Published in final edited form as:

J Chromatogr B Analyt Technol Biomed Life Sci. 2016 June 01; 1022: 13–20. doi:10.1016/j.jchromb.2016.03.045.

Anticancer 20(R)-dammarane-3 β , 12 β , 20, 25-tetrol-loaded polymeric micelles: preparation, quantification and pharmacokinetics

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Abstract

Polymeric micelles are effective drug-loading sites and often used to formulate poorly water-soluble agents. In the present study, the amphiphilic copolymer methoxy-capped poly(ethyleneglycol)-block-poly(ϵ -caprolactone) (mPEG-b-PCL) was successfully developed for the delivery of 20(R)-dammarane-3 β , 12 β , 20, 25-tetrol (25-OH-PPD), a natural anticancer product from *Panax notoginseng*. The 25-OH-PPD-loaded micelles were characterized by morphological observation and thermodynamic stability testing. The concentrations of 25-OH-PPD were determined by HPLC-MS/MS. The optimum MRM transition of 25-OH-PPD was selected at m/z 479.4.0 $\rightarrow$ 461.4. The chromatographic separation was achieved on a SB-C18 column (1.8 μ m, 2.1 $\times$ 50 mm) with an optimized gradient mobile phase system. The extraction recoveries of plasma and various tissue homogenates were within the range of 81.1% to 110.4% and the matrix effects ranged from 81.9% to 106.7%. The intra- and inter-day precision values (RSD%) were less than 12.0%, with accuracies ranging from 85.2% to 114.2%. In addition, 25-OH-PPD was found to be stable in different biological matrix after three freeze-thaw cycles, at room temperature and at -70°C for 4 weeks. The pharmacokinetics of 25-OH-PPD-loaded micelles was evaluated in rats. The micelles appeared as transparent liquid, stable and uniform spheres with an average particle size of $35.4\pm 4.2\text{nm}$. The maximum concentration of 25-OH-PPD in micelles was much lower than in free drug preparation. However, the drug in the micelles was released steadily, with a $t_{1/2}$ of $9.1\pm 4.0\text{h}$, significantly longer than in free drug ($3.3\pm 1.4\text{h}$). However, the drug concentrations in tissues after the micelle administration were lower than the levels after administration of the free

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Conflict of Interest

These authors declare no conflict of interest.

drugs. In summary, the micelles were characterized by long circulation and sustained release, with an ability to avoid uptake by the reticuloendothelial system, providing a promising approach to deliver intravenous 25-OH-PPD for therapy.

Keywords

Panax notoginseng; 20(*R*)-dammarane-3 β ,12 β ,20, 25-tetrol; polymeric micelles; LC-ESI-MS/MS; pharmacokinetics

1. Introduction

Chemotherapeutic agents play a key role in cancer therapy. Despite advances in targeted therapy, conventional drugs such as natural products and their derivatives are still irreplaceable for cancer therapy and prevention [1]. Compared with their synthetic counterparts, natural products represent a rich source of new therapeutic and preventive drugs [2]. *Panax notoginseng*, one of the popular traditional Chinese medicines, shows great beneficial effects in ischemic cardiovascular and cerebrovascular diseases and intracerebral hemorrhage [3,4]. Further, the ginsenosides derived from ginseng and the roots and leaves of *P. notoginseng* display remarkable antioxidant, anti-proliferative, and pro-apoptotic activities in different models of human cancer [5-8]. More recently, 20(*R*)-dammarane-3 β , 12 β , 20, 25-tetrol (25-OH-PPD) has been isolated from the leaves of *P. notoginseng* [9]. It has been shown to possess potent anti-cancer activity, resulting in cell-cycle arrest and induced apoptosis, at least partially via inhibition of the MDM2 oncogene and related pathways [10,11]. Further investigations have demonstrated that the anticancer mechanisms of the three derivatives of 25-OH-PPD are mediated via inhibition of Wnt/ β -catenin pathway and activation of caspase-signaling pathways [12,13].

Although it is a promising anticancer agent, 25-OH-PPD shows poor solubility in water and oil, underscoring the need for development of appropriate pharmaceutical dosage forms for the evaluation of its *in vivo* efficacy and future clinical testing [14]. Different formulations have been developed. For instance, a self-microemulsifying drug delivery system (SMEDDS) has been used to deliver 25-OCH₃-PPD, the prodrug of 25-OH-PPD, which greatly enhances its bioavailability by improving solubility and lymphatic transport [15]. A targeted drug delivery system (TDDS) can make significant impact on cancer chemotherapy. Nanoparticles, such as polymeric micelles, have been widely investigated, demonstrating advantages in targeting transport of anticancer drugs. Drugs with poor hydrophilicity can be embedded into the hydrophobic cores, while the hydrophilic shell allows stabilization of micelles and protects them from rapid clearance. Compared with ordinary preparations, micelles medium, and enable precise molecular targeting. The poly (lactic-co-glycolic acid) (PLGA) has been used to encapsulate 25-OCH₃-PPD [16]. Compared with the free drug, the 25-OCH₃-PPD-loaded PEG-PLGA showed a better oral bioavailability and antitumor efficacy *in vitro* and *in vivo*. Additionally, the oral bioavailability of *P. notoginseng* saponins is also increased greatly with a water-in-oil nanoemulsion formulation [17].

In the present study, a passive target delivery system consisting of mPEG-b-PCL copolymers was prepared and optimized to encapsulate 25-OH-PPD and enhance its water solubility.

Meanwhile, the plasma pharmacokinetics and tissue distribution of 25-OH-PPD were also evaluated after administration of 25-OH-PPD-loaded micelles in rats.

2. Materials and Methods

2.1. Chemicals and reagents

The test compound 25-OH-PPD was isolated from the fruits of *Panax ginseng* as described previously [10,14]. The mPEG3000-b-PCL2500 (Mw/Mn 1.09) was purchased from Advanced Polymer Materials Inc. (Montreal, QC, Canada). Diazepam (used as the internal standard, 99.0% purity) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ammonium formate was purchased from Sigma (St Louis, MO, USA). Acetonitrile and methanol (HPLC grade) were obtained from Fisher Scientific (Atlanta, GA, USA). High-purity water (18.2 MΩ.cm) was produced by a Milli-Q reagent water system (Millipore Inc, Bedford, MA, USA). All other chemicals and solvents used for sample preparation and HPLC analysis were of analytical grade and obtained from commercial sources.

2.2. Preparation of 25-OH-PPD-loaded PEG-b-PCL micelles

The micelles were prepared using thin film hydration method as established previously [18]. Briefly, the mPEG-b-PCL copolymers and 25-OH-PPD (25:1, w/w) were co-dissolved in acetonitrile and subjected to sonication in a single-neck flask. The solution was evaporated using a rotary evaporator (Shanghai Yarong Biochemistry Equipment Apparatus Company Co. Ltd, Shanghai, China) at 37°C for 30 min, leading to the formation of a dry solid film. The film was warmed to 50°C, supplemented with 5% glucose solution at 50°C and vortexed for 30 s. After sonication for 3 min, a clear micellar solution was obtained. Finally, the solution was filtered through a 0.22-μm cellulose nitrate membrane filter to remove precipitates.

2.3. Analysis of particle sizes, zeta potential, and morphology

The average particle sizes and size distribution were measured by dynamic light scattering (DLS) using Nano Series Zen 4003 Zeta sizer (Malvern, UK). The polydispersity index (PDI) ranged between 0 and 1. Zeta potential was determined using the same instrument. Each sample was analyzed at a temperature of 25°C in triplicate. The uranyl acetate solution (1%, w/v) was used to stain micelles encapsulating 25-OH-PPD. After drying at room temperature, a thin film was formed and the shape of micelles were observed under a transmission electron microscope (TEM-2100, JEOL, Tokyo, Japan) [19].

2.4. Determination of encapsulation efficiency

The encapsulation efficiency (EE) of 25-OH-PPD-loaded micelles was estimated using a previously described method [18]. In brief, 1.0 mL (1 mg/mL) of drug-loaded micelles was poured onto a 0.22-μm membrane filter to remove the non-encapsulated drug, and the filtered fluid (0.1 mL) was collected and mixed with pure methanol (10 mL). The solution was appropriately diluted prior to LC-MS/MS analysis. All samples were analyzed in triplicate. Encapsulation efficiency (EE) and drug-loading coefficient (DL) were calculated using the following equations:

$$EE\% = \frac{25\text{-OH-PPD weight measured in micelles}}{25\text{-OH-PPD weight added}} \times 100\%$$

$$DL\% = \frac{25\text{-OH-PPD weight measured in micelles}}{\text{Total weight of micelles added} + 25\text{-OH-PPD weight added}} \times 100\%$$

2.5. Determination of stability

The dilution method was used to evaluate thermodynamic stability of drug-loaded micelles [18]. Briefly, the 25-OH-PPD-loaded micelles were diluted with distilled water to different concentrations. An hour later, the solution was centrifuged at 10,000 rpm for 10 min and the supernatant was filtered with a 0.22- μ m filter membrane. The particle sizes and size distribution were analyzed as above, and the drug concentrations were analyzed by LC-MS/MS.

2.6. Quantification of 25-OH-PPD using LC-MS/MS

2.6.1. Instrumentation and LC-MS/MS conditions—An Agilent 1200 chromatography system coupled to a 6460 triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) in electro spray ionization (ESI) mode was used to separate and quantify the test compound. Tuning parameters were optimized for both 25-OH-PPD and IS using their pure standards. The source temperature was set at 300°C. Optimized fragmentor and collision energy values were 100, 3V for 25-OH-PPD and 110, 30V for IS, respectively, while the capillary voltage was set at 3500V. The gas flow was set at 8L/min and the nebulizer at 30 psi, using high purity nitrogen as the collision gas.

The chromatographic separation was achieved on a SB-C18 (1.8 μ m, 2.1 $\times$ 50 mm) analytical column (Agilent Technologies, Palo Alto, CA, USA) protected by an Agilent gasket. An optimized gradient mobile phase system was composed of water containing 10 mM of aqueous ammonium formate (phase A) and acetonitrile (phase B) at a flow rate of 0.4 mL/min. The gradient was set as follows: phase B was increased from 50% to 95% within the first 1.5 min, held for 0.1 min, decreased to 50 % within next 2.4 min (total gradient time: 4.0 min). All the data were acquired and analyzed using the Agilent B. 06.00 series (Agilent Technologies).

2.6.2. Sample preparation—A liquid-liquid extraction method was used to extract 25-OH-PPD from biological matrices. Briefly, rat plasma (50 μ L) or tissue homogenate (100 μ L) was placed in an Eppendorf tube and mixed with IS (1 μ g/mL, 5 μ L for plasma, 10 μ L for homogenates). The mixture was extracted with ethyl acetate (200 μ L for plasma, 400 μ L for homogenates) by vortexing and centrifugation at 18,000 g for 20 min at 4°C. The supernatant was transferred to another tube and evaporated to dryness in a vacuum desiccator at room temperature. The residue was reconstituted with the mobile phase (100 μ L) and an aliquot of the solution (2 μ L) was injected onto the LC-MS/MS system.

2.6.3. Method validation—The drug concentrations used for establishing the standard curves were 10, 50, 200, 1,000, 5,000, and 10,000 ng/mL in control (drug-free) pooled rat plasma and homogenates of liver, heart, spleen, lungs, kidneys, and brain, respectively. The quality control (QC) samples were prepared with the final concentrations of low (20 ng/mL), medium (1,000 ng/mL) and high (8,000 ng/mL), respectively. The intra-day and inter-day accuracy and precision of the method were assessed by evaluating three QC samples on three validation days (n=3). The percentage recoveries of 25-OH-PPD were determined by comparing the mean peak areas of extracted samples with those of pure compound dissolved in corresponding blank matrix after sample preparation. The matrix effects were evaluated by comparing the peak areas of post-extraction spiked samples with that of unextracted analyte in acetonitrile at the same concentrations. The stability was evaluated by analyzing QC samples at different concentrations (low, medium, and high) at room temperature, freeze-thaw cycles, and long-term storage (−70°C, 4 weeks).

2.7. Pharmacokinetic and tissue distribution studies

2.7.1. Animals—Healthy male Sprague-Dawley rats (weighing 220±10 g) were purchased from the Vital River Laboratories (Beijing, China) and fed with a commercial diet and tap water *ad libitum*. The rats were housed in the animal room maintained at 25°C, < 70% of humidity, and 12 h/12 h light/dark cycle for at least three days before experiments. All the experimental procedures were approved by the Animal Ethics Committee of Beijing Friendship Hospital and followed the Guiding Principles for Care and Use of Laboratory Animals of China.

2.7.2. Drug dosing and biological sampling—For the pharmacokinetic study, the rats were randomly assigned to two groups (5 rats/group). A single dose of 5mg/kg 25-OH-PPD-loaded micelles or free drugs were administered to rats intravenously [20]. Under anesthesia, approximately 120 µL of blood sample was collected from the left femoral veins of the rats into heparinized tubes at 0 (pro-drug), 2, 15, and 30 min, and 1, 2, 4, 8, 12, 24, 36, and 48 h after a single bolus injection of the test compounds. The blood samples were immediately centrifuged at 3000 rpm for 10 min, and the plasma (50 µL) was precisely collected and stored at −70°C until analysis.

To evaluate the effect of formulation on the tissue distribution of the test compound, the rats were intravenously administered 5mg/kg 25-OH-PPD-loaded micelles. At 0.5, 2, 8 and 24 h (n=5 for each time point) after drug dosing, the rats were sacrificed and various tissues (heart, liver, spleen, lung, kidneys and brain) were collected. All tissues samples were immediately trimmed off extraneous fat and connective tissue, blotted, weighed, homogenized in ice-cold PBS, and stored at −70°C until analysis. All the plasma and tissue samples were then processed as described above for drug analysis.

2.7.3. Pharmacokinetic data analysis—The average plasma drug concentrations following intravenous administration were analyzed and used for pharmacokinetic analyses. Major pharmacokinetic parameters were calculated using the DAS 2.0 program (Mathematical Pharmacology Professional Committee of China, Shanghai, China) [21]. The elimination half-life ($t_{1/2\beta}$), area under the concentration-time curve (AUC), and mean

residence time (MRT) were calculated. The two groups were compared using Student's *t*-test or one-way ANOVA test. A P-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Characterization of drug-loaded micelles

After several tests, the amphiphilic copolymer mPEG3000-b-PCL2500 was determined as an ideal vehicle for 25-OH-PPD. The micelle solution was transparent liquid and uniformly spherical. Polymeric micelle's diameter should be no greater than 100 nm in size, to prevent extravasation of the particles through gaps of endothelial cells into target tissues, and efficiently evade renal clearance and reticuloendothelial capture [22]. In the present study, the average particle size was 35.4 ± 4.2 nm (Figure 1).

The polydispersity index (PDI) value was shown in table 1. The loading efficiency of the micelles for 25-OH-PPD was around 4%; the encapsulation efficiency was $90.1 \pm 3.2\%$. The stability profiles of the micellar system are shown in Table 1. There were no changes in mean particle size, size distribution and drug concentrations of the 25-OH-PPD-loaded micelles obtained after dilution compared with that of undiluted micelles, which were consistent with the results of low CMC value of mPEG-b-PCL. The micelles were characterized by small particle size and thermal stability, enabling the formulation of water-soluble intravenous injection of 25-OH-PPD.

3.2. Optimization of LC–MS/MS conditions

Quadrupole full scans were carried out in both positive and negative ionization modes to optimize the ESI conditions of 25-OH-PPD and IS. A higher intensity was observed in the positive ion mode. The full-scan mass spectra showed a predominantly protonated molecular ion $[M+H]^+$ at m/z 497.4 for 25-OH-PPD and m/z 285.0 for IS. The ion fragment, m/z 461.4, was dominant and used for 25-OH-PPD quantification (Table 2). Similarly, the optimum MRM transition of IS was selected at m/z 285.0 $\rightarrow$ 193.1 (Figure 2). No interference was observed in plasma or tissue at the retention times of the analyte and IS. Both 25-OH-PPD and IS were eluted rapidly and selectively, with retention times approximately 1.32 and 2.68 min, respectively.

3.3. Assay validation of LC–MS/MS

Representative MRM chromatograms of 25-OH-PPD in rat plasma and tissues are shown in Figure 3. The method was linear in the range 10–10,000 ng/mL, with the linear least squares regression equation ($1/x^2$) correlation coefficients of the standard curves for 25-OH-PPD in plasma and various tissue homogenates above 0.99 (Table 3). The extraction recoveries were within the range of 81.1%–110.4% and there were no significant enhancing and suppressing matrix effects (81.9%–106.7%), indicating that ethyl acetate offered good extraction procedure for 25-OH-PPD in biological matrices [23, 24]. Meanwhile, the extraction recoveries and matrix effects of the internal standard were within the ranges of 84.0%–90.3% and 84.7%–91.1%, respectively. The intra- and inter-day precision values (RSD%) were both less than 12.0%, while the assay accuracies ranged from 85.2 to 114.2% (Table 4). The data for the stability during sample processing indicated that 25-OH-PPD was stable after three

freeze-thaw cycles, at room temperature and at -70°C for 4 weeks. The analytical validations followed the FDA guidance on bioanalytical method validation and met all the requirements for determining 25-OH-PPD in plasma and different tissues.

3.4. Pharmacokinetics of drug-loaded micelles

The plasma concentration-time profiles following intravenous administration of 25-OH-PPD-loaded micelles or free drugs at an equivalent dose of 5mg/kg are shown in Figure 4. The micelles were rapidly distributed into various tissues after administration, so the blood concentration was relatively low. Although the maximum concentration of 25-OH-PPD after micelle administration was significantly lower compared with the free drug, it was released steadily with concentrations of 378.3 ± 220.9 ng/mL at 24 h, to 72.1 ± 38.0 ng/mL at 48 h. By contrast, the concentration of 25-OH-PPD after administration of free drug decreased quickly, from 44.3 ± 33.7 ng/mL at 24 h, to undetectable levels at 36 h. Further, there were significant differences in the *in vivo* pharmacokinetics between the micelles and free drug. The elimination half-lives and mean residence times of 25-OH-PPD were 9.1 ± 4.0 h and 13.3 ± 1.7 h for micelles, 3.3 ± 1.4 h and 3.7 ± 1.2 h for free drugs, respectively. The parameters of 25-OH-PPD-loaded micelles manifested sustained-release characteristics.

3.5. Biodistribution of drug-loaded micelles

Compared with the free drug, the micelles showed a similar AUC value, but a significant reduction in C_{max} , suggesting that 25-OH-PPD specifically concentrates in target organs rapidly after delivery by drug-loaded micelle (Figure 5). In addition, the drug-loaded micelles were concentrated in target organs (*e.g.*, spleen) and released in the metabolic course, thus slowing the clearance rate of 25-OH-PPD in blood. High concentration of 25-OH-PPD (approximate 10 $\mu\text{g/g}$ in spleen and liver, respectively, following a dose of 10 mg/kg) in target organs after intravenous injection of free drugs has been reported in literature [25]. However, the drug-loaded micelle exhibited a low concentration in these organs (approximate 0.5 $\mu\text{g/g}$ in spleen and liver, respectively, following a dose of 5 mg/kg), suggesting the long half-life of micelles, which were not easily engulfed by the reticuloendothelial system or eliminated by liver and kidneys.

Micelles may improve solubility, prolong the circulation time, delay release of the micelles exhibited prolonged circulation time. Further investigations are still needed to evaluate its accumulation in solid tumor tissues and antitumor efficacy *in vivo*.

4. Conclusion

In the present study, we successfully employed amphiphilic copolymer mPEG-b-PCL to encapsulate 25-OH-PPD, and the drug-loaded micelles greatly increased the solubility of the compound. The micelles were characterized by a long half-life, sustained drug release, and ability to avoid reticuloendothelial uptake. Therefore, the polymeric micelles are a promising approach to deliver 25-OH-PPD intravenously.

Acknowledgements

This work was supported by Chinese Medicine Research Project of Beijing 14ZY16 (to J.Y), Beijing Municipal Science & Technology Commission Z141100002214019 (to S. S.), the National Institutes of Health (NIH) grant R01 CA186662 (to R.Z.) and the American Cancer Society (ACS) grant RSG-15-009-01-CDD (to W.W.). The content is solely the responsibility of the authors, and do not necessarily represent the official views of the National Institutes of Health.

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Figure 1. Characterization of 25-OH-PPD-loaded micelles. (A) Representative particle size distribution of micelles by dynamic light scattering; (B) Transmission electron microscopy image.

Figure 2. Product ion mass spectra of (A) 25-OH-PPD and (B) diazepam (IS), and their respective structures.

Figure 3.

Representative multiple reaction monitoring chromatograms obtained after the intravenous administration of 5mg/kg 25-OH-PPD-loaded micelles: (A) rat plasma (358.0 ng/mL), (B) spleen (619.6 ng/mL) and (C) kidneys (982.8 ng/mL)..

Figure 4. Plasma concentration-time profiles of 25-OH-PPD-loaded micelles (drug-loaded micelles) and 25-OH-PPD (free drugs) in rats following a single intravenous administration of 5mg/kg (n=5/group).

Figure 5. Mean concentrations of 25-OH-PPD in various tissues after intravenous administration of 5 mg/kg 25-OH-PPD-loaded micelles (n=5).

Table 1

Stability of 25-OH-PPD-loaded micelles evaluated by dilution method (n=3).

Dilution times	Z-Average (nm)	PDI	Entrapment efficiency (%)
0	36.19 ± 2.12	0.19 ± 0.04	90.88 ± 1.65
10	36.48 ± 2.71	0.17 ± 0.03	90.72 ± 2.73
50	38.14 ± 1.58	0.20 ± 0.02	90.51 ± 1.93
100	39.55 ± 3.34	0.21 ± 0.03	88.47 ± 2.62

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Table 2

Optimized mass spectrometric conditions.

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision energy (V)	Polarity
25-OH-PPD	479.4	461.4	100	3	Positive
Diazepam (I.S)	285.0	193.1	110	30	Positive

Table 3

Calibration curves and linear ranges of 25-OH-PPD in different biological samples.

Bio-samples	Calibration curve	Correlation coefficient (r)	Linear range(ng/mL)
Plasma	$Y=0.9684x+0.0346$	0.9906	10-10000
Heart	$Y=1.1517x+0.0361$	0.9912	10-10000
Liver	$Y=0.8499x+0.0303$	0.9934	10-10000
Spleen	$Y=1.0699x+0.0088$	0.9909	10-10000
Lung	$Y=1.2742x+0.0201$	0.9937	10-10000
Kidney	$Y=0.8415x+0.0143$	0.9942	10-10000
Brain	$Y=0.9882x+0.0183$	0.9978	10-10000

Table 4

Precision, accuracy, recovery, matrix effects and stability of 25-OH-PPD assay in rat plasma and tissue homogenates (n=3).

Biological matrix	QC (ng/mL)	Intra-day		Inter-day		Extraction recovery (mean±SD, %)	Matrix effect (mean±SD, %)	Stability ((mean ± SD, %)		Long-term (-70°C, 4W)
		Precision (RSD, %)	Accuracy (mean, %)	Precision (RSD, %)	Accuracy (mean, %)			Room temperature	Freeze-thaw cycle	
Plasma	10	6.1	103.3	5.5	98.7	86.6±6.8	88.5±5.7	91.8±1.0	93.8±1.9	92.3±3.0
	20	10.0	99.5	10.3	92.9	83.2±6.8	85.0±5.0	95.0±1.6	92.3±1.4	91.4±7.1
	1000	12.0	97.9	8.6	105.6	105.1±2.4	103.8±7.7	96.5±1.8	97.42±0.60	89.4±5.2
	8000	6.3	89.1	5.8	103.6	83.5±3.2	88.3±5.8	98.3±0.5	97.38±0.76	88.0±1.6
Heart	10	6.7	102.4	6.2	103.6	91.2±2.6	92.2±5.0	99.2±0.9	101.9±4.9	93.4±1.7
	20	9.3	109.1	6.5	114.2	81.8±1.3	85.0±2.2	93.3±4.9	99.9±2.3	93.8±8.4
	1000	6.8	93.2	7.3	94.7	106.1±7.2	89.6±8.3	98.5±5.0	101.1±3.2	94.9±9.8
	8000	10.4	88.7	6.4	85.2	84.7±2.4	81.9±0.6	90.2±2.9	89.8±3.7	89.0±4.6
Liver	10	8.4	94.7	10.2	99.6	88.0±7.9	90.1±5.5	91.4±8.7	93.4±9.4	90.8±6.9
	20	8.9	96.2	8.3	97.0	81.1 ± 4.5	94.2 ± 8.2	95.9±2.8	98.5±4.4	95.6±3.9
	1000	7.4	96.8	10.0	98.6	81.7 ± 2.1	106.7±12.8	104.5±2.7	89.4±8.6	87.4±6.8
	8000	6.8	92.9	9.2	92.5	91.1±2.2	93.6±1.5	89.5±3.0	90.5±2.5	89.7±2.7
Spleen	10	10.3	103.9	5.1	99.3	88.1±11.7	89.0±8.4	96.6±4.1	101.1±6.9	91.9±5.8
	20	10.0	104.5	10.9	105.8	81.4±6.5	86.7±2.9	110.5±3.6	102.1±3.2	98.8±4.0
	1000	9.2	93.3	8.7	95.6	88.2±2.3	88.8±2.2	95.7±8.3	98.7±6.3	95.7±3.5
	8000	7.4	92.6	6.4	89.8	80.2±1.6	87.4±6.7	91.5±2.5	89.6±2.8	89.7±0.4
Lung	10	10.8	103.9	5.3	97.9	86.3±7.3	87.3±6.8	90.3±7.8	97.7±6.8	96.5±7.5
	20	4.8	104.2	5.4	107.7	104.4±1.5	92.6±4.5	102.1±4.5	99.3±8.5	92.8±2.8
	1000	1.8	98.1	6.8	91.2	92.9±3.3	103.6±11.5	99.8±7.6	89.0±7.1	93.2±8.0
	8000	3.6	86.8	6.5	89.1	83.1±2.4	91.1±12.8	89.5±1.7	90.0±3.1	88.3±3.0
Kidney	10	7.3	98.4	4.4	97.6	85.7±6.6	86.9±4.9	100.4±9.4	100.5±5.4	91.5±4.3
	20	8.1	103.8	8.6	98.8	85.6±11.0	91.8±5.4	105.3±3.5	96.8±4.5	90.4±3.3
	1000	1.0	99.7	5.3	104.6	110.4±4.2	106.3±3.3	101.9±4.0	96.9±5.0	96.0±4.8

Biologic al matrix	QC (ng/mL)	Intra-day		Inter-day		Extraction recovery	Matrix effect (mean±SD, %)	Stability ((mean ± SD, %)		
		Precision (RSD, %)	Accurac y (mean, %)	Precision (RSD, %)	Accurac y (mean, %)			Room temperature	Freeze-thaw cycle	Long-term (-70°C, 4W)
	8000	7.0	91.9	5.2	96.8	87.7±6.4	90.9±9.3	89.8±1.2	94.8±4.9	89.0±1.6
Brain	10	6.2	94.2	6.7	96.9	87.2±9.1	84.2±2.8	94.2±7.1	90.6±6.7	88.6±1.0
	20	7.6	104.3	7.4	110.1	82.5±7.7	83.5±1.3	108.4±6.3	94.2±5.5	93.2±4.8
	1000	8.7	99.6	8.1	102.8	98.5±1.9	103.7±2.9	103.4±8.1	92.2±5.2	90.1±1.7
	8000	2.3	94.8	2.0	94.6	83.8±8.6	89.6±1.5	88.1±2.6	93.5±1.2	90.0±2.5

Table 5

PK parameters of 25-OH-PPD in rats following intravenous administration of drug-loaded micelles and free drug (n=5, mean $\pm$ SD).

PK Parameters	25-OH-PPD-loaded micelles	Free drugs
$C_{\max}$ (ng. mL ⁻¹)	7228.7 $\pm$ 599.9	14958.4 $\pm$ 2458.8
$t_{1/2}$ (h)	9.1 $\pm$ 4.0 [#]	3.3 $\pm$ 1.4
MRT(h)	13.3 $\pm$ 1.7 [#]	3.7 $\pm$ 1.2
AUC($-\infty$) (ng.h.mL ⁻¹)	21472.6 $\pm$ 10155.5	24020.8 $\pm$ 6093.7
AUC _{0-t} (ng.h.mL ⁻¹)	23947.5 $\pm$ 10323.6	25922.3 $\pm$ 5949.1

[#]
 $p < 0.05$, compared with free drugs