Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 989–996

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

# PK and tissue distribution of docetaxel in rabbits after i.v. administration of liposomal and injectable formulations

Ling Zhao, Yu-meng Wei, Xiao-dong Zhong, Yan Liang, Xue-mei Zhang, Wei Li, Bi-bo Li, Ya Wang, Yu Yu*

*Research Laboratory of Medicinal chemistry and Biomaterials, Chongqing Pharmaceutical Engineering Research Center, School of Pharmacy, Chongqing Medical University, No. 1 Yixue Yuan Road, Chongqing 400016, PR China*

ARTICLE INFO

*Article history:*
Received 10 November 2008
Received in revised form 12 January 2009
Accepted 13 January 2009
Available online 20 January 2009

*Keywords:*
Docetaxel
Liposome
Injection
Pharmacokinetics
Tissue distribution
HPLC

ABSTRACT

A simple and sensitive HPLC method was established and validated for the determination of docetaxel (DTX) in rabbit plasma and tissue samples. Biosamples were spiked with paclitaxel as an internal standard and pre-treated by solid phase extraction (SPE). Sample separation was performed on a reverse-phase HPLC column at 30 °C by using a mobile phase of acetonitrile–methanol–0.02 M ammonium acetate buffer (pH 5.0) (20:47.5:32.5, v/v/v) at flow rate of 1.0 mL/min The UV absorbance of the samples was measured at the wavelength of 230 nm. The standard curves were linear over the ranges of 0.02525–2.525 μg/mL for plasma, 1.010–202.00 μg/g for lung, 0.202–20.20 μg/g for spleen, liver and kidney, 0.202–10.10 μg/g for heart and stomach, 0.0505–2.02 μg/g for brain, respectively. The limits of quantification (LOQ) were 10.0 ng/mL in the plasma samples and 20.0 ng/g in the tissue samples, respectively. The analysis method was successfully applied to pharmacokinetics and tissue distribution studies of DTX liposomes and DTX injection after i.v. administration to the rabbits. The results showed that the liposome carrier led to a significant difference in pharmacokinetics and tissue distribution profile compared to the conventional DTX injection.



## 1. Introduction

Taxanes play an important role in the treatment of solid tumors. As a second-generation semi-synthetic taxane derivative, docetaxel (DTX) has shown significant antitumor activities against various human cancers such as non-small cell lung cancer and breast cancer. Unfortunately, clinical use of DTX is limited due to its serious adverse effects including neutropenia, peripheral neuropathy and hypersensitivity reactions. As a result, current research is mainly focused on developing new drug delivery systems of DTX to improve therapeutic index and reduce adverse reactions. Various drug delivery systems have been reported recently, such as DTX loaded liposomes [1–2], *N*-palmitoyl chitosan anchored DTX liposomes [3], fibrinogen-coated olive oil droplets [4–5], PEGylated liposomes [6–7], PEGylated immunoliposomes [8] and nanoparticle–aptamer bioconjugates [9]. Although they have some advantages, there are still some barriers for those formulations to meet the requirement for clinical use and industrial production.

Increasing evidence has suggested that, when the drug is incorporated into liposomes, its pharmacokinetics may be completely altered [10–11]. Therefore, it is necessary for us to study pharmacokinetics and tissue distribution of DTX loaded liposomes in animal model. Among the analytical techniques for biosamples, HPLC is the most common means to determine pharmacokinetics of the liposomes containing drug [10–12]. Based on the literature review, HPLC method has been used for the quantitative determination of DTX in biological fluids such as plasma and urine and methods for sample pre-treatment mainly included liquid–liquid extraction (LLE) and solid phase extraction (SPE) [13–16]. Only one report has been found by using LLE pre-treatment to analyze DTX in rat tissues without an internal standard [17]. Furthermore, the details for HPLC methods were not provided. At present, there are very few studies on the determination of DTX in rabbit plasma and tissue with pre-treatment of SPE.

In our study, DTX liposomes were prepared by solid dispersion-effervescent technique. The characteristics of liposomes, such as particle size and surface charges, were different from previous literatures [1–3,6–7]. The aim of this study was to establish and validate a simple and sensitive HPLC method to determine DTX in rabbit plasma and tissue samples with pre-treatment of SPE. The method was used successfully to study pharmacokinetics and tissue distribution of DTX liposomes and DTX injection after a single i.v. administration to the rabbit. Their pharmacokinetic parameters were also compared to discuss the pharmacokinetic and tissue distribution differences between DTX liposomes and DTX injection.

* Corresponding author. Tel.: +86 23 66868777; fax: +86 23 68485161.
*E-mail address:* yuyu3519@163.com (Y. Yu).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.01.016

## 2. Experimental

### 2.1. Materials and animals

DTX (batch, 20060901) and internal standard paclitaxel (batch, 20061001) were purchased from Chongqing Meilian Pharmaceutical Co., Ltd. (Chongqing, PR China). DTX injection (batch, 20070509) was obtained from Jiangsu Hengrui Pharmaceutical Co., Ltd. (Jiangsu, PR China). Phosopholipon 90H (HSPC) (batch, 00270) was obtained from Nattermann–Phospholipid (Cologne, Germany). Cholesterol (batch, 20060807) was provided by Nanjing Xinbai Medicine Co., Ltd. (Jiangsu, PR China). Carbonic acid monosodium salt (NaHCO3) (batch, 20060506) was obtained from Hunan Huari Medicine Co., Ltd. (Hunan, PR China). Monohydrate citric acid (batch, 20060709) was obtained from Shanghai Zhanzhi Medicine Co., Ltd. (Shanghai, PR China). Polysorbate 80 (Standard of injection, EP, batch, 20060905) was from Beijing Huiyou chemical Co., Ltd. (Beijing, PR China).

HPLC grade methanol and acetonitrile were obtained from Jiangsu Hanbang Co., Ltd. (Jiangsu, PR China). Dialysis membrane bag with molecular of 8000–10,000, analytical grade ammonium acetate, ethanol and other reagents were purchased from Chongqing Chemical Regent Co. Ltd. (Chongqing, PR China). Water used throughout the study was prepared by double distillation of water and filtered through Millipore membrane with 0.22 μm.

Animal experiments were performed on fifteen healthy albino rabbits ($2.0 \pm 0.1$ kg) from the Laboratory Animal Center of Chongqing Medical University. Animals were allowed free access to food and water in this study. Animal experiments were approved by the Chongqing Medical University animal ethical experimentation committee (Chongqing, PR China).

### 2.2. Preparation of DTX liposomes

DTX liposomes were prepared by the solid dispersion-effervescent technique. Briefly, DTX (12 mg), HSPC (240 mg), cholesterol (120 mg), monohydrate citric acid (1440 mg) and polysorbate 80 (9.6 mg) were dissolved in 30 mL of ethanol. The above solution was filtered through a 0.22 μm hydrophobic membrane, and transferred to a 100 mL round bottom flask. Under a constant stirring at 300 rpm at 45 °C, the organic solvent was completely removed to form solid granules (proliposomes), which were dried at 4 °C under vacuum condition for 24 h and stored in sealed containers under a nitrogen atmosphere in a dark place at 4–8 °C.

The proliposomes containing DTX were hydrated under constant shaking for a period of 20 min prior to use, by introduction of an aqueous solution of $NaHCO_3$ (5.0%, w/v) at $25 \pm 5$ °C, which was prepared followed by filtration through a 0.22 μm membrane with addition of appropriate amount of activated carbon.

### 2.3. HPLC analysis

#### 2.3.1. Chromatographic conditions

The determination of DTX in various samples was carried out by using a modification of the HPLC procedures described by Garg and Ackland [18]. The HPLC system consisted of an Agilent series 1100 Chemstation, Agilent 1100 VWD absorbance detector. Sample separation was performed on a Phenomenex LUNA C18 column (5 μm, 250 mm × 4.6 mm) (Phenomenex, USA) with a guard column (Phenomenex C18, 4.0 mm × 3.0 mm). The mobile phase consisting of acetonitrile–methanol–ammonium acetate buffer (pH 5; 0.02 M) (20:47.5:32.5, v/v/v) was prepared daily and degassed by sonication in an ultrasonic bath before use. Column temperature was 30 °C and the flow rate was 1 mL/min, respectively. The UV absorbance of the sample (20 μL) was measured at the wavelength of 230 nm. Data collection and processing were performed using Agilent series 1100 Chemstation software.

#### 2.3.2. Calibration standard and quality control samples

Blood samples were collected from experimental rabbits and stored at −20 °C until DTX was assayed. The blank plasma (1 mL) was spiked with 50 μL of various known concentrations of DTX solutions and 50 μL of paclitaxel solution (10.0 μg/mL in methanol) as the internal standard for the determination of DTX to obtain a series of DTX standard solutions with the concentrations of 0.02525, 0.101, 0.2525, 0.505, 1.2625, and 2.525 μg/mL. Quality control (QC) samples were prepared by blank plasma at low, medium and high DTX concentrations.

Various blank tissues (heart, liver, spleen, lung, kidney, stomach and brain) were removed, weighed and homogenized with 0.9% saline (4 mL/g). Tissue samples with various DTX concentrations (0.0505–202.00 μg/g) were prepared by adding 50 μL of various known concentrations of DTX solutions and 50 μL of paclitaxel solution (10.0 μg/mL in methanol) to 1.0 mL different tissue homogenates. The DTX concentration ranges may differ in various tissues. QC samples were prepared from blank tissue homogenates at low, medium and high DTX concentrations.

Samples were pre-treated by SPE procedure described in Section 2.4. Plasma and tissue samples were quantified using the ratio of the peak area of DTX to that of paclitaxel as the assay response.

#### 2.3.3. Validation of analysis method

The selectivity, linearity, precision, accuracy, extraction recovery and stability were investigated to validate the HPLC method.

Selectivity was investigated by comparing the chromatography of blank rabbit plasma or blank tissue homogenate of the lung as a representative sample, blank rabbit plasma or blank lung homogenate spiked with DTX and (or) paclitaxel, and a plasma sample or a lung tissue sample. The standard curves were obtained through analysis of calibration standard biosamples and plot of peak area ratios ($R$) of DTX and paclitaxel versus the corresponding DTX concentration ($C$). Intra-day and inter-day accuracy and precision was determined by analysis of QC samples at low, medium and high concentration on the same day and on different days. The extraction recoveries of DTX at three QC levels were calculated by comparing the peak area of DTX in extracted biosamples with those obtained by directly determining DTX standard solutions at the same concentration. The stability of DTX in rabbit plasma and tissue samples was tested on five freeze–thaw cycles with QC samples at three concentration levels. These biosamples were frozen at −20 °C and thawed at room temperature on days 0, 2, 4, 6, and 8.

### 2.4. Pharmacokinetics study

Experimental rabbits were randomly divided into three groups (five rabbits per group). Group 1 was treated with DTX liposomes containing 2 mg of DTX per kg body weight via the ear vein, while a corresponding dose of DTX injection was administered to group 2. Group 3 offered blank plasma and blank tissue for establishment of the analysis method. Blood samples (2 mL) were drawn from the marginal ear vein into heparinized centrifuge tubes just before dosing (0 h) and after 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h, the samples were then centrifuged at 5000 rpm for 5 min to separate the plasma.

After 24 h, these rabbits were immediately sacrificed following blood collection; various tissues (heart, liver, spleen, lung, kidney, stomach and brain) were rapidly removed, washed with 0.9% saline solution, then wiped with filter paper, weighed and homogenized with 0.9% saline (4 mL/g).

To 1.0 mL of the above plasma or tissue homogenates, 3 mL protein precipitation agent (methanol–ammonium acetate buffer (pH

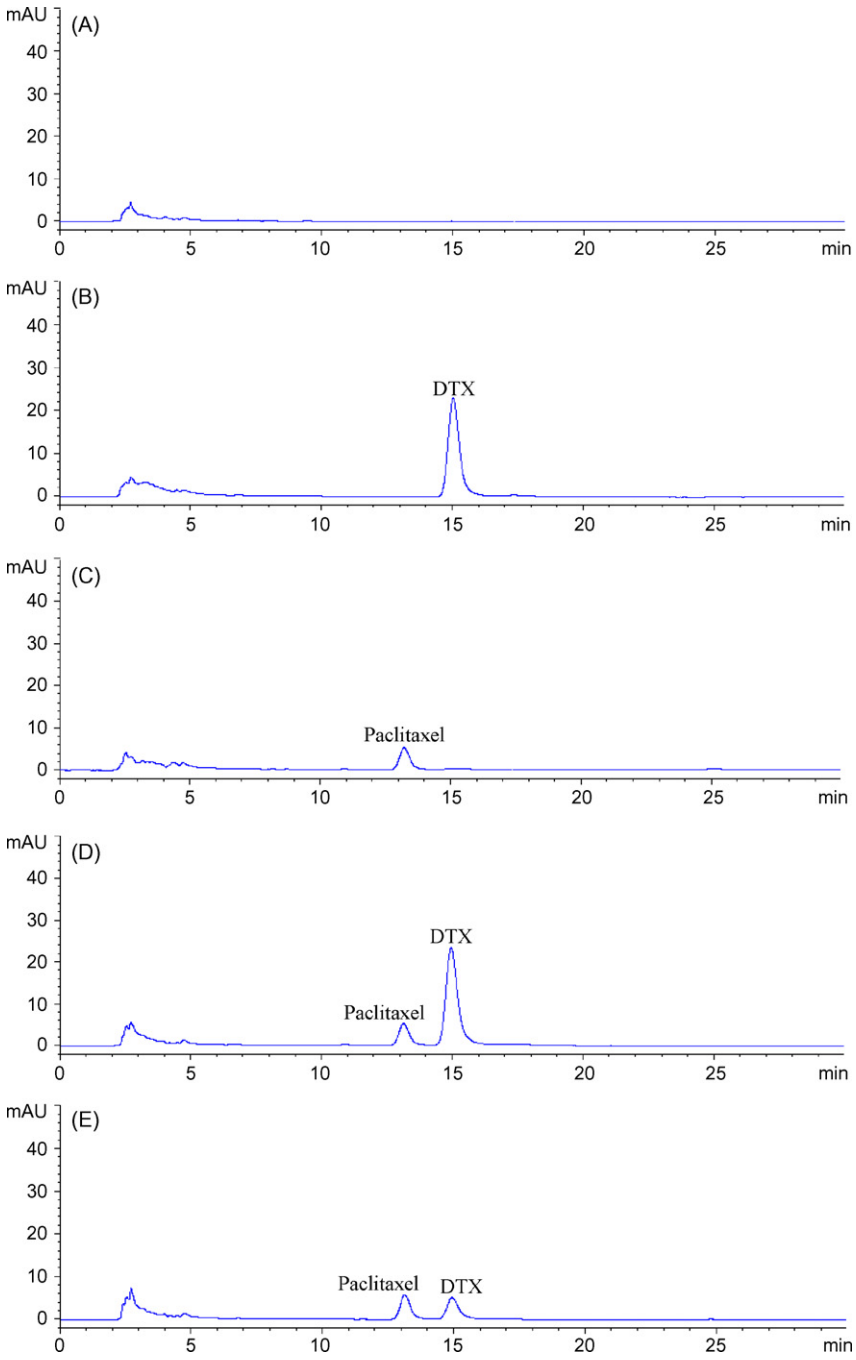


**Fig. 1.** Representative HPLC chromatograms obtained for blank plasma (A), blank plasma spiked with DTX 5.05 μg/mL (B), blank plasma spiked with paclitaxel (internal standard) 0.50 μg/mL (C), blank plasma spiked with DTX 5.05 μg/mL + paclitaxel 0.50 μg/mL (D) and a plasma biosample collected from a rabbit at 0.083 h after i.v. administration of DTX liposomes at a dosage of 2 mg/kg (DTX concentration = 0.64 μg/mL) + paclitaxel (0.50 μg/mL) (E).

5.0; 6 M) (1:2, v/v)) and 50 μL of internal standard solution containing paclitaxel (10.0 μg/mL) were added and mixed for 5 min by vortex and then treated by sonication at 40 °C for 10 min to extract DTX and paclitaxel. Following centrifugation at 12000 rpm for 10 min, the clear supernatant was transferred to a glass tube, which was diluted (four-fold, v/v) with water. The diluted mixture was slowly transferred into each SPE column (Phenomenex C18) that was first conditioned with 2 mL of methanol followed by 2 mL of ammonium acetate buffer (pH 5.0; 0.01 M). After washing SPE column in turn with 2 mL of ammonium acetate buffer (pH 5; 0.01 M), methanol–ammonium acetate buffer (pH 5; 0.01 M) (1:9, v/v) and methanol–ammonium acetate buffer (pH 5; 0.01 M) (2:8, v/v), each column was dried under the vacuum for approximately 1 min and both compounds were eluted with 2 mL mixture solution of acetonitrile–methanol (1:1, v/v) into the glass tubes and the eluate was evaporated to dryness at 40 °C under a stream of nitrogen. The dry sample was reconstituted with 200 μL of mobile phase and vortex-mixed, and centrifuged at 12000 rpm for 5 min. Then 20 μL of the clear supernatant was injected into chromatographic system.

### 2.5. Pharmacokinetic analysis and statistics

Pharmacokinetic parameters of DTX in rabbit plasma were calculated by the 3p97 software provided by the Chinese Pharmacological Society. The most suitable pharmacokinetic model was determined in terms of the range of the coefficient of determina-

tion ($r^2$) and comparisons of Akaike's information criterion values (AIC) [19–20]. Further pharmacokinetic analysis was performed to calculate the area under the curve (AUC), the elimination half-lives ($t_{1/2}$), apparent volume of distribution ($V$) and the clearance rate (CL).

The significance of the difference was analyzed by ANOVA Models with Statistical Program for Social Sciences (SPSS 15.0), and a significant level of less than 0.01 was considered statistically significant.

## 3. Result and discussion

### 3.1. Characterization of liposomes

DTX liposomes prepared in this study were milky white suspension. Particle size and zeta-potential of liposomes determined in the Malvern ZEN3600 instrument (Malvern Instruments, UK) were $1011 \pm 22$ nm and $-23.7 \pm 0.26$ mV, respectively. The entrapment efficiency of DTX liposomes measured by using a dialysis method as described by Immordino et al. [6] was $90.12 \pm 0.36\%$.

### 3.2. Chromatography and sample preparation

#### 3.2.1. Chromatography

Several authors have previously reported a HPLC method for the determination of DTX in biosamples using a reverse-phase column and the various ratio of acetonitrile and ammonium acetate buffer (pH 5.0; 0.02 M) as mobile phase [16,18]. However, under our experimental conditions, this mobile phase consisting of acetonitrile and ammonium acetate buffer (pH 5.0; 0.02 M) was found to be serious tailing for paclitaxel and DTX peaks. Thus, the mobile phase was modified to add the appropriate volume of methanol. The resulting system offered good resolution and decreased tailing for both the DTX and paclitaxel peaks. Interestingly, it was observed that the location of the paclitaxel peak and the DTX peak was just switched with and without methanol. These authors state that as the polarity of the mobile phase was increased by the introduction of methanol, the retention time of DTX was significantly prolonged compared to that of paclitaxel. The presumption was validated in this study, specifically, the relative retention times of DTX/paclitaxel was increased with increasing methanol volume ratio. In order to improve peak symmetry, the optimization of volume ratio of the mobile phase was carried out. As a result, acetonitrile–methanol–ammonium acetate buffer (pH 5.0; 0.02 M) (20:47.5:32.5, v/v/v) was used as a mobile phase to provide satisfactory results for this study.

#### 3.2.2. Sample preparation

At present, biosamples of DTX in the plasma or urine were often pre-treated by using LLE and SPE methods, but rabbit tissue samples pre-treated by using the SPE method have not been reported yet. In the previous literature on LLE method [17], the rat plasma or tissue samples were prepared with an acetonitrile as protein precipitation and the supernatant after precipitation was evaporated at approximately 45 °C under a gentle stream of nitrogen. Then the residue was dissolved in 1 mL mobile phase followed by filtration for determination with 50 μL sample solution loaded into the HPLC system. In this study, the above LLE method was not effective in removing endogenous substances which interfere with the quantification of DTX and paclitaxel in rabbit plasma and tissue samples. The extraction recoveries obtained from tissue QC samples were not ideal. To some extent, these failures may be caused by the differences in animal species. In this experiment, it was found that ammonium acetate buffer (pH 5.0) concentration and the ratio of methanol and ammonium acetate buffer had a significant effect on extraction recoveries of DTX and paclitaxel in rabbit tissue samples. If the methanol–ammonium acetate buffer (pH 5.0; 6 M) (1:2, v/v) was used as protein precipitant in our study. SPE method would not only remove the interfering substances in rabbit plasma or rabbit tissue samples, but also offer good extraction recoveries of DTX and paclitaxel in rabbit biosamples. Therefore, SPE method was chosen to prepare rabbit plasma and rabbit tissue biosamples in this investigation.

### 3.3. Validation of analysis method

#### 3.3.1. Selectivity and chromatography

The typical chromatograms obtained from blank rabbit plasma or blank rabbit tissue homogenate of the lung as a representative sample, blank rabbit plasma or blank rabbit lung homogenate spiked with DTX and (or) paclitaxel, and a plasma sample collected at 0.083 h after i.v. administration of DTX liposomes or a lung sample was shown in Figs. 1 and 2, respectively. No significant interfering peaks at or near the retention time of DTX or paclitaxel were observed, which indicated that the samples were highly purified by the SPE method. Therefore, selectivity of the chromatographic conditions is good.

#### 3.3.2. Linearity, limit of quantification and limit of detection

The typical equation of the standard curves of the peak area ratio ($R$) to the concentration ($C$), the linear range and correlation coefficient ($r$) of DTX in plasma and tissue homogenates were summarized in Table 1. Standard curves were linear over the ranges 0.02525–2.525 μg/mL in plasma and 0.0505–202.00 μg/g in tissue homogenates and excellent correlation between analyzed peak area ratio and concentration of the drug was observed with $\geq$0.999. The assay method offered the limits of quantification (LOQ) of 10.0 ng/mL in rabbit plasma and 20.0 ng/g in tissue homogenate samples, and the limit of detection (LOD) of DTX in plasma and tissue samples were 3 ng/mL and 6 ng/g, respectively.

#### 3.3.3. Accuracy and precision

The accuracy and precision of analysis method were assessed with QC samples at low, medium and high concentration and the results were shown in Table 2. It can be seen from Table 2 that the analysis method established in this study is accurate and precise with intra- and inter-day R.S.D. below 10% for these biosamples. The accuracy of plasma and tissue samples ranged from 92.8 to 113.4% and 80.7 to 116.5%, respectively. According to the requirements of Chinese Pharmacopoeia (2005 edition, part II) [21], these values were within the acceptable range, therefore, the analysis method is accurate and precise for determination of DTX in rabbit plasma and tissues.

#### 3.3.4. Extraction recovery

The extraction recovery from DTX biosamples was calculated by comparing peak area with QC samples ($n = 3$) at low, medium and high concentration levels. The extraction recovery of DTX in plasma, heart, liver, spleen, lung, kidney, stomach and brain were within the range of 84.1–91.7%, 86.2–92.3%, 81.3–95.9%, 70.8–84.6%, 83.9–97.6%, 86.4–99.4%, 88.1–95.4%, 87.4–94.7%, and the extraction recovery of paclitaxel (internal standard) in plasma, heart, liver, spleen, lung, kidney, stomach and brain were 95.3, 90.4, 94.9, 97.8, 95.1, 93.1, 103.0, and 96.4%, respectively.

#### 3.3.5. Stability

The stability of DTX in rabbit plasma and tissue was studied after five freeze–thaw cycles and the results are given in Table 3. The coefficient of variation ranged from 3.10 to 9.28%. The results showed that DTX were stable for at least 8 days in rabbit plasma and tissue stored at −20 °C.

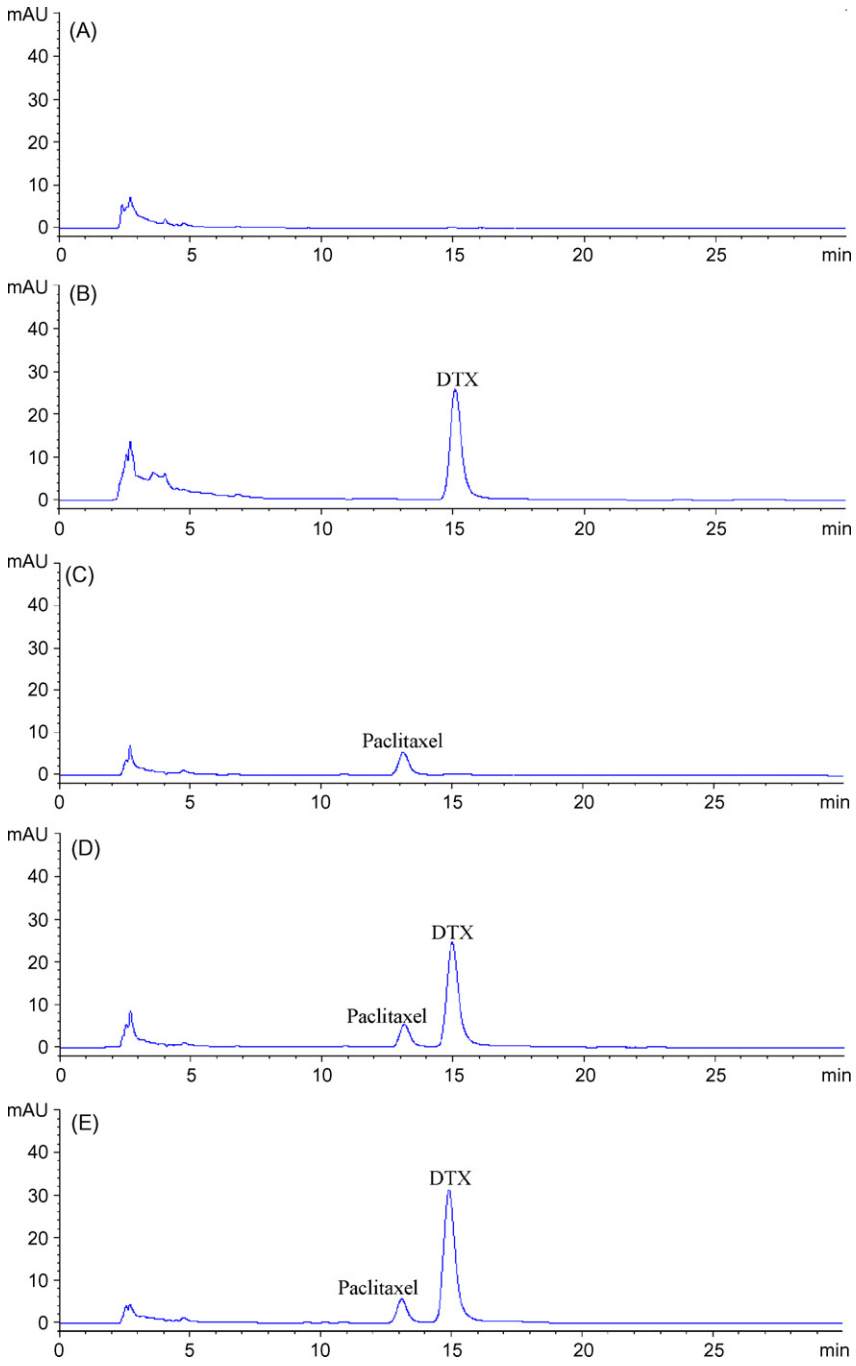


**Fig. 2.** Representative HPLC chromatograms obtained for blank rabbit lung (A), blank lung tissue spiked with DTX (20.2 μg/g) (B), blank lung tissue spiked with paclitaxel (internal standard) (2.0 μg/g) (C), blank lung tissue homogenates spiked with DTX (20.2 μg/g) + paclitaxel (2.0 μg/g) (D) and a lung tissue sample (DTX concentration was 19.5 μg/g) collected from a rabbit at 24 h after i.v. administration of DTX liposomes at a dosage of 2 mg/kg + paclitaxel (2.0 μg/g) (E).

**Table 1**
Linear ranges, standard curves and correlation coefficients of DTX in different biosamples[a].

| Biosamples | Linear ranges (μg/mL or μg/g)[b] | Standard curves | Correlation coefficients ($r$) |
|---|---|---|---|
| Plasma | 0.02525–2.525 | $R = 1.3863C - 0.004$ | 0.9993 |
| Lung | 1.010–202.00 | $R = 0.2888C + 0.093$ | 0.9996 |
| Spleen | 0.202–20.20 | $R = 0.3265C + 0.132$ | 0.9996 |
| Liver | 0.202–20.20 | $R = 0.2792C + 0.130$ | 0.9990 |
| Kidney | 0.202–20.20 | $R = 0.3135C + 0.062$ | 0.9998 |
| Heart | 0.202–10.10 | $R = 0.3410C + 0.135$ | 0.9996 |
| Stomach | 0.202–10.10 | $R = 0.3717C - 0.012$ | 0.9990 |
| Brain | 0.0505–2.02 | $R = 0.3400C + 0.096$ | 0.9998 |

[a] The values are arithmetic means, $n = 3$.
[b] The unit of drug concentration in plasma: μg/mL. The unit of drug concentration in tissue: μg/g.

**Table 2**
Intra-day and inter-day accuracy and precision of DTX in rabbit plasma and tissue homogenates quality control samples by HPLC-UV method ($n = 6$)[a].

| Added concentration (μg/mL or μg/g)[b] | Intra-day | | | Inter-day | | |
|---|---|---|---|---|---|---|
| | Measured concentration (μg/mL or μg/g)[b] | Accuracy (%) | (R.S.D.) (%) | Measured concentration (μg/mL or μg/g)[b] | Accuracy (%) | (R.S.D.) (%) |
| Plasma | | | | | | |
| 0.02525 | 0.0286 | 113.4 | 9.31 | 0.0275 | 109.6 | 9.77 |
| 0.101 | 0.1038 | 103.9 | 7.84 | 1.0080 | 99.8 | 8.12 |
| 1.2625 | 1.1715 | 92.8 | 5.14 | 1.2777 | 101.2 | 5.91 |
| Heart | | | | | | |
| 0.202 | 0.1640 | 81.2 | 7.85 | 0.1685 | 83.4 | 8.55 |
| 1.01 | 1.1524 | 114.1 | 6.51 | 1.0979 | 108.7 | 7.74 |
| 5.05 | 5.2639 | 104.2 | 4.79 | 4.9642 | 98.3 | 5.61 |
| Liver | | | | | | |
| 0.202 | 0.2006 | 99.3 | 8.31 | 0.2069 | 102.4 | 8.90 |
| 2.02 | 1.8833 | 93.2 | 7.10 | 1.8281 | 90.5 | 7.63 |
| 20.2 | 19.7708 | 97.9 | 3.88 | 20.3414 | 100.7 | 4.52 |
| Spleen | | | | | | |
| 0.202 | 0.1838 | 91.0 | 5.62 | 0.1729 | 85.6 | 6.67 |
| 2.02 | 2.2664 | 112.2 | 5.04 | 2.1028 | 104.1 | 6.32 |
| 20.2 | 20.1838 | 99.9 | 3.71 | 19.6546 | 97.3 | 3.88 |
| Lung | | | | | | |
| 1.01 | 1.0736 | 106.3 | 6.37 | 1.0221 | 101.2 | 7.15 |
| 20.20 | 21.8386 | 108.1 | 5.29 | 22.8260 | 110.3 | 5.54 |
| 202.0 | 200.586 | 99.3 | 3.46 | 211.090 | 104.5 | 3.81 |
| Kidney | | | | | | |
| 0.202 | 0.1630 | 80.7 | 7.81 | 0.1640 | 81.2 | 8.54 |
| 2.02 | 2.2581 | 111.8 | 5.37 | 2.0644 | 102.2 | 6.05 |
| 20.2 | 20.3212 | 100.6 | 4.51 | 20.0182 | 99.1 | 4.92 |
| Stomach | | | | | | |
| 0.202 | 0.2353 | 116.5 | 8.89 | 0.2139 | 105.9 | 9.25 |
| 2.02 | 1.7736 | 87.8 | 7.41 | 1.7978 | 89.0 | 7.81 |
| 10.1 | 8.8981 | 88.1 | 6.55 | 8.6557 | 85.7 | 8.04 |
| Brain | | | | | | |
| 0.0505 | 0.0409 | 80.9 | 4.41 | 0.0422 | 83.6 | 3.96 |
| 0.404 | 0.3470 | 85.9 | 3.36 | 0.3547 | 87.8 | 3.57 |
| 2.02 | 2.1117 | 104.5 | 2.89 | 2.0503 | 101.5 | 3.07 |

[a] The values are arithmetic means, $n = 6$.
[b] The unit of drug concentration in plasma: μg/mL; the unit of drug concentration in tissue: μg/g.

**Table 3**
Stability of DTX in rabbit plasma and tissue on five freeze–thaw cycles determined by HPLC-UV method ($n = 6$)[a].

| Samples | Added concentration (μg/mL or μg/g)[b] | Measured concentration (μg/mL or μg/g)[b] | Variation coefficient (%) |
|---|---|---|---|
| Plasma | 0.02525 | 0.0267 | 8.99 |
| | 0.101 | 0.0912 | 7.79 |
| | 1.2625 | 1.2243 | 6.40 |
| Heart | 0.202 | 0.1795 | 7.58 |
| | 1.01 | 0.9779 | 5.79 |
| | 5.05 | 4.9524 | 3.70 |
| Liver | 0.202 | 0.1896 | 9.28 |
| | 2.02 | 2.1024 | 6.60 |
| | 20.2 | 20.5871 | 3.10 |
| Spleen | 0.202 | 0.1894 | 7.39 |
| | 2.02 | 2.0522 | 5.70 |
| | 20.2 | 21.3556 | 3.90 |
| Lung | 1.01 | 1.0514 | 6.90 |
| | 20.20 | 21.9851 | 4.40 |
| | 202.0 | 209.6871 | 3.81 |
| Kidney | 0.202 | 0.1710 | 9.12 |
| | 2.02 | 2.1894 | 7.00 |
| | 20.2 | 20.8745 | 4.10 |
| Stomach | 0.202 | 0.2248 | 8.99 |
| | 2.02 | 1.8071 | 7.20 |
| | 10.1 | 8.9025 | 6.60 |
| Brain | 0.0505 | 0.0449 | 4.01 |
| | 0.404 | 0.3570 | 4.90 |
| | 2.02 | 2.1047 | 3.26 |

[a] The values are arithmetic means, $n = 6$.
[b] The unit of drug concentration in plasma: μg/mL; the unit of drug concentration in tissue: μg/g.

**Table 4**
Plasma concentration of DTX in rabbits determined by HPLC-UV method after i.v. administration of DTX liposomes and DTX injection (arithmetic means ± S.D., $n = 5$).

| Time (h) | Plasma concentration for DTX liposomes (μg/mL) | Plasma concentration for DTX injection (μg/mL) |
|---|---|---|
| 0.083 | 0.651 ± 0.091 | 0.585 ± 0.137 |
| 0.25 | 0.075 ± 0.011 | 0.322 ± 0.084 |
| 0.5 | 0.059 ± 0.006 | 0.200 ± 0.069 |
| 1 | 0.093 ± 0.011 | 0.146 ± 0.036 |
| 2 | 0.050 ± 0.010 | 0.144 ± 0.033 |
| 4 | 0.045 ± 0.007 | 0.139 ± 0.019 |
| 6 | 0.043 ± 0.007 | 0.102 ± 0.022 |
| 8 | 0.037 ± 0.004 | 0.098 ± 0.021 |
| 12 | 0.032 ± 0.004 | 0.070 ± 0.024 |
| 24 | 0.010 ± 0.0 | 0.037 ± 0.015 |

### 3.4. Pharmacokinetics and tissue distribution study

#### 3.4.1. Plasma pharmacokinetics study

After a single i.v. administration of DTX liposomes and DTX injection of 2 mg/kg in rabbits, the plasma drug concentration were shown in Table 4 and the main pharmacokinetic parameters were summarized in Table 5. From Table 4, it can be seen that the drug concentration in plasma rapidly decreased during the first 0.25 h, which is consistent with Marchettini's study that a rapid clearance of the drug from the systemic circulation was observed during the first 15 min after i.v. injection of DTX solution [17]. In addition, the plasma drug concentration was detectable up to 24 h in rabbits using the analysis method established in this study. DTX plasma concentrations obtained at 0.083 h after i.v. administration were 0.651 ± 0.091 μg/mL for DTX liposomes and 0.5851 ± 0.1365 μg/mL for DTX injection, respectively. Moreover, plasma drug concentration in the case of DTX liposomes declined rapidly after attaining peaking value, whereas plasma concentration of DTX injection declined gradually. Also the liposomal DTX concentration in the plasma was markedly lower than injection at each time point except for first 0.083 h.

Based on the analysis of models and parameters, a three-compartment model presented the best fit to the plasma drug concentration time curves obtained in rabbits. The half-lives of DTX liposomes and DTX injection were 11.027 and 10.714 h, respectively. The volume of distribution of DTX liposomes (0.272 mL/kg) was significantly smaller than that of DTX injection (2.378 mL/kg). The AUC of DTX injection was about 2.0 times greater than that of the DTX liposomes.

#### 3.4.2. Tissue distribution study

The tissue distribution of DTX after i.v. administration of DTX liposomes and DTX injection at 24 h were shown in Fig. 3. These results demonstrated that the liposome carrier altered the tissue distribution pattern of DTX in rabbits significantly in comparison with the DTX injection. After i.v. administration of liposomal DTX, the drug concentration in lung was the highest (19.62 ± 3.32 μg/g) among all tissues or plasma. Compared with DTX injection, DTX concentration in the lung after i.v. administration of DTX liposomes was enhanced from 1.42 ± 0.30 to 19.62 ± 3.32 μg/g (about 14-fold). In other word, for DTX liposomes, the liposome carrier could delivery DTX mainly into lung tissue after i.v. administration. In addition, with liposome carrier, the drug concentration in the liver and spleen was slightly higher in comparison with that of DTX injection ($p < 0.01$). Many literatures also suggested that drug encapsulated by liposomes produced a higher drug concentration in RES-rich organs such as the liver and spleen [22–23].

**Table 5**
The comparative pharmacokinetic parameters after i.v. administration of DTX loaded liposomes and DTX injection in rabbit ($n = 5$).

| Samples | $t_{1/2}$ (h) | $C_{max}$ (μg/mL) | AUC (μg h/mL) | CL (mL/(kg h)) | $V$ (mL/kg) |
|---|---|---|---|---|---|
| DTX liposomes | 11.027 | 0.651 | 0.893 | 1.788 | 0.272 |
| DTX injection | 10.714 | 0.585 | 2.130 | 0.766 | 2.378 |

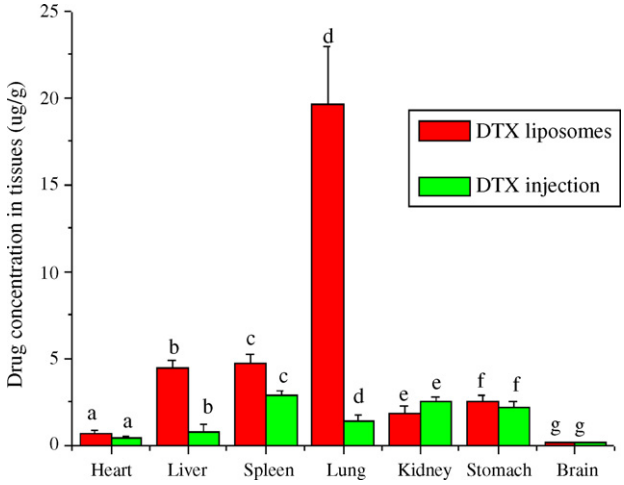


**Fig. 3.** Distribution in tissues in rabbits after intravenous administration of DTX liposomes and DTX injection at 24 h. These values are arithmetic mean ± S.D., $n = 5$. [a] $p = 0.151$, [b] $p = 0.001$, [c] $p = 0.008$, [d] $p = 0.000$, [e] $p = 0.002$, [f] $p = 0.857$, and [g] $p = 0.650$.

As shown in Fig. 3, liposomal DTX concentrations in heart, stomach and brain were similar to DTX injection ($p > 0.01$). On the other hand, drug concentration in the kidney for DTX liposomes was lower than that for DTX injection ($p < 0.01$). The results suggested that liposomes could greatly increase drug accumulation in the lung; however, it did not increase drug concentration in the heart, kidney, stomach and brain after i.v. administration of DTX liposomes in a rabbit model. As evident from these values of biodistribution, it could be concluded that the DTX liposomes were used for treatment of lung cancer to reduce toxicity to other tissues and improve therapeutic index.

## 4. Conclusion

The reverse-phase HPLC method established and validated in this study is simple, selective, sensitive, accurate and precise for determination of DTX in rabbit plasma and tissue samples. This validated HPLC assay method has been successfully applied to the pharmacokinetic and tissue distribution studies of DTX after i.v. administration of DTX loaded liposomes and DTX injection in a rabbit model. The assay method is also suitable for quantitative determination of DTX in biosamples for preclinical and clinical experimental studies of DTX liposomes. Furthermore, it was found that pharmacokinetics and tissue distribution behavior of DTX in rabbits after i.v. administration of DTX loaded liposomes and DTX injection were significantly altered.

## Acknowledgments

This work received financial support from National Natural Science Foundation (project nos.: 30772595, 30371632, 30171070) of the People's Republic of China and Chongqing Education Commission.

## References

[1] Y.P. Li, L.L. Cheng, W.W. Gu, C.N. Patent 200510029634.0, 21 March (2007).
[2] D.K. Wang, W.L. Li, C.X. Zhang, W.T. Zhang, L. Lin, C.N. patent 200710007668.9, 24 October (2007).
[3] L. Ge, J.B. Zhu, F. Xiong, B. Ni, J. Pharm. Pharmacol. 59 (2007) 661–667.

[4] A.S. Jakate, C.M. Einhaus, A.P. DeAnglis, G.S. Retzinger, P.B. Desai, Cancer Res. 63 (2003) 7314–7320.
[5] C.M. Einhaus, A.C. Retzinger, A.O. Perrotta, M.D. Dentler, A.S. Jakate, P.B. Desai, G.S. Retzinger, Clin. Cancer Res. 10 (2004) 7001–7010.
[6] M.L. Immordino, P. Brusa, S. Arpicco, B. Stella, F. Dosio, L. Cattel, J. Control. Release 91 (2003) 417–429.
[7] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, J. Control. Release 65 (2000) 271–284.
[8] Q.W. Wang, H.L. Lu, C.C. Song, H. Liu, C.G. Xu, World J. Gastroenterol. 11 (2005) 4003–4007.
[9] A.D. Ellington, J.W. Szostak, Nature 346 (1990) 818–822.
[10] L. Jia, M. Garza, H. Wong, D. Reimer, T. Redelmeier, J.B. Camden, S.D. Weitman, J. Pharm. Biomed. Anal. 28 (2002) 65–72.
[11] R.N. Kotwania, P.C. Gokhalea, P.V. Bodhea, B.G. Kirodiana, N.A. Kshirsagar, S.K. Pandyab, Int. J. Pharm. 28 (2002) 11–15.
[12] L. Embree, K.A. Gelmon, A.W. Tolcher, N.J. Hudon, J.R. Heggie, C. Dedhar, M.S. Webb, M.B. Bally, L.D. Mayer, J. Pharm. Biomed. Anal. 16 (1997) 675–687.
[13] J.M. Extra, F. Rousseau, R. Bruno, M. Clavel, N. Lebail, M. Marty, Cancer Res. 53 (1993) 1037–1042.
[14] H. Rosing, V. Lustig, F.P. Koopman, W.W. ten Bokkel Huinink, J.H. Beijnen, J. Chromatogr. B 696 (1997) 89–98.
[15] M.R. Rouini, A. Lotfolahi, D.J. Stewart, J.M. Molepo, F.H. Shirazi, J.C. Vergniol, E. Tomiak, F. Delorme, L. Vernillet, M. Giguere, R. Goel, J. Pharm. Biomed. Anal. 17 (1998) 1243–1247.
[16] J. Ciccolini, J. Catalin, M.F. Blachon, A. Durand, J. Chromatogr. B 759 (2001) 299–306.
[17] P. Marchettini, O.A. Stuart, F. Mohamed, D. Yoo, P.H. Sugarbaker, Cancer Chemother. Pharm. 49 (2002) 499–503.
[18] M.B. Garg, S.P. Ackland, J. Chromatogr. B 748 (2000) 383–388.
[19] H.G. Boxenbaum, S. Riegelman, R.M. Elashoff, J. Pharmacokinet. Pharm. 2 (1974) 123–148.
[20] K. Yamaoka, T. Nakagawa, T. Uno, J. Pharmacokinet. Pharm. 6 (1978) 165–175.
[21] The Pharmacopoeia of the People's Republic of China. Part II. The Pharmacopoeia Commission of PRC, 2005, pp. Appendix 174.
[22] P.C. Gokhale, B. Radhakrishnan, S.R. Husain, D.R. Abernethy, R. Sacher, A. Dritschilo, A. Rahman, Br. J. Cancer 74 (1996) 43–48.
[23] J. Cheng, N. Wen, F. Xiong, S.J. Chen, J.B. Zhu, J. Drug Target 14 (2006) 717–724.