1, Ginkgolide J; 2, Ginkgolide C; 3, bilobalide; 4, Ginkgolide B; 5, Ginkgolide A; 6, quercetin; 7, kaempferol; 8, isorhamnetin.

**Abbreviation:** GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves.

**Table 4** Effects of Different Stabilizers on the Solubility of FAs and TLs in GBCCM (mean  $\pm$  SD, n = 3)

Stabilizers	Compound 1	Concentration (mg/L)	Compound 2	Concentration (mg/L)
PVP K30	FAs	1250.20 $\pm$ 132.25	TLs	4.97 $\pm$ 0.06
SDS		120.50 $\pm$ 10.30		7.26 $\pm$ 0.11
HPMC E5		24.10 $\pm$ 2.15		4.37 $\pm$ 0.08
Poloxamer 188		2.20 $\pm$ 0.05		5.29 $\pm$ 0.06
HPC-SL		46.35 $\pm$ 1.84		5.07 $\pm$ 0.03
PBS (pH6.8)		2.15 $\pm$ 0.02		3.32 $\pm$ 0.03

**Abbreviations:** FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves; PVP K30, Polyvinylpyrrolidone Kollidon<sup>®</sup> 30; SDS, Sodium dodecyl sulfate; HPMC E5, Hydroxypropyl methylcellulose E5; HPC-SL, Hydroxypropyl cellulose-SL; PBS, Phosphate Buffered Saline.

TLs. It was also reported that the combined application of stabilizers could significantly reduce drug particles diameter and improve the long-term stability.<sup>17</sup> Therefore, PVP K30 and SDS were mainly selected in the formulation screening.

## Preparation of GBCCM NC-SD

Previous studies found that the effective concentration of GBCCM including FAs (2.7 mg/kg) and TLs (1.7 mg/kg) in rats was about 4.4 mg/kg.<sup>1</sup> In consideration of the poor water solubility of GBCCM, this study was to improve the overall solubility and bioavailability through the combination of excipient solubilization, nanocrystalline and solid dispersion technologies. The solubility of FAs was very low, belonging to practically insoluble substances, and there was almost no difference in different pH media. For this kind of compound with extremely poor solubility, nanocrystalline and solid dispersion technology had been widely used as a means of low cost and easy industrialization. For example, Pietta et al prepared quercetin nanocrystals with three stabilizers and found that their oral bioavailability was significantly higher than that of quercetin API suspension.<sup>18</sup> Li et al prepared a quercetin solid dispersion (QSD) and found that quercetin existed in QSD in an amorphous state, greatly improving the bioavailability.<sup>19</sup> PVP K30 was an amorphous polymer stabilizer, which existed in a network structure in the solution. Drugs were easy to enter and maintain a high dispersion state.<sup>17</sup>

In this study, the PVP K30 and SDS were used as stabilizers and solubilizers to obtain nanocrystalline suspension by high-pressure homogenization. Then, GBCCM NC-SD with PVP K30 as carrier could be obtained by lyophilizing the nanocrystalline suspension. In the freeze-drying process, protective agents were often added to the nanocrystalline suspension.<sup>20,21</sup> In order to prevent the preparation from being made into a large size in the later stage, this study mainly investigated the freeze-drying effect without protective agents.

The contents of FAs and TLs in GBCCM NC-SD were 32.30% and 21.42% respectively, among which the contents of KMF and BB were the highest (Table 5). The drug loading capacity of GBCCM NC-SD was as high as 53.72%.

## Characterization of GBCCM NC-SD

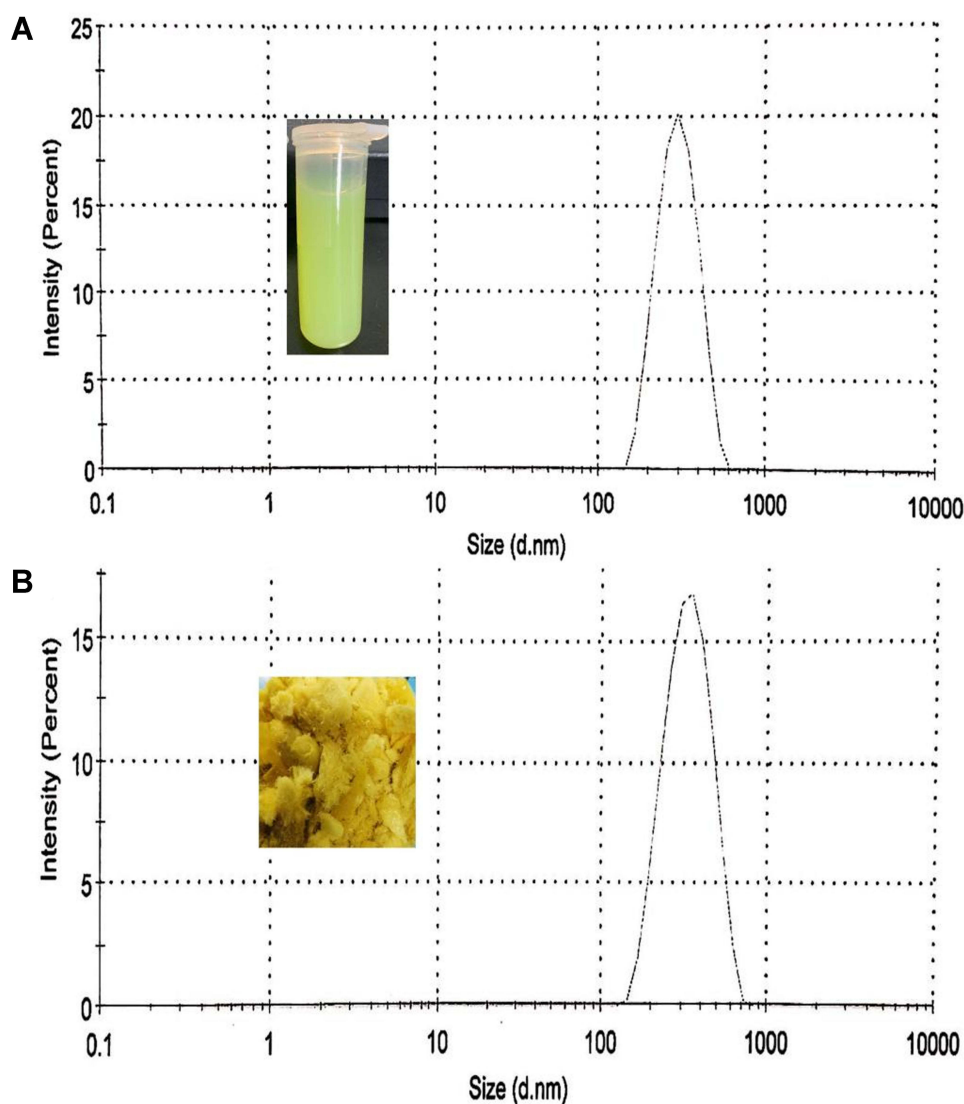
### Particle Size and Zeta Potential

The particle size distribution and the physical photos of GBCCM nanocrystalline suspension before (A) and after (B) freeze-drying are shown in Figure 3. The nanocrystalline suspension was yellow-green liquid with opalescence; GBCCM NC-SD obtained after freeze-drying was yellow uniform powder. The particle size distribution of nanocrystalline suspension was slightly narrower than that after freeze-drying.

**Table 5** Contents of FAs and TLs in GBCCM NC-SD

	GJ /%	GC /%	BB/%	GA/%	GB/%	Total/%		QCT/%	KMF/%	ISR/%	Total/%
TLs	0.21 $\pm$ 0.01	2.19 $\pm$ 0.03	12.01 $\pm$ 0.12	3.43 $\pm$ 0.03	3.58 $\pm$ 0.02	21.42 $\pm$ 0.08	FAs	13.82 $\pm$ 0.08	15.83 $\pm$ 0.13	2.65 $\pm$ 0.01	32.30 $\pm$ 0.11

**Abbreviations:** FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves; NC-SD, nanocrystalline solid dispersion; GJ, Ginkgolide J; GC, Ginkgolide C; BB, bilobalide; GB, Ginkgolide B; GA, Ginkgolide A; QCT, quercetin; KMF, kaempferol; ISR, isorhamnetin.



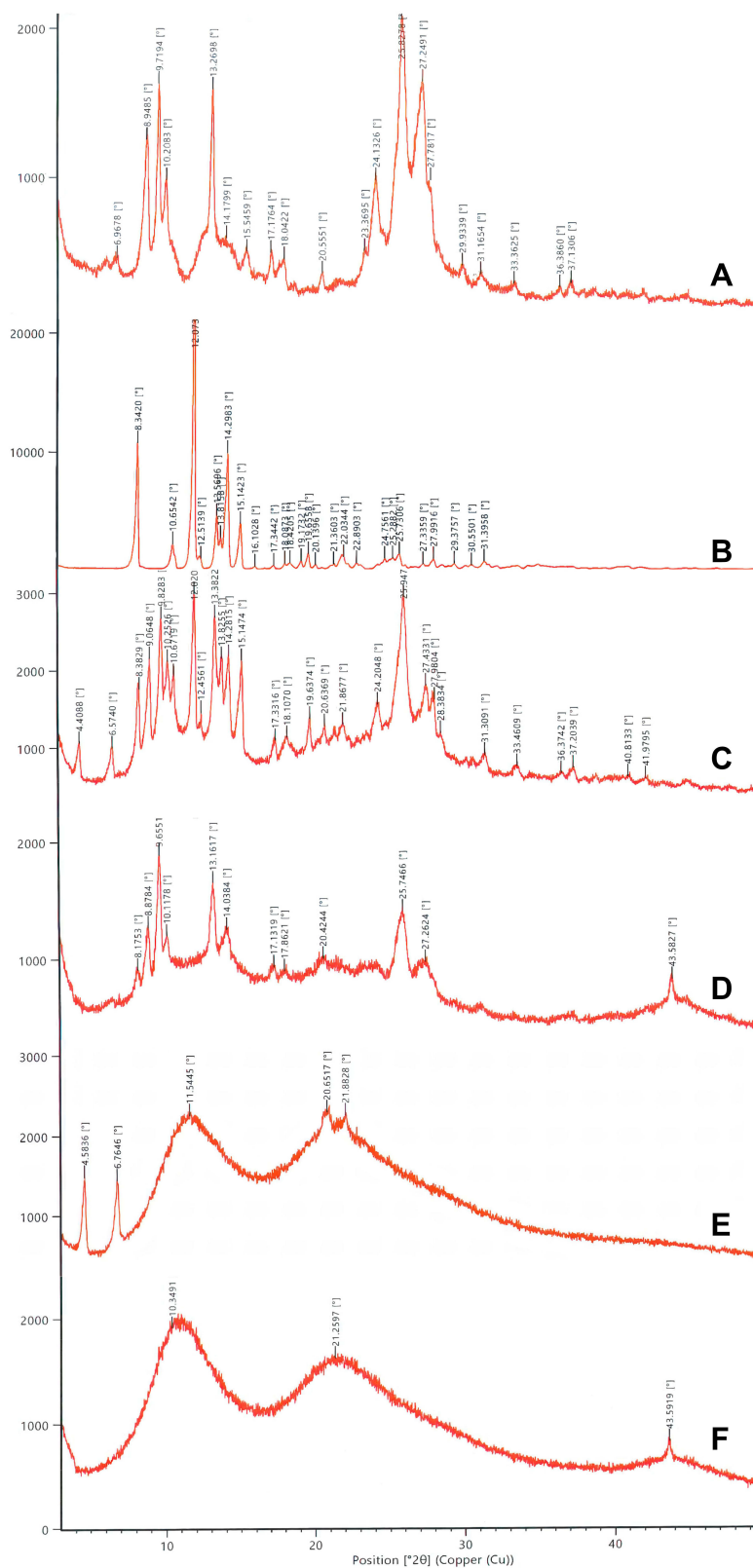
**Figure 3** The particle size distribution and the physical photos of GBCCM nanocrystalline suspension before (A) and after (B) freeze-drying. **Abbreviation:** GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves.

The average particle size, PDI and zeta potential of GBCCM nanocrystalline suspension and GBCCM NC-SD obtained after freeze-drying were  $305.5 \pm 22.1$  nm,  $0.27 \pm 0.02$ ,  $-30.9 \pm 1.2$  mV and  $335.9 \pm 32.8$  nm,  $0.29 \pm 0.02$  and  $-28.4 \pm 0.7$  mV, respectively. Although the particle size and PDI of GBCCM NC-SD after redissolution increased, there was no significant difference compared with GBCCM nanocrystalline suspension.

### Powder X-Ray Diffraction Analysis (PXRD)

In order to confirm the crystalline state of GBCCM NC-SD, PXRD analyses of FAs, TLs, physical mixture of GBCCM and stabilizers, GBCCM NC-SD, the stabilizers physical mixture and freeze-dried product of the physical mixture of stabilizers were performed (Figure 4). The XRPD pattern of the physical mixture of GBCCM and stabilizers (Figure 4C) could be seen as the superposition of the characteristic diffraction peaks of FAs (Figure 4A), TLs (Figure 4B) and the stabilizers mixture (Figure 4E). It could be seen from the PXRD pattern of GBCCM NC-SD (Figure 4D) that the characteristic peaks of the stabilizers mixture and TLs disappear, and only the characteristic diffraction peaks of FAs, which may be weakened due to the joint action of stabilizer and homogenization.<sup>22,23</sup> In other words, only FAs crystals existed in GBCCM NC-SD, and TLs had been transformed into amorphous powder. A new diffraction peak appeared at  $2\theta = 43.5^\circ$  (Figure 4D), which was speculated to be formed by stabilizer. This hypothesis was confirmed by PXRD





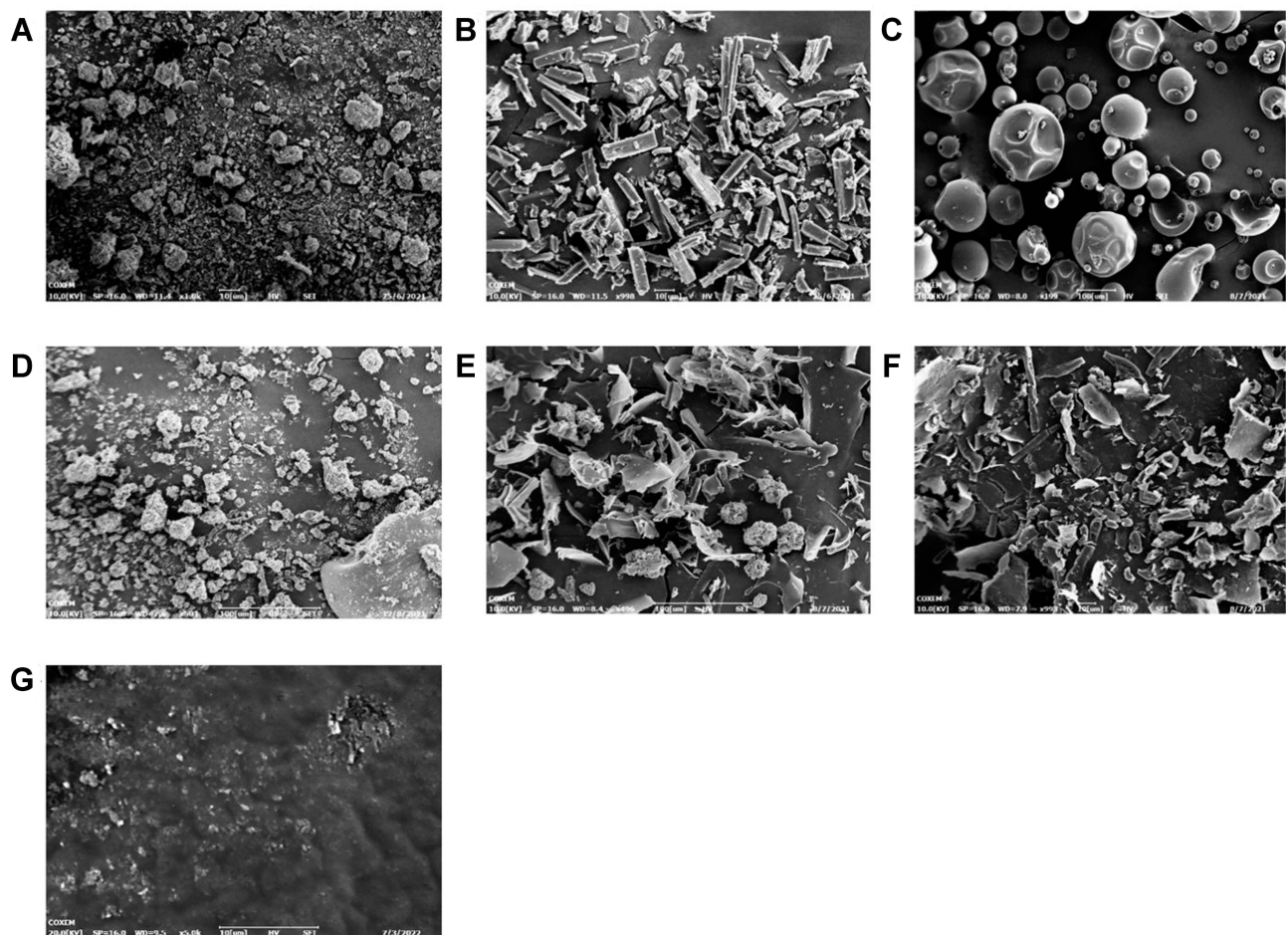
**Figure 4** PXRD analyses of FAs (A), TLs (B), physical mixture of GBCCM and stabilizers (C), GBCCM NC-SD (D), the physical mixture of stabilizers (E) and freeze-dried product of the physical mixture of stabilizers (F).

**Abbreviations:** PXRD, powder X-ray diffractometry; FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves NC-SD, nanocrystalline solid dispersion.

analysis of the stabilizer mixture before and after lyophilization (Figure 3E). It was found that the diffraction peak disappeared at  $2\theta = 4.5^\circ$  and  $6.7^\circ$ , and the new diffraction peak appeared at the same position with Figure 4D.

### Scanning Electron Microscopy (SEM)

FAs, TLs, physical mixture of stabilizers, physical mixture of GBCCM and stabilizers, lyophilized physical mixture of GBCCM and stabilizers, lyophilized centrifugal precipitation of GBCCM nanosuspension and GBCCM NC-SD were observed by SEM, as shown in Figure 4. As could be seen, FAs was rod-like crystals of various sizes, some of which clustered together to form spheroids with a size of about 1–30  $\mu\text{m}$  (Figure 5A). TLs was a uniform short rod-like crystal with a size of about 10–30  $\mu\text{m}$  (Figure 5B). The physical mixture of stabilizers was spherical particles with a size of about 10–150  $\mu\text{m}$  (Figure 5C). In Figure 5D, we could see spherical or unsmooth rod-shaped crystals of FAs, smooth rod-shaped crystals of TLs and spherical granular of stabilizers. However, after the physical mixture of GBCCM and stabilizers was freeze-dried, broken excipients and spherical or rod-shaped crystals of FAs could be observed, and there were no rod-shaped crystals of TLs (Figure 5E). It showed that crystals of TLs might transform into amorphous powder in the process of high-pressure homogenization and freeze-drying. Due to the interference of a variety of amorphous fragments in GBCCM NC-SD, it was difficult to find FAs crystals (Figure 5F). Therefore, the GBCCM nanocrystalline suspension was centrifuged at 15,000 r/min for 15 min, the precipitation was freeze-dried and observed by SEM. It could be found that the nanoscale spherical or rod-shaped crystals of FAs (Figure 5G).



**Figure 5** SEM of FAs (1,000 $\times$ , **A**), SEM of TLs (1,000 $\times$ , **B**), SEM of physical mixture of stabilizers (200 $\times$ , **C**), SEM of physical mixture of GBCCM and stabilizers (500 $\times$ , **D**), SEM of lyophilized physical mixture of GBCCM and stabilizers (500 $\times$ , **E**), SEM of lyophilized centrifugal precipitation of GBCCM nanosuspension (1,000 $\times$ , **F**) and SEM of GBCCM NC-SD (5,000 $\times$ , **G**).

**Abbreviations:** SEM, Scanning electron microscopy; FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves NC-SD, nanocrystalline solid dispersion.

## In vitro Drug Release Study

When investigating the dissolution of GBCCM, PM and GBCCM NC-SD in vitro, the dissolution medium was 0.5% SDS instead of water, because the intestinal bile and other secretions in the body also had solubilizing effect, which was similar to the solubilizing effect of 0.5% SDS.<sup>24</sup>

Figure 6 showed the dissolution profiles of FAs and TLs in GBCCM, PM and GBCCM NC-SD in PBS buffer (pH 6.8) containing 0.5% SDS over a time period of 60 min. FAs in GBCCM NC-SD displayed a significant increase in the cumulative dissolution ( $52.92 \pm 1.38\%$ ,  $P < 0.01$ ) at 60 min compared with it in GBCCM ( $12.77 \pm 1.25\%$ ) and PM ( $14.27 \pm 1.56\%$ ). Similarly, the cumulative dissolution rate of TLs in GBCCM NC-SD was also improved ( $99.21 \pm 1.82\%$  vs  $90.91 \pm 3.24\%$ ,  $92.91 \pm 2.52\%$ ,  $P < 0.05$ ) at 60 min. Compared with GBCCM, the cumulative dissolution of PM was improved to some extent, but there was no significant difference, which might be due to the strong solubilization effect of the stabilizer at high concentration, but weak at low concentration.

In general, the cumulative dissolution of FAs and TLs in GBCCM NC-SD was significantly higher than that in GBCCM and PM ( $P < 0.01$ ). Increased in vitro dissolution of GBCCM NC-SD could be explained as followings: As a polymeric surfactant, PVP K30 could make the drug easy to be wetted by water, and had a certain solubilizing effect.<sup>19</sup> The significant reduction of FAs in drug particle size increased the specific surface area and apparent solubility, and reduced the thickness of the diffusion layer, thus facilitating drug dissolution.<sup>7</sup> The TLs changed from crystal to amorphous state and was highly dispersed in the carrier, which increased the dissolution surface area and reduced the dissolution lattice energy.<sup>25</sup>

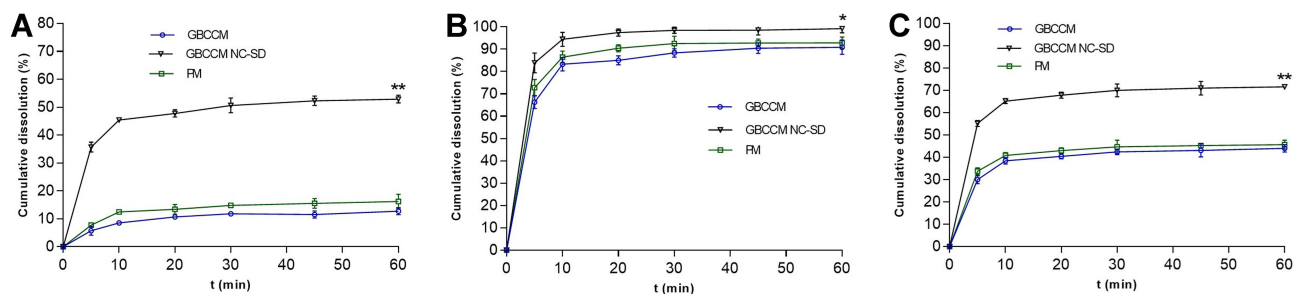
## In vivo Bioavailability

### Verification of the Methodology

For this LC-MS/MS method, the representative chromatograms of GBCCM are shown in Figure 7 and suggest that the retention time of each compound was hardly affected by endogenous substances in the rat plasma. In addition, for the blank plasma sample, the response enhancement of FAs and TLs was not observed, which suggested that the carry-over was negligible and there was no crosstalk between MS channels. The linear ranges for the determination of FAs and TLs were 0.1–300 ng/mL and 1.0–1000 ng/mL, respectively. The RSDs of intra-day and inter-day precision were all  $< 8.52\%$ . The recovery of each compound was 96.3–106.5%. This method was fully validated using the rat plasma with a simple and low plasma volume protein precipitation procedure to support the pharmacokinetic study in rats.

### Pharmacokinetic Parameters of Each Component Under Different Administration Routes

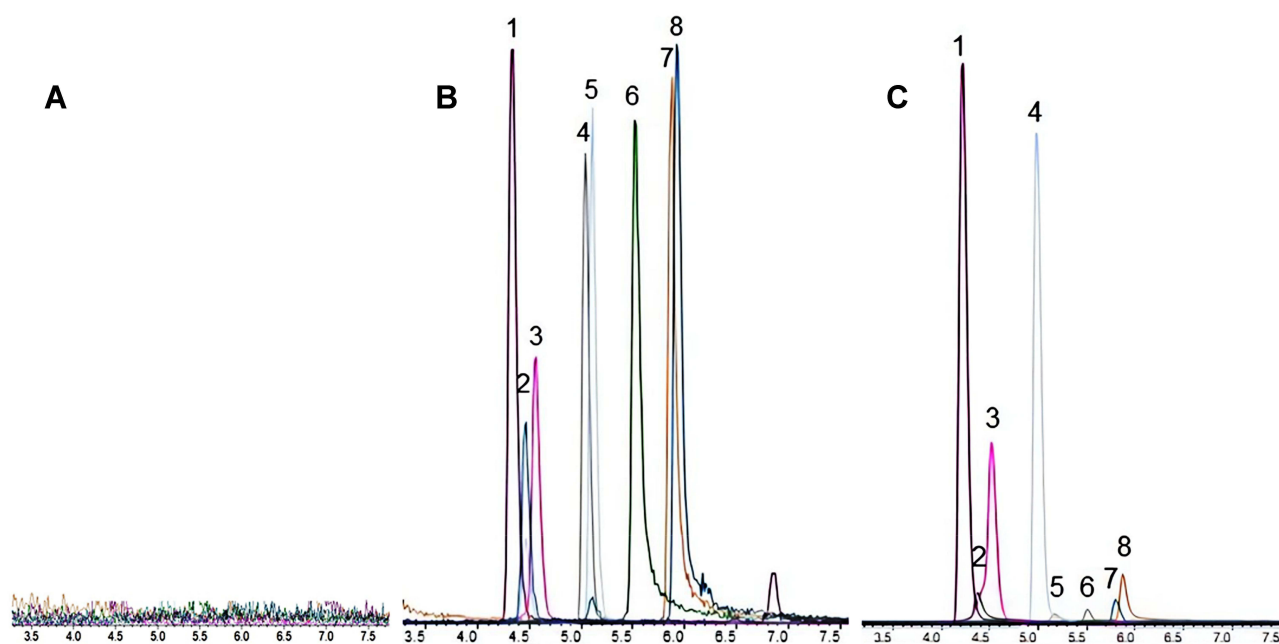
In recent years, there had been many studies on the pharmacokinetics of nanocrystals, dispersions and liposomes made of GBE.<sup>26–28</sup> Flavonoids in GBE mainly existed in the form of flavonoid glycosides.<sup>29</sup> Compared with FAs, they had large polarity, poor membrane permeability, and complex in vivo absorption and metabolism mechanisms.<sup>30</sup> Besides flavonoid glycosides and TLs, there were about 70% unknown components in GBE, which might have a great impact on the pharmacokinetic characteristics of each component. Compared with GBE composed of complex components, the



**Figure 6** In vitro dissolution curves of FAs and TLs in GBCCM, PM and GBCCM NC-SD. (A, FAs; B, TLs; C, FAs+TLs) (mean $\pm$ SD, n=3).

**Notes:** \* $P < 0.05$  and \*\* $P < 0.01$  VS GBCCM.

**Abbreviations:** FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves NC-SD, nanocrystalline solid dispersion; PM, physical mixture of GBCCM and stabilizer.



**Figure 7** Representative LC-MS chromatograms of GBCCM. (A) blank plasma, (B) blank plasma added to mixed reference substance and (C) a blood sample from a rat after GBCCM injection. 1, bilobalide; 2, Ginkgolide J; 3, Ginkgolide C; 4, Ginkgolide A; 5, Ginkgolide B; 6, quercetin; 7, kaempferol; 8, isorhamnetin.

**Abbreviations:** LC-MS, liquid chromatograph-mass spectrometer; GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves.

pharmacokinetic results of GBCCM containing high-purity FAs and TLs were easier to repeat due to avoiding the influence of many factors.

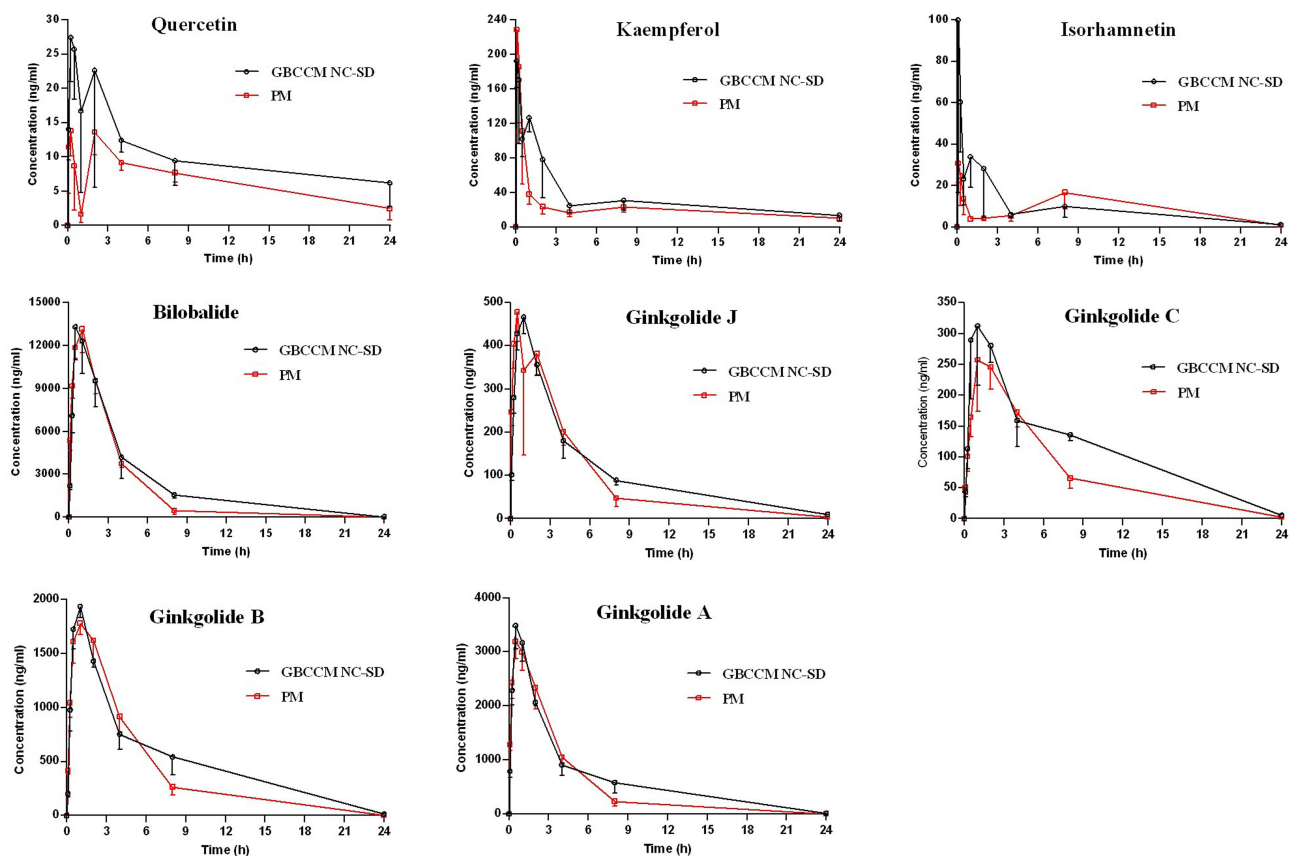
The plasma concentration-time curves of FAs and TLs in GBCCM NC-SD and PM in rats after oral administration are presented in Figure 8, and the associated pharmacokinetic parameters were summarized in Table 6. It could be seen from the figure that the FAs in GBCCM NC-SD group and PM group both had double peaks or multi peaks, the same as those in GBE, which might be caused by intestinal liver circulation.<sup>31</sup>

As could be seen from Table 6, the area under the curve ( $AUC_{0-t}$ ) and absolute bioavailability (F) of QCT and KMF in GBCCM NC-SD group were significantly increased compared with PM group, and peak concentration ( $C_{max}$ ) of QCT was increased to about 2 times, while other pharmacokinetic parameters showed no significant change. In comparison with PM group, multispectral refraction topography ( $MRT_{0-t}$ ) of all TLs in GBCCM NC-SD group was significantly prolonged. In addition,  $AUC_{0-t}$  and F of BB, GC and GB were significantly increased. The enhanced oral bioavailability of FAs and TLs in GBCCM NC-SD group could be attributed to the increased apparent solubility and dissolution rate as determined by the aforementioned in vitro studies. In particular, the pharmacokinetic indexes such as  $AUC_{0-t}$ ,  $MRT_{0-t}$ , half-life ( $T_{1/2}$ ) and  $C_{max}$  of GB in GBCCM NC-SD group were significantly prolonged, indicating that the solid dispersion technology greatly improved the PK properties of GB. As the most active platelet activating factor antagonist among TLs, the improvement of the PK properties of GB might promote the antihypertensive activity of GBCCM.

### Integrated Pharmacokinetic Results

GBCCM mainly contained eight components, including three FAs and five TLs, of which the pharmacokinetic properties were quite different. For example,  $T_{1/2}$  of FAs was basically between 8 and 20 h, belonging to slow metabolism compounds. However, the  $T_{1/2}$  of TLs was basically between 2 and 4 h, which belonged to the fast-metabolic compounds.<sup>10</sup> The pharmacokinetic properties of any single component could not be used to characterize the overall pharmacokinetic behavior of GBCCM. The multi-component integration based on the self-defined weight coefficient of the area under the curve ( $AUC_{0-t}$ ) was used to obtain the integrated pharmacokinetic parameters that took into account the characteristics of each component of GBCCM, which reflected the “Holistic View” of traditional Chinese medicine





**Figure 8** Plasma concentration-time curves of FAs and TLs in GBCCM NC-SD and PM in rats after oral administration (mean $\pm$ SD, n=5).

**Abbreviations:** FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of *Ginkgo biloba* leaves NC-SD, nanocrystalline solid dispersion; PM, physical mixture of GBCCM and stabilizer.

theory.<sup>9,10</sup> The self-defined weight coefficient method highlighted the influence of the ingredients with larger exposure levels, such as KMF and BB, on the overall exposure of GBCCM.

The plasma concentration of each component was determined at 8 time-points during the 24h after administration, and the AUC<sub>0-t</sub> was calculated by DSA software. By substituting it into formula (1) and (2), the corresponding self-defined weight coefficients of each component in the two types of components could be obtained. The results of self-defined weight coefficients of FAs and TLs were shown in Table 7. By substituting the blood drug concentration at different time points after administration and the self-defined weight coefficients of the eight components into formula (3) and (4), the integrated blood drug concentration of FAs and TLs at different time points could be obtained. It could be observed from Figure 9 that the integrated plasma drug concentration-time curves of FAs and TLs were basically consistent with that of KMF and BB, respectively.

The integrated pharmacokinetic parameters are summarized in Table 8. For FAs, the integrated T<sub>1/2</sub> of the three groups was between 10.38 and 19.34 h, and there was no significant difference (P > 0.05). The integrated maximum concentration (T<sub>max</sub>) and C<sub>max</sub> of PM group and GBCCM NC-SD group also had no significant difference (P > 0.05). The integrated AUC<sub>0-t</sub> was 600.43 and 402.90 ng/mL/h respectively, which was significantly increased in NC-SD group (P < 0.05). The absolute bioavailability of integrated FAs was only 1.19% and 0.79% respectively. Although the GBCCM NC-SD group was significantly improved (P < 0.05), the absolute value was still low. It was speculated that the apparent dissolution had little effect on the absolute bioavailability of FAs, while the complex intestinal absorption, intestinal bacterial metabolic mechanism and in vivo metabolic process might have a greater impact.<sup>32,33</sup>

For TLs, integrated T<sub>1/2</sub> of the three groups was between 1.13 and 2.47 h. Among them, the GBCCM NC-SD group was significantly longer than the PM group (P < 0.05), and both were significantly longer than the GBCCM group (P <



**Table 6** Pharmacokinetic Parameters of FAs and TLs in GBCCM NC-SD Group, GBCCM Group and PM Group (mean  $\pm$  SD, n = 5)

Administration	Compounds	AUC <sub>0-t</sub> (ng/mL/h)	MRT <sub>0-t</sub> (h)	t <sub>1/2</sub> (h)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	F
	<b>Quercetin</b>						
Injection	GBCCM group	6540.65 $\pm$ 1343.39	6.97 $\pm$ 0.67	9.77 $\pm$ 3.19	4338.19 $\pm$ 2586.53	0.03 $\pm$ 0.00	–
Oral	GBCCM NC-SD group	240.80 $\pm$ 45.72**	9.07 $\pm$ 2.40	13.65 $\pm$ 10.14	33.97 $\pm$ 10.97*	0.80 $\pm$ 0.74	0.61 $\pm$ 0.12**
Oral	PM group	153.93 $\pm$ 26.06	7.98 $\pm$ 1.16	10.14 $\pm$ 4.45	17.05 $\pm$ 3.40	1.32 $\pm$ 0.95	0.39 $\pm$ 0.07
	<b>Kaemferol</b>						
Injection	GBCCM group	11,036.00 $\pm$ 2780.82	1.09 $\pm$ 0.24	13.79 $\pm$ 5.70	66,120.20 $\pm$ 9706.33	0.03 $\pm$ 0.00	–
Oral	GBCCM NC-SD group	802.45 $\pm$ 136.96**	7.09 $\pm$ 1.55	10.38 $\pm$ 10.16	215.93 $\pm$ 56.69	0.12 $\pm$ 0.08	1.21 $\pm$ 0.21**
Oral	PM group	526.29 $\pm$ 52.13	7.60 $\pm$ 1.11	18.97 $\pm$ 15.92	247.73 $\pm$ 36.42	0.15 $\pm$ 0.09	0.79 $\pm$ 0.08
	<b>Isorhmnetin</b>						
Injection	GBCCM group	1005.85 $\pm$ 267.96	0.99 $\pm$ 0.33	6.92 $\pm$ 7.05	6898.21 $\pm$ 1944.62	0.03 $\pm$ 0.00	–
Oral	GBCCM NC-SD group	229.26 $\pm$ 69.05	5.16 $\pm$ 1.12	5.23 $\pm$ 1.70	105.13 $\pm$ 78.82	0.15 $\pm$ 0.09	3.80 $\pm$ 1.14
Oral	PM group	214.20 $\pm$ 81.33	7.38 $\pm$ 0.50	8.00 $\pm$ 5.08	33.85 $\pm$ 7.65	0.15 $\pm$ 0.09	3.55 $\pm$ 1.35
	<b>Bilobalide</b>						
Injection	GBCCM group	16,428.10 $\pm$ 1908.04	1.73 $\pm$ 0.53	1.23 $\pm$ 0.45	18,741.20 $\pm$ 2834.73	0.03 $\pm$ 0.00	–
Oral	GBCCM NC-SD group	59,036.60 $\pm$ 9060.30*	3.83 $\pm$ 0.08**	2.38 $\pm$ 0.15	13,592.10 $\pm$ 1872.28	0.70 $\pm$ 0.27	59.89 $\pm$ 12.24*
Oral	PM group	44,045.50 $\pm$ 4873.48	3.00 $\pm$ 0.43	1.99 $\pm$ 0.40	12,950.90 $\pm$ 1658.87	0.80 $\pm$ 0.27	44.69 $\pm$ 4.94
	<b>Ginkgolide J</b>						
Injection	GBCCM group	1358.75 $\pm$ 508.35	3.23 $\pm$ 0.37	2.24 $\pm$ 1.56	979.12 $\pm$ 356.04	0.06 $\pm$ 0.06	–
Oral	GBCCM NC-SD group	2638.21 $\pm$ 287.24	4.88 $\pm$ 0.28**	3.19 $\pm$ 0.67	472.86 $\pm$ 27.94	0.90 $\pm$ 0.22	32.36 $\pm$ 3.52
Oral	PM group	2248.97 $\pm$ 324.94	3.84 $\pm$ 0.46	3.13 $\pm$ 0.66	501.58 $\pm$ 66.79	0.70 $\pm$ 0.27	27.59 $\pm$ 3.99
	<b>Ginkgolide C</b>						
Injection	GBCCM group	8639.81 $\pm$ 4699.96	1.66 $\pm$ 0.37	3.45 $\pm$ 1.74	11,680.30 $\pm$ 1247.76	0.03 $\pm$ 0.00	–
Oral	GBCCM NC-SD group	2679.71 $\pm$ 286.87**	5.64 $\pm$ 0.44*	4.03 $\pm$ 0.56	336.06 $\pm$ 88.41	1.30 $\pm$ 0.67	5.17 $\pm$ 0.55**
Oral	PM group	1852.26 $\pm$ 132.88	4.80 $\pm$ 0.51	3.31 $\pm$ 0.57	277.40 $\pm$ 68.47	1.40 $\pm$ 0.55	3.57 $\pm$ 0.26
	<b>Ginkgolide B</b>						
Injection	GBCCM group	5697.68 $\pm$ 2736.88	1.88 $\pm$ 0.56	1.39 $\pm$ 0.20	5354.40 $\pm$ 477.19	0.03 $\pm$ 0.00	–
Oral	GBCCM NC-SD group	12,258.00 $\pm$ 1929.48*	4.93 $\pm$ 0.35**	3.12 $\pm$ 0.43*	1973.01 $\pm$ 55.33 **	0.90 $\pm$ 0.22	35.86 $\pm$ 5.64*
Oral	PM group	10,094.80 $\pm$ 1064.39	3.92 $\pm$ 0.30	2.62 $\pm$ 0.12	1782.21 $\pm$ 100.58	0.90 $\pm$ 0.22	29.53 $\pm$ 3.11
	<b>Ginkgolide A</b>						
Injection	GBCCM group	4449.33 $\pm$ 1998.88	1.71 $\pm$ 0.59	1.39 $\pm$ 0.65	6434.23 $\pm$ 1038.68	0.03 $\pm$ 0.00	–
Oral	GBCCM NC-SD group	15,990.10 $\pm$ 2712.64	4.29 $\pm$ 0.32**	2.96 $\pm$ 0.34	3543.07 $\pm$ 335.26	0.60 $\pm$ 0.22	59.90 $\pm$ 10.16
Oral	PM group	13,152.90 $\pm$ 1094.70	3.22 $\pm$ 0.28	2.18 $\pm$ 0.39	3274.23 $\pm$ 237.53	0.60 $\pm$ 0.22	49.27 $\pm$ 4.10

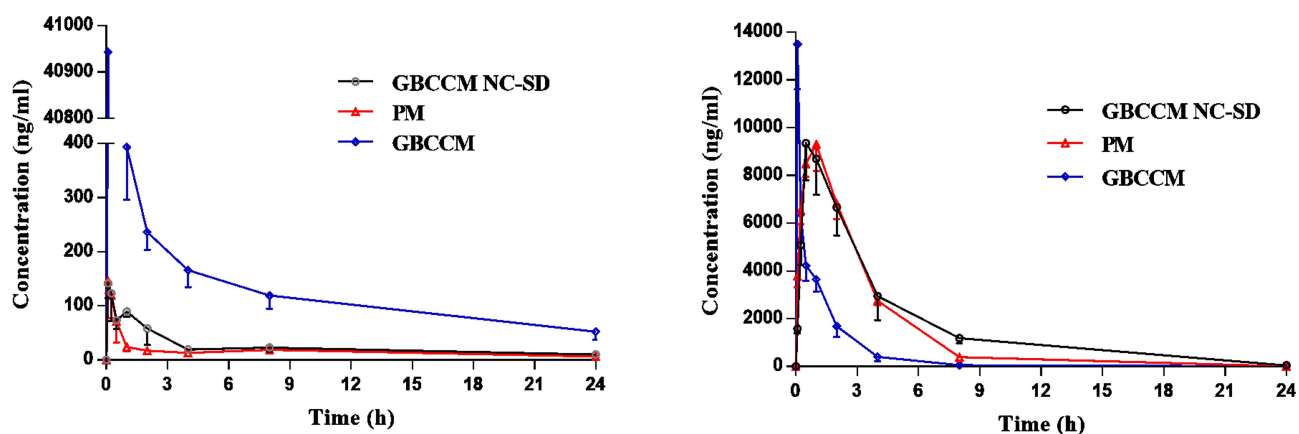
Notes: \*P < 0.05 and \*\*P < 0.01 VS PM group.

Abbreviations: FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of Ginkgo biloba leaves NC-SD, nanocrystalline solid dispersion; PM, physical mixture of GBCCM and stabilizer; AUC<sub>0-t</sub>, area under the curve; MRT<sub>0-t</sub>, mean residence time; t<sub>1/2</sub>, half-life time; C<sub>max</sub>, mean peak concentration; T<sub>max</sub>, peak time; F, absolute bioavailability.

**Table 7** AUC<sub>0-t</sub> and Corresponding Weighting Coefficients ( $\omega_i$ ,  $\omega_j$ ) of FAs and TLs in Rats After Intra-gastric or Intravenous

Adminstration	Parameters	QCT	KMF	ISR	Parameters	BB	GJ	GC	GB	GA
GBCCM NC-SD	AUC <sub>0-t</sub> (mg h L <sup>-1</sup> )	240.80	802.45	229.26	AUC <sub>0-t</sub> (mg h L <sup>-1</sup> )	59,036.60	2638.21	2679.71	12,258.00	15,990.10
	$\omega_i$	0.19	0.63	0.18	$\omega_j$	0.64	0.03	0.03	0.13	0.17
PM	AUC <sub>0-t</sub> (mg h L <sup>-1</sup> )	153.93	546.29	214.20	AUC <sub>0-t</sub> (mg h L <sup>-1</sup> )	50,045.50	2248.97	1852.26	10,094.80	13,152.90
	$\omega_i$	0.17	0.60	0.23	$\omega_j$	0.65	0.03	0.02	0.13	0.17
GBCCM	AUC <sub>0-t</sub> (mg h L <sup>-1</sup> )	6540.65	11,036.00	1005.85	AUC <sub>0-t</sub> (mg h L <sup>-1</sup> )	20,428.10	1358.75	8639.81	5697.68	4449.33
	$\omega_i$	0.35	0.59	0.06	$\omega_j$	0.51	0.03	0.21	0.14	0.11

**Abbreviations:** FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of Ginkgo biloba leaves NC-SD, nanocrystalline solid dispersion; PM, physical mixture of GBCCM and stabilizer; AUC<sub>0-t</sub>, area under the curve; GJ, Ginkgolide J; GC, Ginkgolide C; BB, bilobalide; GB, Ginkgolide B; GA, Ginkgolide A; QCT, quercetin; KMF, kaempferol; ISR, isorhamnetin.



**Figure 9** The integrated blood drug concentration-time curve of FAs and TLs.

**Abbreviations:** FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of Ginkgo biloba leaves NC-SD, nanocrystalline solid dispersion; PM, physical mixture of GBCCM and stabilizer.

0.05). The integrated  $T_{max}$  and  $C_{max}$  of the GBCCM NC-SD group and PM group had no significant difference ( $P > 0.05$ ). The integrated  $AUC_{0-t}$  of the GBCCM NC-SD group and PM group were 42,310.62 and 31,414.31 ng/mL/h respectively, and the absolute bioavailability of TLs could be calculated to be 62.19% and 45.61%, respectively, which were significantly increased ( $P < 0.05$ ), indicating that the solid dispersion technology significantly improved the PK properties of TLs.

The bioavailability of the GBCCM NC-SD was significantly higher than that of GBCCM, mainly due to the improved dissolution by nanocrystalline and dispersion technology, the intestinal adhesion of nanoparticles and the impact on the overall intestinal absorption.<sup>34,35</sup> However, the increase in the apparent solubility did not significantly affect other pharmacokinetic parameters such as  $C_{max}$  and  $T_{max}$ , which might be related to the complex in vivo absorption, distribution, metabolism and excretion mechanism of FAs and TLs in GBCCM.<sup>36-38</sup> The relevant mechanisms needed to be further studied.

## Conclusions

Based on CAD technology, a convenient chromatographic method for simultaneous detection of eight components in GBCCM was developed. At the same time, a formulation intermediate based on nanocrystalline solid dispersion technology was prepared, in which FAs was mainly in the form of nanocrystals with a particle size of about 335 nm, and TLs was mainly dispersed in it in the form of amorphous. Through in vitro dissolution study, it was found that the cumulative dissolution of FAs and TLs in GBCCM NC-SD was increased from 12.77% to 52.92% ( $P < 0.01$ ) and 90.91%

**Table 8** The Integrated Pharmacokinetic Parameters of FAs and TLs in Rats After Intragastric or Intravenous

Parameters	GBCCM Group	GBCCM NC-SD Group	PM Group	GBCCM Group	GBCCM NC-SD Group	PM Group
	FAs			TLs		
$t_{1/2}$ (h)	11.24±3.72	10.38±9.87	19.34±11.58	1.13±0.07	2.47±0.14*, **	2.17±0.29*
$T_{max}$ (h)	0.03±0.00	0.12±0.08**	0.15±0.09**	0.03±0.00	0.70±0.27**	0.90±0.22**
$C_{max}$ (ng/mL)	40,943.18±6596.46	152.40±42.95	158.49±24.07	13,497.32±1890.40	9558.86±1242.33	9332.84±1080.87
$AUC_{0-t}$ (ng/mL/h)	8809.60±2014.44	600.43±90.16**	402.90±43.22	11,551.99±1930.31	42,310.62±8428.64*	31,414.31±5238.27
F		1.19±0.33*	0.79±0.18		62.19±14.30*	45.61±6.41

**Notes:** \* $P < 0.05$  and \*\* $P < 0.01$  VS PM group; \* $P < 0.05$ , \*\* $P < 0.01$  VS GBCCM group.

**Abbreviations:** FAs, flavonoid aglycones; TLs, terpenoid lactones; GBCCM, component-based Chinese medicine of Ginkgo biloba leaves NC-SD, nanocrystalline solid dispersion; PM, physical mixture of GBCCM and stabilizer;  $AUC_{0-t}$ , area under the curve;  $MRT_{0-t}$ , mean residence time;  $t_{1/2}$ , half-life time;  $C_{max}$ , mean peak concentration;  $T_{max}$ , peak time; F, absolute bioavailability.

to 99.21% ( $P < 0.05$ ) respectively. A UPLC-QTRAP-MS/MS method was developed to determine the plasma concentrations of eight active ingredients. By defining the weight coefficient, a classified integrated pharmacokinetic study model was established, and the integrated pharmacokinetic parameters of GBCCM in rats with different administration modes were obtained by statistical moment method. In comparison with PM, the integrated pharmacokinetics  $AUC_{0-t}$  of FAs and TLs in GBCCM NC-SD were significantly increased ( $P < 0.05$ ), and the  $T_{1/2}$  of TLs was also significantly prolonged ( $P < 0.05$ ). The parameters obtained from the classified integrated pharmacokinetic model constructed in this study could characterize the overall in vivo behavior of similar components in GBCCM to the greatest extent, and provide PK data support for the development of a new antihypertensive drug.

## Acknowledgments

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## Disclosure

Hongbao Liang, Chenghong Sun, Zhong Feng, Xianzhen Wang, Lingpeng Kong, Feng Zhu, Jingchun Yao, Xiaomei Yuan, Zhong Liu, Guimin Zhang work for Shandong New Time Pharmaceutical Co., Ltd and/or Lunan Pharmaceutical Group Co., Ltd. The authors declare that they have no other conflicts of interest in this work.

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