



## Original Article

Determination of 6258-70, a new semi-synthetic taxane, in rat plasma and tissues: Application to the pharmacokinetics and tissue distribution study <sup>☆</sup>Simin Zhao, Yuanyuan Zhang, Ping Ju, Liqiang Gu, Rui Zhuang, Longshan Zhao, Xing Tang, Kaishun Bi, Xiaohui Chen <sup>\*</sup>

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## ABSTRACT

Cancer is the leading cause of death all over the world. Among the chemotherapy drugs, taxanes play an important role in cancer treatment. 6258-70 is a new semi-synthetic taxane which has a broad spectrum of antitumor activity. A fast and reliable high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was developed for quantification of 6258-70 in rat plasma and tissues in this paper. After extraction by liquid-liquid extraction method with methyl tert-butyl ether, the samples were separated on a Kinetex C<sub>18</sub> column (50 mm × 2.1 mm, 2.6 μm, Phenomenex, USA) within 3 min. The method was fully validated with the matrix effect between 87.7% and 99.5% and the recovery ranging from 80.3% to 90.1%. The intra- and inter-day precisions were less than 9.5% and the accuracy ranged from -3.8% to 6.5%. The reliable method was successfully applied to the pharmacokinetics and tissue distribution studies of 6258-70 after intravenous administration in rats. The pharmacokinetic results indicated that the pharmacokinetic behavior of 6258-70 in rats was in accordance with linear features within tested dosage of 1 to 4 mg/kg, and there was no significant difference between the two genders. The tissue distribution study showed that 6258-70 had an effective penetration, spread widely and rapidly and could cross blood-brain barrier. The results of pharmacokinetics and tissue distribution may provide a guide for future study.

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## 1. Introduction

The incidence of cancer is increasing, and cancer is becoming the leading cause of death all over the world [1,2]. Plenty of cancer chemotherapy drugs were adopted while taxanes were the most popular and active ones in the last two decades. Taxanes are potent microtubule poisons that promote microtubule to assemble and prevent its depolymerization, which is essential for mitotic function of cancer cell [3–5]. Among these taxanes, paclitaxel and docetaxel are the most widely used drugs as the front-line treatment or combination drugs for the therapy of ovarian cancer, breast cancer, non-small cell lung cancer and cervical cancer [6].

However, the long-term use of taxanes is limited contributed to two major reasons. First, as taxanes have high substrate affinity for multidrug-resistance (MDR) proteins, in particular the ATP-dependent drug efflux pump P-glycoprotein (P-gp), it can lead to MDR reaction. In addition, because of poor solubility of taxanes, Tween 80 and Cromoplor EL need to be used, which can cause

hypersensitivity reactions and cumulative fluid retention [7–9]. According to the study of structure-activity relationship, the active compound which is derived from taxanes by modifying C-2, C-10 and C-3' position shows a great improvement towards MDR and keeps the same activity at the same time [10–12]. These derivative compounds from taxanes include cabazitaxel, larotaxel, and tesa-taxel, which are under clinical test [13–16]. Thus, it is very meaningful to modify structure of taxanes in order to obtain new drugs with lower toxicity and higher effect, especially against the drug-resistant human cancer.

6258-70 (Fig. 1A) is a new semi-synthetic taxane derivated with modification at the C-7, C-10 and C-3' position from docetaxel (DTX) (Fig. 1B). As one member of taxanes, 6258-70 has the same advantages as other taxanes, including good active and broad spectrum of antitumor activity. In addition, according to the study on structure-activity relationship [10], as phenyl is replaced with isobutenyl at C-3' position, 6258-70 shows poorer affinity to P-gp compared with docetaxel. These advantages make 6258-70 meaningful for deeper research.

According to ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals [17], preclinical pharmacokinetic study plays an important role in the development of new drugs. The pharmacokinetic parameters in animal species can be used to make critical

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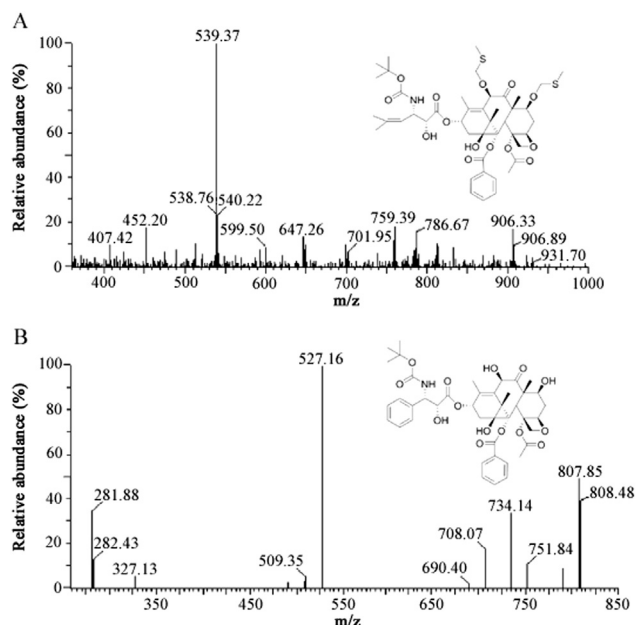


Fig. 1. Chemical structures and full scan mass spectra of (A) 6258-70 and (B) docetaxel.

decisions supporting the safety and efficacy of drugs. Selective and reliable analytical methods for the quantitative evaluation of a drug are critical for the conduct of preclinical, biopharmaceutics and clinical pharmacology research. However, as far as we know, there have been no reports on the pharmacokinetics and tissue distribution studies of 6258-70. Thus, a fast and reliable high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) bioanalytical method was developed and successfully applied to the quantification of 6258-70 in rat plasma and tissues for the first time in this paper. The pharmacokinetics and

Table 1

Calibration curves of 6258-70 in rat plasma and tissue homogenate.

Samples	Slope $\times 10^{-2}$	Intercept $\times 10^{-2}$	R	Linear ranges (ng/mL)
Plasma	1.24	0.87	0.999	4–4000
Liver	1.18	0.43	0.997	4–4000
Heart	1.08	0.52	0.998	4–4000
Spleen	0.72	3.94	0.997	4–4000
Lung	0.93	5.11	0.993	4–4000
Kidney	0.98	3.93	0.994	4–4000
Intestine	1.19	1.94	0.992	4–4000
Stomach	1.07	3.51	0.996	4–4000
Muscle	1.11	0.60	0.997	4–4000
Bladder	1.05	2.34	0.998	4–4000
Brain	0.92	4.52	0.999	4–4000
Fat	1.66	0.84	0.999	4–4000
Pancreas	1.23	0.76	0.996	4–4000
Testicle	0.83	1.51	0.997	4–4000
Uterus	0.86	0.42	0.997	4–4000

tissue distribution properties of 6258-70 after injection were demonstrated and discussed in this work, which will be useful for future studies.

## 2. Experimental

### 2.1. Chemical reagents and animals

6258-70 (purity > 98.0%), docetaxel (DTX, purity > 98%) (IS) and 6258-70 injection were supplied by Professor Xing Tang from Shenyang Pharmaceutical University. Acetonitrile, methanol and ammonium acetate of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Distilled water was provided by Wahaha Co., Ltd. (Hangzhou, China) and used throughout the study. Other chemical reagents were of analytical grade.

Sprague–Dawley (SD) rats (mean weight  $220 \pm 10$  g) were

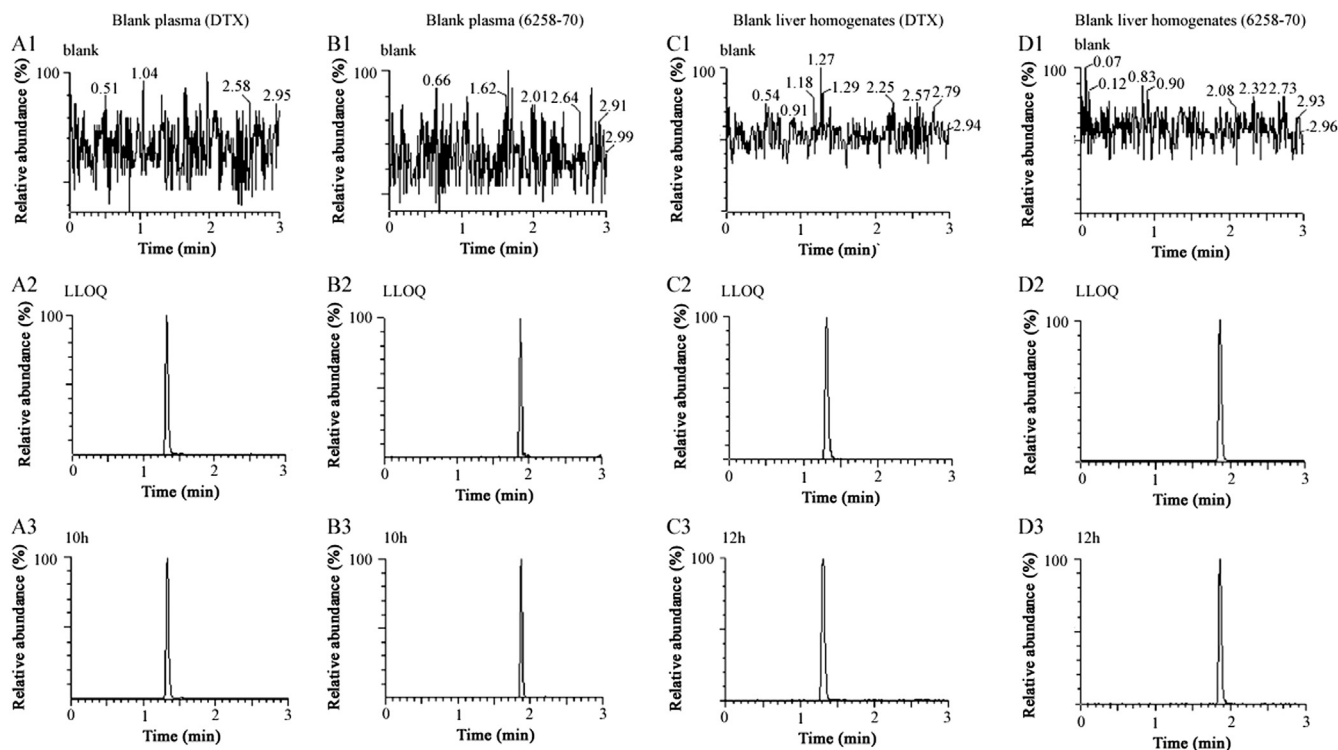


Fig. 2. MRM chromatograms of blank rat matrix (plasma or liver homogenate), blank rat matrix spiked with 6258-70 and IS, and rat plasma sample at 10 h or liver homogenate at 12 h.

**Table 2**Precision, accuracy, recovery and matrix effect of HPLC–MS/MS method for analysis of 6258-70 and IS in rat plasma and liver ( $n=6$ ).

Type of matrix	Compound	Concentration (ng/mL)	Accuracy (RE, %)	Precision		Matrix effect (Mean $\pm$ SD, %)	Recovery (Mean $\pm$ SD, %)
				Intra-day (RSD, %)	Inter-day (RSD, %)		
Plasma	6258-70	10	-2.2	9.4	9.5	93.8 $\pm$ 9.1	84.3 $\pm$ 7.3
		200	-3.2	8.7	9.3	89.9 $\pm$ 4.8	90.1 $\pm$ 11.1
		3200	0.1	6.9	6.8	99.5 $\pm$ 4.7	88.0 $\pm$ 5.1
	DTX	5000	-	-	-	98.1 $\pm$ 7.1	80.3 $\pm$ 5.8
Liver	6258-70	10	-3.2	3.0	4.2	90.0 $\pm$ 1.3	88.0 $\pm$ 5.1
		200	6.5	6.5	3.6	87.7 $\pm$ 2.1	88.5 $\pm$ 6.2
		3200	-3.8	3.9	4.7	88.2 $\pm$ 3.3	86.4 $\pm$ 4.3
	DTX	4000	-	-	-	96.3 $\pm$ 3.9	85.2 $\pm$ 4.6

**Table 3**Stability of the analytes in rat plasma and liver homogenate ( $n=3$ ).

Type of matrix	Concentration (ng/mL)	Room temperature for 4 h		-80 °C for 30 days		Three freeze-thaw cycles		8 h after prepared at 10 °C	
		RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)
Plasma	10	-8.7	5.2	-3.9	7.6	-8.5	5.9	-8.1	5.1
	200	1.8	14.1	-5.6	6.8	-2.7	14.5	-9.9	5.2
	3200	4.5	2.0	-1.0	9.7	5.7	8.1	3.2	5.9
Liver	10	2.8	4.1	11.3	1.3	-14.4	3.2	-2.7	8.8
	200	-7.5	14.0	-7.9	1.1	-3.8	2.2	-4.9	3.0
	3200	-5.6	9.7	-4.9	3.9	-6.3	3.2	-3.3	8.5

obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All procedures for animals were in accordance with the Guideline for Animal Experimentation of Shenyang Pharmaceutical University and the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China.

## 2.2. HPLC–MS/MS conditions

An ACQUITY Ultra-Performance Liquid Chromatography system (Waters Corp., Milford, MA, USA) equipped with a Xevo-TQ mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) interface and triple quadrupole mass analyzer was applied to the assay.

Chromatographic separation was accomplished on a Kinetex C<sub>18</sub> column (50 mm  $\times$  2.1 mm, 2.6  $\mu$ m, Phenomenex, USA) within 3 min. Gradient elution was applied with mobile phase A (acetonitrile) and phase B (5 mM ammonium acetate in water) at a flow rate of 0.2 mL/min. After an injection of 5  $\mu$ L, phase A was linearly increased from 50% to 95% in 1.0 min, and kept for 1.0 min. Then, it was decreased to 50% during the following 0.2 min, and kept for 0.8 min. The column and autosampler tray temperatures were kept constant at 40 °C and 10 °C, respectively.

Multiple reaction monitoring (MRM) in positive ESI mode was performed to quantify 6258-70 and IS, respectively. The fragment transitions for 6258-70 and IS were  $m/z$  906.6  $\rightarrow$  539.4 and 808.5  $\rightarrow$  527.3, respectively. The cone voltage and collision energy were 20 V and 10 eV for 6258-70, 16 V and 8 eV for IS. Other parameters are as follows: capillary voltage, 3.0 kV; cone gas (N<sub>2</sub>), 50 L/h; desolvation gas (N<sub>2</sub>), 400 L/h; collision gas (Ar<sub>2</sub>), 0.18 L/h; source temperature, 150 °C; desolvation temperature, 400 °C.

## 2.3. Preparation of stock solutions and calibration standard solutions

Stock solutions of 6258-70 and IS were prepared with methanol, both at a concentration of 0.1 mg/mL. The stock solution of the analyte was serially diluted to get the working solutions with

methanol. IS working solutions of 5000 ng/mL and 4000 ng/mL were diluted from the stock solution by methanol. All the solutions were stored under -20 °C.

Calibration standard samples (4, 10, 25, 100, 400, 1600, 4000 ng/mL) were obtained by adding 20  $\mu$ L of working solutions to 50  $\mu$ L blank rat plasma or 100  $\mu$ L tissue homogenates, and quality control (QC) samples (10, 200, 3200 ng/mL) were prepared in the same way from another stock solution.

## 2.4. Preparation of 6258-70 injection and sampling

6258-70 injection for rats was prepared by dissolving the compound in Tween 80 (1:26, m/m), and then diluted with ethanol-physiological saline (13:87, m/v) to the final concentration. All procedures for animals were in accordance with the Guideline for Animal Experimentation of Shenyang Pharmaceutical University and the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China.

In the pharmacokinetic study, 36 SD rats (50% of each sex) were randomly assigned to three groups. After intravenous administration with 6258-70 at a single dose of 1, 2 and 4 mg/kg, respectively, blood samples of 0.2 mL were collected from the suborbital vein into heparinized tubes at 0.083, 0.17, 0.33, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h. An immediate centrifugation at 1520 g was performed for 5 min and about 100  $\mu$ L of plasma was obtained. All the plasma samples were stored at -80 °C until determination.

For the tissue distribution study, 24 SD rats (50% of each sex) were randomly divided into 4 groups (six rats per group). 6258-70 was administered to rats at a dose of 2 mg/kg via intravenous administration. Liver, heart, spleen, lung, kidney, stomach, intestine, muscle, brain, bladder, testicle, uterus, fat and pancreas were collected at 0.25, 2, 6, and 12 h after administration, respectively. Tissue samples were washed in ice-cold 0.9% saline solution and wiped with filter paper. After that, all tissues were shredded and then about 0.33 g of the tissue samples (taking all the tissue

samples and recording the weight if the total weight of the tissue was less than 0.33 g) were weighed and homogenized with 1 mL of methanol, and then the supernatant was stored at  $-80^{\circ}\text{C}$  until the analysis.

### 2.5. Sample preparation

An aliquot of 50  $\mu\text{L}$  plasma or 100  $\mu\text{L}$  tissue homogenate was spiked with 20  $\mu\text{L}$  IS working solution, 20  $\mu\text{L}$  methanol and 100  $\mu\text{L}$  distilled water. The mixture was extracted with 2 mL methyl tert-butyl ether by vortex mixing for 3 min. After centrifugation at 1520 g for 5 min, the upper organic layer was transferred to a tube and evaporated to dryness at  $35^{\circ}\text{C}$  under nitrogen. The residue was redissolved in 200  $\mu\text{L}$  methanol, and an aliquot of 5  $\mu\text{L}$  was injected into the HPLC–MS/MS system.

### 2.6. Method validation

The method was fully validated in accordance with United States Food and Drug Administration (US FDA) guidelines and related reference [18,19]. Selectivity was investigated using six sources of the appropriate blank matrix (plasma or liver homogenate), one spiked sample (lower limit of quantification, LLOQ), and one sample at 10 h (plasma) or 12 h (liver homogenate) after dosing for monitoring interference of endogenous components in matrix or metabolites.

For calibration curves, which ranged from 4 to 4000 ng/mL, a least-squares linear regression with a weighted factor ( $1/C^2$ ) was adopted to plot the response ratio of 6258-70/IS versus the nominal concentration. The LLOQ was defined as the concentration with, at least, a signal-to-noise ratio of 10, and with an acceptable accuracy within  $\pm 20\%$  and the precision below 20%.

Intra- and inter-day precision and accuracy were determined by analyzing QC samples at low, medium and high concentrations on three different days with six replicates at each concentration per day. Precision was defined as the relative standard deviation (RSD%) and accuracy was defined as relative error (RE%).

The recovery of the analyte was determined at three QC levels with six replicates by comparing the peak areas from extracted samples with those from extracted blank samples spiked with the analyte at the same concentration. Matrix effects were calculated by the peak ratio of blank sample extracts spiked with the analyte to pure standard solution containing equivalent amounts of the compounds. The recovery and the matrix effect of IS were determined in the same way at the concentration of 5000 ng/mL for pharmacokinetics and 4000 ng/mL for tissue distribution.

Stability studies in plasma and liver homogenate were also conducted at three QC levels under several different storage conditions: at room temperature for 4 h, at  $-80^{\circ}\text{C}$  for 30 days, after three freeze-thaw cycles, and 8 h after preparation at  $10^{\circ}\text{C}$ .

The carry-over effect was investigated by injecting a blank plasma sample after the injection of an upper limit of quantification sample (ULQQ), and peak area found in the first blank sample should be less than 20% of the peak area at LLOQ of the analyte.

### 2.7. Statistical analysis

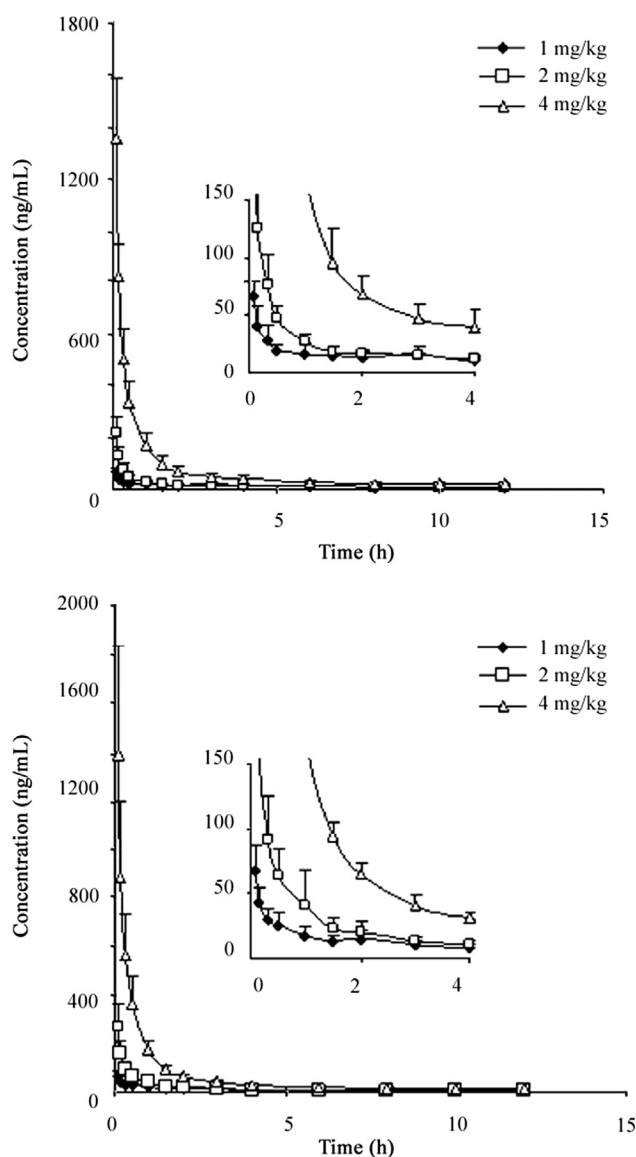
The pharmacokinetic parameters of 6258-70 were obtained by the non-compartmental analysis of plasma concentration versus time profiles using the DAS 2.1 software package (Chinese Pharmacological Society). All the data were expressed as mean  $\pm$  standard deviation (SD). The pharmacokinetic parameters in the two gender groups were compared using SPSS software (ver.16.0; SPSS, Chicago, IL) in independent samples *T*-test or Mann–Whitney test. When *p* was less than 0.05, it could be deemed that there was significant difference.

## 3. Results and discussion

### 3.1. HPLC–MS/MS optimization

The standard solutions of 6258-70 and IS, at the concentration of 1  $\mu\text{g}/\text{mL}$  respectively, were infused into the mass spectrometer to full scan in positive and negative modes. The results showed that the ion  $[\text{M}+\text{H}]^+$  at  $m/z$  906.6 and 808.5 exhibited the highest ionization response for 6258-70 and IS, respectively with most abundant stable fragment ions of  $m/z$  906.6  $\rightarrow$  539.4 and 808.5  $\rightarrow$  527.3, respectively. Therefore, 906.6 and 808.5 were selected as parent ions and 539.4 and 527.3 were chosen as daughter ions.

The composition of mobile phase has a significant influence on chromatographic behavior and appropriate ionization. Acetonitrile was chosen as the organic phase for higher responses. The addition of formic acid was found to increase the response of 6258-70, while the peak was likely to be bifurcation during the gradient elution. Compared with formic acid, addition of 5 mM ammonium acetate in water led to an enhanced response and good peak



**Fig. 3.** Mean concentration–time curves of 6258-70 in rat plasma after intravenous administration at different doses of 1, 2 and 4 mg/kg ( $n=6$ ) for (A) male and (B) female rats.

**Table 4**Main pharmacokinetic parameters of 6258-70 after intravenous administration at three doses for different genders in rats (mean  $\pm$  SD;  $n=6$ ).

Parameters	Male			Female		
	1 mg/kg	2 mg/kg	4 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg
AUC <sub>0-t</sub> ( $\mu\text{g h/L}$ )	149.0 $\pm$ 35.6	188.2 $\pm$ 34.3	941.3 $\pm$ 179.3	109.4 $\pm$ 26.3	214.9 $\pm$ 74.6	903.2 $\pm$ 200.2
AUC <sub>0-<math>\infty</math></sub> ( $\mu\text{g h/L}$ )	261.4 $\pm$ 41.0	266.2 $\pm$ 32.8	1139.0 $\pm$ 204.8	167.0 $\pm$ 36.8	270.6 $\pm$ 94.8	1049.7 $\pm$ 233.5
$t_{1/2}$ (h)	8.1 $\pm$ 2.0	8.0 $\pm$ 2.4	7.9 $\pm$ 1.7	7.9 $\pm$ 2.9	6.1 $\pm$ 1.4	7.5 $\pm$ 2.5
$C_{\text{max}}$ (ng/mL)	66.0 $\pm$ 13.6	217.7 $\pm$ 58.6	1358.8 $\pm$ 230.9	67.6 $\pm$ 20.6	263.9 $\pm$ 94.7	1388.4 $\pm$ 447.9
CL <sub>z</sub> (L/h/kg)	3.9 $\pm$ 0.7	7.6 $\pm$ 1.0	3.6 $\pm$ 0.7	6.3 $\pm$ 1.7	8.1 $\pm$ 2.7	4.0 $\pm$ 1.2
V <sub>z</sub> (L/kg)	44.9 $\pm$ 9.8	89.1 $\pm$ 35.6	40.9 $\pm$ 10.5	68.5 $\pm$ 22.6	68.1 $\pm$ 16.1	41.7 $\pm$ 11.4
MRT <sub>0-t</sub> (h)	4.4 $\pm$ 0.5	2.8 $\pm$ 0.5	6.0 $\pm$ 1.6	3.5 $\pm$ 0.4	2.8 $\pm$ 0.5	4.9 $\pm$ 0.2

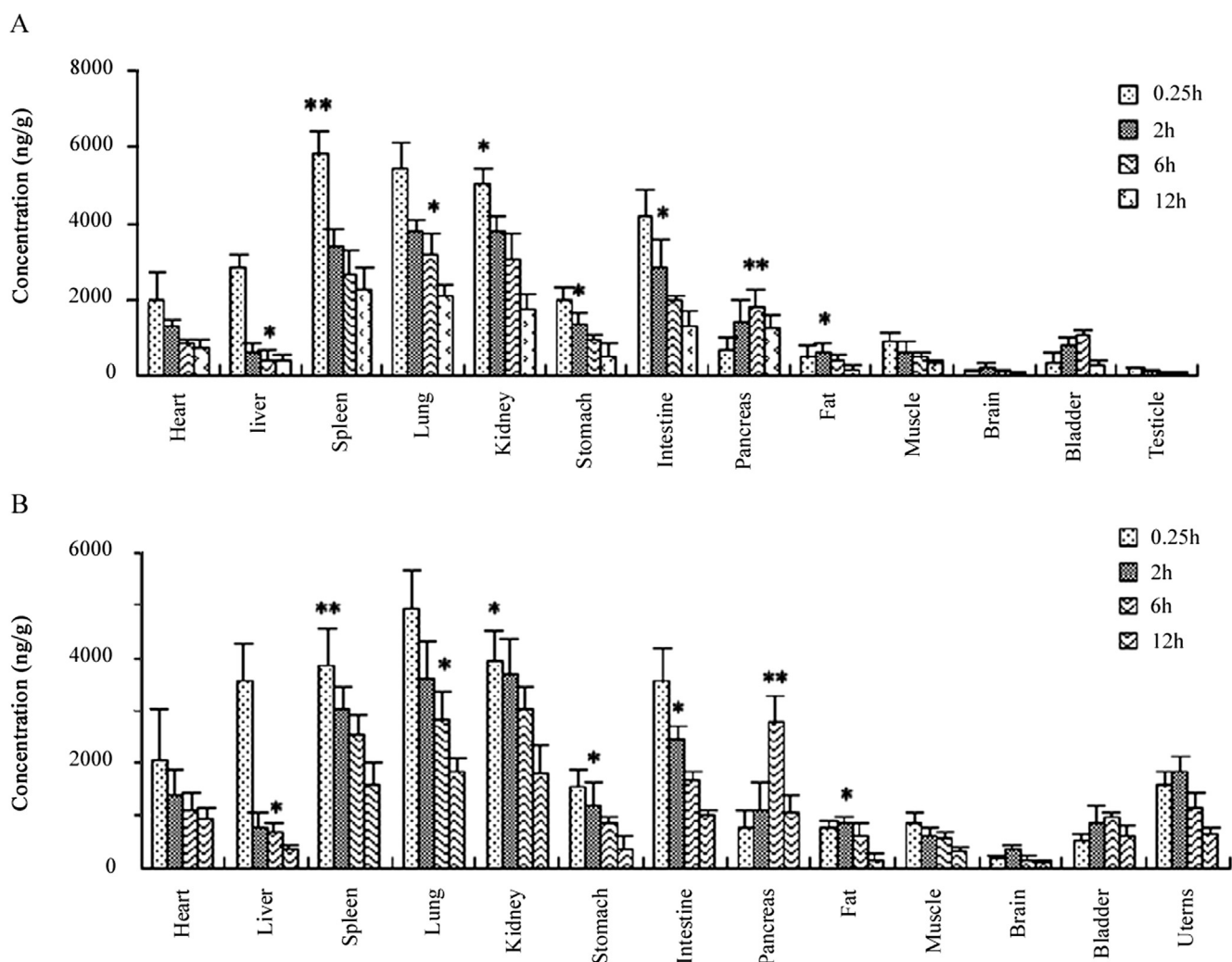
shape. Thus, the composition of mobile phase was acetonitrile and 5 mM ammonium acetate.

### 3.2. Sample preparation

Although protein precipitation is faster and simpler, the over-run matrix effect for 6258-70 was unignored. Liquid-liquid extraction was surveyed. Several solvents such as ethyl acetate, ether and methyl tert-butyl ether were tested, among which methyl tert-butyl ether provided a better recovery more than 80%. Moreover, the addition of 100  $\mu\text{L}$  distilled water was used to get a cleaner sample with appropriate matrix effect.

### 3.3. Method validation

Typical chromatograms of blank rat matrix (plasma or liver homogenate), blank rat matrix spiked with 6258-70 and IS, and rat plasma sample at 0.5 h or liver homogenate at 12 h after administration are shown in Fig. 2. No interference was observed at retention time of 6258-70 (1.88 min) or the IS (1.33 min). The calibration curves showed good linearity over the concentration ranging from 4 to 4000 ng/mL for 6258-70. Typical calibration curves for 6258-70 in rat plasma and different tissues are listed in Table 1. All the correlation coefficients ( $R$ ) were  $\geq 0.99$ . The LLOQ of the assay was 4 ng/mL in rat plasma and liver homogenates,



**Fig. 4.** Tissue distribution of 6258-70 after intravenous administration for (A) male and (B) female rats at dose of 2 mg/kg. \* means  $p < 0.05$  and \*\* means  $p < 0.01$ , indicating that there was a significant difference of the concentration of drug in tissue between the two genders.

with RSD 4.1% and RE 1.0% in plasma, and with RSD 11.0% and RE 5.2% in liver. Intra- and inter-day precision, accuracy and recovery for the analyte and IS are summarized in Table 2. The matrix effect of 6258-70 and IS in spiked rat plasma and liver homogenate was between 87.7% and 99.5%, indicating that no significant matrix effect was observed for 6258-70 or IS. All standards met the criteria of < 15% deviation from nominal concentration. The results of the stability tests are listed in Table 3. The analyte and IS were found to be stable in the plasma samples and liver samples at room temperature for 4 h, at  $-80^{\circ}\text{C}$  for 30 days, after three freeze-thaw cycles, and 8 h after prepared at  $10^{\circ}\text{C}$ . No peak area more than 20% of the lowest calibrator was detected in the blank sample injected after ULOQ, resulting in a negligible carry-over effect. These results show the method is reliable enough.

#### 3.4. Pharmacokinetic study and tissue distribution

The validated method was successfully applied to the pharmacokinetic study of 6258-70 at three different doses after intravenous administration. The mean plasma concentration vs time curves of 6258-70 between female and male at three doses are shown in Fig. 3, while related pharmacokinetic parameters are listed in Table 4. The linear regression analysis about experimental data showed priority to the  $\ln\text{AUC}_{0-t}$  and dosage,  $\ln\text{AUC}_{0-\infty}$  and dosage, as well as  $\ln C_{\max}$  and dosage between female and male rats, and the coefficient ( $R^2$ ) were above 0.86. This result demonstrated that the pharmacokinetic behavior of 6258-70 in vivo was in accordance with linear features within tested dosage. Pharmacokinetic parameters of two gender groups at three dosages after intravenous administration,  $\text{AUC}_{0-t}$ ,  $\text{AUC}_{0-\infty}$ ,  $C_{\max}$ ,  $V_z$  and  $CL_z$ , were compared via an independent-samples *T*-test after logarithmic transformation.  $t_{1/2}$  and  $\text{MRT}_{0-t}$  were measured using Mann-Whitney test. With statistical analysis, the results indicated that dosage did not affect the pharmacokinetic parameters ( $p > 0.05$ ) of 6258-70 between two genders at three dosages. These pharmacokinetic properties may afford some references to dosing region of future toxicity and toxicokinetic test.

The tissue distribution of 6258-70 at 2 mg/kg after intravenous administration are presented in Fig. 4. The results indicated that 6258-70 distributed widely and rapidly, having an effective penetration and wider spectrum of antitumor active. As Fig. 4 presents, the content of 6258-70 was higher in rich-blood tissues, such as heart, liver, spleen, lung and kidney. In addition, 6258-70 could also be observed in the brain, uterus, gastrointestinal tract and pancreas. The amount of the drug across the blood-brain-barrier (BBB) depends on the lipophilicity and molecular weight [20]. 6258-70 with highly lipophilic property can easily transport across brain endothelial cells by passive diffusion. Meanwhile, there was some 6258-70 in gastrointestinal tract and pancreas, which may have an effect on patients' life quality. It may be taken into consideration when we choose prescription design and formulation.

As regard to gender differences, the concentration of 6258-70 in collected tissues at measured time between two genders was analyzed via an independent-samples *T*-test after logarithmic transformation, and results are listed in Fig. 4. The results indicated there were significant differences at different time in some collected tissues, including liver, spleen, lung, kidney, intestine and pancreas, especially in reproductive organs, which maybe related to the differences in fat, weight, enzymes, as well as hormone. In addition, as taxanes are metabolized by CYP3A, it is possible for 6258-70 metabolized in this way, while the active and content of CYP3A are different between two genders [21–26].

There are some researches on derivative compounds from taxanes, such as DTX, TM-2 and Larotaxel [27–29]. Compared with DTX, 6258-70 has a longer  $t_{1/2}$  and a higher  $CL_z$  [20].

Pharmacokinetic behavior of TM-2 and 6258-70 in vivo was in accordance with linear features within tested dosage and they have the similar  $t_{1/2}$ . Compared with Larotaxel, 6258-70 has a longer  $t_{1/2}$ . Besides, both 6258-70 and Larotaxel are distributed widely and rapidly. The content of 6258-70 and Larotaxel was higher in rich-blood tissues, such as heart, liver, spleen, lung and kidney, and could also be observed in the BBB.

#### 4. Conclusions

It is the first time of developing a method for quantification of 6258-70, a new semi-synthetic taxane, in rat plasma and tissues. The fully validated method was successfully applied to the pharmacokinetics and tissue distribution studies of the two genders. The pharmacokinetic results indicated that the pharmacokinetic behavior of 6258-70 in rats was in accordance with linear features within tested dosage and there was no significant difference between two genders. The tissue distribution study showed 6258-70 had an effective penetration, spreading widely and rapidly. In addition, 6258-70 accumulated most in rich-blood tissues and could cross BBB. Besides, there are some differences in liver, spleen, lung, intestine, pancreas, fat and reproductive organs between the two genders. Thus, it may need more attention to this question in future study. The method and results of pharmacokinetics and tissue distribution in this paper may be useful to further study of 6258-70.

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