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Development and validation of a nylon6 nanofibers mat-based SPE coupled with HPLC method for the determination of docetaxel in rabbit plasma and its application to the relative bioavailability study

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ABSTRACT

A simple and sensitive HPLC method was established and validated for the determination of docetaxel (DTX) in rabbit plasma. Biosamples were spiked with paclitaxel (PCX) as an internal standard (I.S.) and pre-treated by solid-phase extraction (SPE). The SPE procedure followed a simple protein digestion was based on nylon6 electrospun nanofibers mats as sorbents. Under optimized conditions, target analytes in 500 μL of plasma sample can be completely extracted by only 2.5 mg nylon6 nanofibers mat and eluted by 100 μL solvent. The HPLC separation was obtained on C18 column and UV detector was used to quantify the target analytes. The extraction recovery was more than 85%; the standard curve was linear over the validated concentrations range of 10–5000 ng/mL and the limit of detection was 2 ng/mL. The inter-day coefficient of variation (CV%) of the calibration standards was below 5.0% and the mean accuracy was in the range of 92.8–113.4%. Moreover, analysing quality control plasma samples in 3 days, the results showed that the method was precise and accurate, for the intra- and inter-day CV% within 10% and the accuracy from 96.0% to 114.0%. The developed and validated method was successfully applied to relative bioavailability study for the preclinical evaluation of a new injectable DTX–sulfobutyl ether beta-cyclodextrin (DTX–SBE-β-CD) inclusion complex freeze-dried powder (test preparation), compared with the reference preparation (DTX injection, Taxotere®) in healthy rabbits. On the basis of the mean AUC(0–*t*) and AUC(0–infinity), the relative bioavailability of the test preparation was found to be 113.1%.

1. Introduction

Docetaxel (DTX) is a new generation anticancer drug as taxane drugs, especially effective for breast, nonsmall-cell lung, ovarian and head and neck cancers [1]. However, DTX like other taxanes such as paclitaxel (PCX) shows very poor solubility in water (≤0.5 mg/L), which limits its clinical application and bioavailability enormously. Hence, the available formulation of DTX for clinical use, e.g. a commercial DTX injection (Taxotere®), contains a high concentration of Tween 80. This Tween 80-based vehicle has been associated with several hypersensitivity reactions and has shown incompatibility with common PVC intravenous administration sets [2]. In order to improve water solubility of DTX and avoid the use of Tween 80, alternative dosage forms have been suggested, including polymeric nanoparticles [3–5], liposomes [6], cyclodextrins [7] and albumin-conjugated formulation [8].

Cyclodextrins are able to form non-covalent complexes with water insoluble drugs by incorporating the drug within the inner hydrophobic core, so that the outer hydrophilic groups of the cyclodextrin interact with water rendering the complex soluble. A number of chemically modified cyclodextrins have been developed to overcome the low solubility and toxicity of the natural cyclodextrins and increase their usefulness [9]. Hydrophilic cyclodextrins have been widely used as pharmaceutical excipients to formulate poorly water soluble drugs with the aim to increase drug apparent solubility in biological media and in some cases which enhance bioavailability [10].

Among them, sulfobutyl ether beta-cyclodextrin (SBE-β-CD) has attracted growing interests because of its improved complexing

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ability, great water solubility and low toxicity [11]. DTX–SBE-β-CD inclusion compound freeze-dried powder has been designed and prepared as a novel carrier for delivery of DTX in our group. In order to carry out the subsequent preclinical evaluation for the new preparation, an assay should be developed and validated to determine DTX in rabbits plasma and to apply it to characterize the pharmacological profile and the bioavailability of the new preparation in rabbits.

Based on the literature review, HPLC method has been used for the quantitative determination of DTX in plasma and methods for sample pretreatment mainly included liquid–liquid extraction (LLE) [8,12–17] and solid-phase extraction (SPE) [18–24]. SPE is today the most popular sample preparation method due to its low consumption of organic solvents, simplicity, high recovery, high-preconcentration factors, easily to be automated and operated. In the SPE procedures, the choice of adsorbents is an important factor for obtaining higher enrichment efficiency of analytes [25].

Compared with microscale adsorbents, the extremely large surface-to-volume ratio and small average micropores make nanofibers a promising high-performance adsorbent material that can achieve a larger specific surface and more active situs for adsorption. Accordingly, the attachment of the target molecules would facilitate and a small amount nanofiber is sufficient for the extraction, which greatly reduced the volume of desorption solvent [26–30]. Electrospinning is recognized as a unique and useful technique to prepare non-woven mats of polymer nanofibers [31].

Our previous research, using nylon6 electrospun nanofibers mat as a SPE sorbent, has demonstrated the highly effective extraction nature of nylon6 nanofibers mat for low-pole compounds in aqueous samples, such as estrogens and phthalate esters in environmental water [26,27]. Compared with packing nanofibers tightly into a tip as SPE columns [28–30], the major advantages of membrane or mat are larger media cross-sectional area and decreased in pressure drop, which allows sample processing at higher flow rates, so it is much easier to deal with larger volume samples to obtain better enrichment coefficient. All the facts mentioned above revealed that nanofibers mat has great analytical potential as an efficient SPE adsorbent.

Therefore, in the current research, a novel SPE procedure based on nylon6 electrospun nanofibers mat as sorbents, coupled with HPLC-UV was developed and validated for analysis of DTX in rabbit plasma. The method was used to study the relative bioavailability of DTX–SBE-β-CD inclusion compound freeze-dried powder (test preparation) after a single i.v. administration to the rabbit, and the commercial DTX injection (Taxotere®) was chosen as the reference preparation.

2. Experimental

2.1. Standards and chemicals

DTX (batch, 200906008) was purchased from Sanwei Pharmaceutical Co., LTD. (Shanghai, China) and PCX was purchased from Sigma–Aldrich (USA). Methanol, acetonitrile and tetrahydrofuran of HPLC grade were obtained from Dikma Instrument Co., LTD. (Beijing, China). Ammonium acetate, 3-methylphenol, diethyl ether, formic acid and sodium bicarbonate of analytical grade were purchased from Sinopharm Chemical Reagent Co., LTD. (Shanghai, China). Nylon6 material was purchased from Debiochem (Nanjing, China). Medical normal saline (batch, 20090927) was obtained from Beijing Shuanghe Medicine Co., LTD. (Beijing, China). SBE-β-CD was obtained as a generous present from Jiangsu Key Laboratory for Medical Supermolecule Material and Application, Nanjing Normal University. Water used throughout the study was double distillated.

DTX–SBE-β-CD inclusion compound freeze-dried powder as test preparation was prepared by our research group. The reference preparation, Taxotere® (batch, 09010311), was purchased from Jiangsu Hengrui Pharmaceutical Co., LTD. (Jiangsu, China). For relative bioavailability study, the test preparation was dissolved in medical normal saline to desired dosage, and the reference preparation was treated according to directions to the corresponding dose as the test preparation.

2.2. Standard and QC solutions

For standards, a stock solution for DTX was prepared at the concentration of 1.02 mg/mL. For QCs, a stock solution of DTX was prepared at 100.0 μg/mL. The I.S. stock solution was prepared at 110.2 μg/mL. All stock solutions were prepared in acetonitrile and stored at 4 °C.

Working solutions to obtain the standard points of the calibration curve and the working solutions to prepare the plasma QC samples (low, medium and high concentration), were obtained by combining different amounts of the stock solutions and further diluted with acetonitrile to obtain DTX at the final concentrations of 0.1, 0.2, 1.0, 2.0, 10.0, 20.0 and 50.0 μg/mL.

The I.S. working solution was prepared at 10 μg/mL by diluting the stock solution with acetonitrile.

2.3. Preparation of standards and QC samples

Control rabbit plasma aliquots (450 μL) were spiked with 50 μL of each working solution to obtain a final dilution of 1:10, giving six calibration standards in the DTX at the final concentrations of 0.01, 0.02, 0.10, 0.20, 1.00, 2.00 and 5.00 μg/mL.

To prepare QC samples, three fractions of plasma were added with an appropriate amount of QC solutions, obtaining QC samples at the final DTX concentrations of 0.02, 0.200 and 5.00 μg/mL (low, medium and high concentration respectively).

Several aliquots of the three concentrations were stored at −20 °C as controls for future assays and to check the short-term stability under storage conditions. The analytes were considered stable at each concentration when the differences between the freshly prepared samples and the stability testing samples were found to be not exceeding 15% of the nominal concentration.

2.4. Preparation of nylon6 nanofibers mat

Nylon6 nanofibers mats were fabricated by electrospinning according to the procedures [27]. The diameters of electrospinning nylon6 nanofibers were at the range of 400–800 nm and the thickness of the fibers mats was in the range of 120–150 μm.

A home-made filter with circular nylon6 nanofibers mat (2.5 mg, diameter about 1.5 mm) fixed tightly was made (Fig. 1). The nanofibers mat was preconditioned with 200 μL of acetonitrile and 200 μL of water for one time.

2.5. Sample preparation

Plasma samples (500 μL) were mixed with 10 μL (100 ng) of I.S. working solution and 2 mg of protease by vortex for 30 s, then hydrolyzed in water bath at 40 °C for 10 min and followed by centrifugation at 10,000 rpm for 5 min. The supernatant was separated and passed through the preconditioned nanofibers mat at the rate of 1.0 mL/min by vacuum pumping. The target analytes retained on the nylon6 nanofibers were eluted with 100 μL methanol into the collecting tubes and the eluate was evaporated to dry at 40 °C under a stream of nitrogen. The dry sample was reconstituted with 50 μL of mobile phase and vortex-mixed, and centrifuged at 12,000 rpm

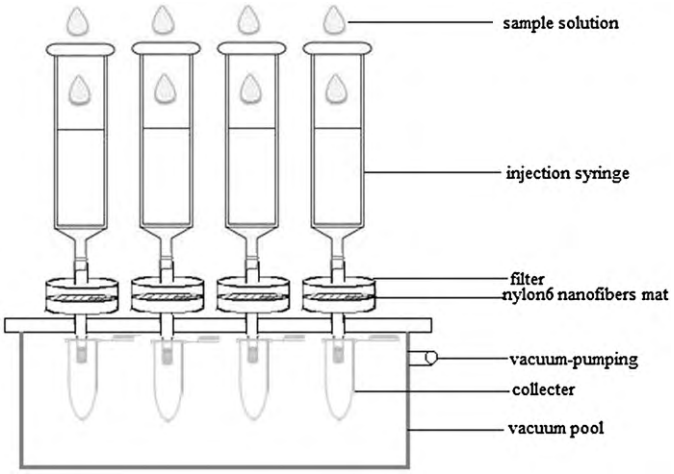

Fig. 1. The solid-phase membrane extraction device based on nylon6 electrospun nanofibers mat.

for 5 min. Then 20 μL of the supernatant was injected into chromatographic system.

2.6. Chromatographic condition

The HPLC system consisted of an LC-10AD pump and a SPD10-Avp UV detector (Shimadzu, Japan). Sample separation was performed on a Dikma Platisil C18 column (150 mm × 4.6 mm, 5 μm particle size, Dikma, China) coupled with a C18 precolumn, 4 mm × 3 mm (Dikma) held at 30 °C. The mobile phases for the chromatographic separation were composed of acetonitrile:35 mM ammonium acetate buffer solution (pH = 5.0): tetrahydrofuran (47:48:5, v/v/v) at a flow rate of 1.0 mL/min. The UV absorbance of the sample (20 μL) was measured at the wavelength of 227 nm. Data collection and processing were performed using Shimadzu LC-Solution software. At the end of the daily analyses, the HPLC column was washed 30 min with acetonitrile/H_2O (1:1) at the flow of 1.0 mL/min.

2.7. Validation study

The present method was set up to quantify DTX in a preclinical explorative relative bioavailability study, so it was applied a short validation protocol performed over during a period of time. The linearity of the calibration curves was validated over 3 days and calculated by the peak-area ratios of DTX/I.S. to the nominal concentration of DTX in the sample. The linearity of the standard curves was determined by a regression analysis and the goodness of the regression by calculating the Pearson's determination coefficient R^2 and by comparison of the true and back-calculated concentrations of the calibration standards.

Intra-day and inter-day accuracy and precision were determined by determining DTX in three replicates of three QC samples at the nominal concentrations of 0.020, 0.200 and 5.000 μg/mL (low, medium and high concentration respectively) for DTX on the same day and on three different days. A standard calibration curve was prepared and processed each day to analyse the QC samples. The precision of the method at each concentration was reported as a coefficient of variation (CV%), expressing the standard deviation as a percentage of the mean calculated concentration, while the accuracy of the measure was determined by expressing the mean calculated concentration as percentage of the nominal concentration.

The detection limit (LOD) was defined as the concentration at which the signal-to-noise ratio was 3. The limit of quantitation (LOQ) was defined as the smallest amount of the analyte that could be measured in a sample with sufficient precision (±15%) and accuracy (range 80–120%).

The extraction recoveries of DTX were calculated in triplicate and at three QC levels (0.020, 0.200 and 5.000 μg/mL). The recovery was calculated by comparing the peak-area ratios of DTX/I.S. from extracted plasma samples with those obtained by directly determining DTX external standard solutions spiked with 10 μL (100 ng) of I.S. working solution at the same concentration.

The stability of DTX in rabbit plasma was evaluated on five freeze–thaw cycles with QC samples at three concentration levels (0.020, 0.200 and 5.000 μg/mL). These plasma samples were frozen at −20 °C and thawed at room temperature on 0 (immediately after preparation), 7, 14, 21, and 30 days. DTX was quantified in the stored samples, using a freshly prepared calibration curve.

2.8. Preparation of the test preparation

DTX–SBE-β-CD inclusion compound freeze-dried powder was prepared by the saturated water solution technique. Briefly, the required amount of DTX (100 mg) was dissolved in 1.0 mL of absolute ethanol, and SBE-β-CD (1.70 g) was dissolved in 5.0 mL of distilled water. The DTX solution was added into the SBE-β-CD solution with constant stirring. After stirring for 2 h, the mixture was serially passed through 0.22 μm pore size membranes, heated to 55 °C by soaking into a water bath to remove ethanol and lyophilized for 48 h to obtain the dried powder of DTX–SBE-β-CD.

2.9. Relative bioavailability study

The experiments were performed on 18 healthy albino rabbits (2.0 ± 0.1 kg, female and male, half and half) from the Laboratory Animal Center of Southeast University (Nanjing, China). Animals were allowed free access to food and water in this study. They were randomly divided into three groups (six rabbits per group, female and male, half and half). Group 1 was treated with the test preparation containing 5 mg of DTX per kg body weight by continuous intravenous infusion via the ear vein within 30 min, while a corresponding dose of the reference preparation was administered by the same way to group 2. Group 3 offered blank plasma and blank tissue for establishment of the analysis method. Blood samples (1.5 mL) were drawn from the marginal ear vein into heparinized centrifuge tubes just before dosing (0 h) and after 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 18 h and 24 h, the samples were then centrifuged at 5000 rpm for 5 min to separate the plasma. Plasma samples were stored frozen at −20 °C until analysis.

2.10. Relative bioavailability analysis and statistics

The pharmacokinetic parameters were calculated using the 3p97 software provided by the Chinese Pharmacological Society. All parameters are reported as mean values ± standard deviation. A non-compartmental analysis was applied. The parameters considered were: C_{max} (maximum plasma concentration), AUC (area under the concentration–time curve from time 0 to the last detectable sample), $t_{1/2}$ (plasma elimination half-life in the terminal phase), MRT (plasma mean residence time) and the relative bioavailability after administration (T/R-ratios, $F = AUC_{test\ preparation}/AUC_{reference\ preparation} \times 100$).

3. Results and discussion

3.1. Sample preparation

3.1.1. Optimization for experimental conditions effect on the enrichment efficiency

In order to achieve high selectivity and extraction efficiency for target analytes and evaluate the enrichment potential of nylon6 nanofibers mat as a solid-phase extraction adsorbent, several important parameters must be considered cautiously. Such parameters include the choice of hydrolase, hydrolysis time, hydrolysis temperature, eluant and its volume, pH and sample flow rate. The effects of different factors on the extraction efficiency were investigated using spiked plasma sample at the final DTX concentrations of 0.5 μg/mL.

In this experiment, three different hydrolases, acid protease, neutral protease and alkalic protease were tested for hydrolyzing plasma samples. Compared with the other hydrolases, neutral protease had the highest efficiency under the same experimental conditions. And then, hydrolysis time and temperature are the important factors for protease hydrolyzing. Hydrolysis degree will gradually increase with the prolonging of the time until complete hydrolysis. At the same time, the activity of hydrolase is affected by temperature. It was found that the optimum hydrolysis time and temperature were 10 min and 40 °C respectively for hydrolyzing plasma samples by neutral protease.

The effect of eluant and its volume on extraction efficiency were also investigated. In this experiment, acetonitrile and methanol were tested for eluting DTX. Compared with acetonitrile, methanol had higher efficiency as the eluting solvent. To ensure the target analytes were eluted from the SPE nanofibers completely, an optimum volume of eluant was investigated through changing the volume of methanol from 50 to 200 μL .The results indicated that the recovery of DTX increased while increasing of the volumes of methanol from 50 to 100 μL. When the volume was more than 100 μL, the recovery kept constant. Therefore, 100 μL methanol was chosen as the optimum eluant.

The pH of sample solution is of great influence on SPE process because it determines the existing state of the target analytes and influences the extraction efficiency [32]. In this paper, the effect of pH on the extraction of the analytes was examined in the range of 3.0–10.0. The experiment results showed that the enrichment efficiencies of target analytes changed with pH were not obvious in the range of 4.0–9.0. While the pH was 3.0, recovery obviously decreased, for nylon6 may become protonated at $pH \leq 3$ owing to –NH– group, and the structure and appearance of the fibers would be destroyed [33]. The recovery was also low when the pH was 10.0. The pH of rabbit plasma hydrolyzed by neutral protease was 6.0–7.0, therefore, high recovery efficiency could be obtained without adjusting pH value.

Volume of sample loaded, as well-known, affects the efficiency of the extraction. In this study, blood samples were drawn from the marginal ear vein of the same rabbit for 11 times, 1.5 mL for every time, before and after dosing (described in Section 2.9). Restricted by the practical situation, 500 μL of plasma for sample preparation was thought reasonable. Just because the volume of sample loaded was relative small in this study, a low flow rate (1.0 mL/min) of sample was favorable to improve the recoveries of analytes, while almost no influence could be observed on the sample preparation time compared with higher flow rate.

3.1.2. Comparison with other preparation methods

At present, biosamples of DTX in the plasma were often pre-treated using LLE and SPE methods. In the literature, DTX was extracted from rat or human plasma using LLE technique with ethyl acetate [13], methyl tertiary butyl ether [12,14,15,17], acetonitrile/*n*-butyl chloride [16]. The volume of plasma was 0.05–1 mL and the organic solvent consumption was 0.6–4.05 mL.C18 [18,19,23], CBA-bonded silica beads [20], CN [21,24] or HLB [22] SPE columns have been used for DTX extracted from plasma samples, and the mass of packing material was at the range of 50–200 mg.

Rabbit plasma samples pre-treated using the SPE method have been reported only by Zhao [18]. In the literature, 1.0 mL rabbit plasma samples were prepared with 3 mL protein precipitation agent, and the supernatant was transferred and diluted (fourfold, v/v) with water. Then the diluted mixture was slowly transferred into SPE column (Phenomenex C18) conditioned in advance. After washing, compounds adsorbed on SPE column were eluted with 2 mL mixture solution of acetonitrile–methanol (1:1, v/v), and the eluate was evaporated to dry at 40 °C under a stream of nitrogen. The residue was dissolved in 200 μL mobile phase, then 20 μL of the supernatant was injected into chromatographic system.

In this study, SPE method based on nylon6 electrospun nanofibers mat as sorbents was developed to prepare rabbit plasma samples. As the sq. results described in Sections 3.3–3.6, the proposed method presented satisfactory analytical performance with good specificity, high recoveries, low LODs and good precision. Moreover, target analytes in 0.5 mL rabbit plasma could be completely extracted by only 2.5 mg nylon6 nanofibers mat and 100 μL methanol eluant. In the whole sample preparation process, the volume of organic solvent consumption was only 0.5 mL, and the sample pretreatment was simple and time-saving relatively. Compared with the literature methods mentioned above, the sample preparation method proposed in this investigation has its own noticeable advantages.

3.2. Specificity and chromatography

The representative chromatograms obtained from blank rabbit plasma, blank rabbit plasma spiked with DTX and/or I.S. and a plasma sample collected at 24 h after i.v. administration of the test preparations are shown in Fig. 2. The retention times for DTX and I.S. were 5.3 and 6.1 min respectively with good resolution and without any interference from endogenous plasma constituents or DTX metabolites at or near the retention time of DTX and I.S., which indicated that the present sample preparation method removed the interfering substances in rabbit plasma efficiently. Therefore, satisfactory specificity of the proposed method was obtained.

3.3. Calibration curves

The peak-area ratios of DTX/I.S. versus the nominal concentrations were plotted and a least-square linear regression weighted by the reciprocal of the concentrations was applied to generate the calibration curves. The calibration curves, prepared on three different days, showed good linearity and acceptable data over a wide range of DTX concentrations 0.010–5.00 μg/mL with $R^2 \geq 0.998$.

The accuracy and precision were determined at each day. Mean accuracy was in the range 92.8–113.4%. The precision expressed as CV% ranged between 2.3% and 4.7%.

3.4. Precision, accuracy and LOQ

The precision and accuracy of the present method were evaluated analysing three replicates of the QC samples at 0.020, 0.200 and 5.000 μg/mL, within a single-run analysis for intra-day study and over three consecutive runs for inter-day study. The accuracy and precision for the determination of DTX in rabbit plasma were shown in Table 1. The method was precise and accurate, with intra- and inter-day CV < 10% and accuracy in the range of 96.0–114.0%. According to the requirements of Chinese Pharmacopoeia (2005

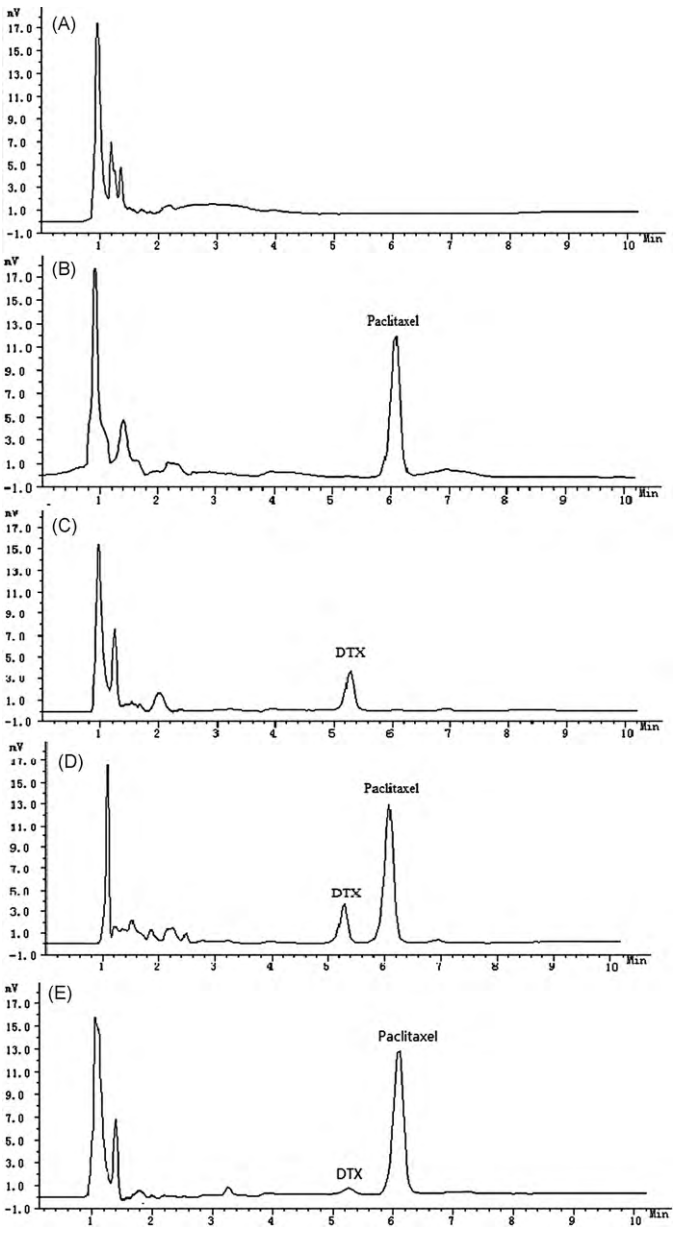

Fig. 2. Representative HPLC chromatograms obtained for blank plasma (A), blank plasma spiked with DTX 2 μg/mL (B), blank plasma spiked with PCX (internal standard) 10 μg/mL (C), blank plasma spiked with DTX 2 μg/mL + PCX 10 μg/mL (D) and a plasma biosample collected from a rabbit at 24 h after i.v. administration of the test preparation at a dosage of 5 mg/kg (DTX concentration = 0.02 μg/mL) + PCX (10 μg/mL) (E).

edition, part II) [32], these values were within the acceptable range. Therefore, the analysis method is accurate and precise for determination of DTX in rabbit plasma.

The LOQ was defined as the lowest concentration that could be measured with a precision within 15% and accuracy between 80% and 120%. Consistent with our aims, we set the LOQ at 10.0 ng/mL, validated through three replicates. The intra-day CV% and accuracy were respectively 9.2 and 91.4%. The LOD was defined as 2.0 ng/mL at which the signal-to-noise ratio was 3.

3.5. Extraction recovery

The recovery was evaluated in triplicate and determined by analysis of QC samples at low, medium and high concentration (0.020, 0.200 and 5.000 μg/mL). The extraction recoveries of DTX at the three QC levels were calculated by comparing the peak area of DTX in extracted biological samples with those obtained by directly determining DTX standard solutions at the same concentration. We verified the absence of significant variations (<15%) for the areas of both the analytes. As a result, the mean extraction recovery for DTX at three QC levels was higher than 85% with reproducibility expressed as CV < 12%.

Table 1
Intra- and inter-day validation of the method for the determination of DTX in rabbit plasma.

	Concentration added (μg/mL)		
	0.020	0.200	5.000
Intra-day			
Day 1			
Mean (N = 3)	0.0216	0.211	4.90
CV%	7.6	5.3	2.1
Accuracy%	100.8	105.5	98.0
Day 2			
Mean (N = 3)	0.0192	0.195	5.03
CV%	6.3	4.5	4.1
Accuracy%	96.0	97.5	100.6
Day 3			
Mean (N = 3)	0.0228	0.201	4.96
CV%	7.2	3.0	3.8
Accuracy%	114.0	100.5	99.2
Inter-day			
Mean (N = 9)	0.0198	0.209	5.10
CV%	9.5	4.8	3.3
Accuracy%	99.0	104.5	102.0

Table 2
Stability of DTX in rabbit plasma after freeze–thaw treatment determined by HPLC-UV method (n = 3).

Concentration added (μg/mL)	Concentration measured (μg/mL)	RSD (%)
0.020	0.0195 ± 0.00232	11.9
0.200	0.205 ± 0.0133	6.5
5.000	5.310 ± 0.414	7.8

3.6. Stability in frozen matrix

The stability of DTX in rabbit plasma was studied after five freeze–thaw cycles, by analysing QC samples at concentrations of 0.020, 0.200 and 5.000 μg/mL (n = 3). The accuracy was in the range 97.6–106.2% and the CV% between 6.5% and 11.9%. The results are shown in Table 2 indicated that DTX was stable for at least 1 month in rabbit plasma stored at −20 °C.

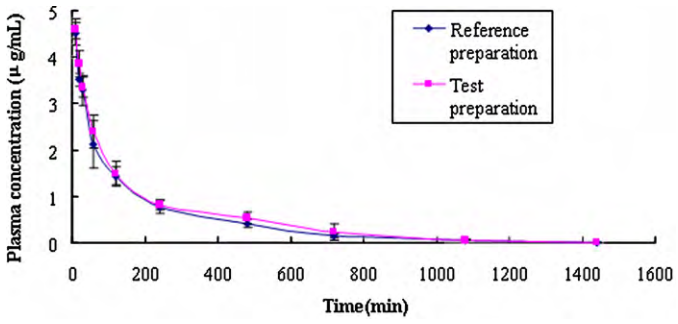

Fig. 3. Mean plasma concentration–time profiles after single i.v. administration of test preparation and reference preparation at a dosage of 5 mg/kg in rabbits (n = 6). The bars shown are mean values with error bars representing ±SD.

Table 3
The comparative pharmacokinetic parameters after i.v. administration of test preparation and reference preparation at a dosage of 5 mg/kg in rabbits ($n = 6$).

Samples	C_{max} (μg mL^{-1})	T_{max} (min)	$t_{1/2}$ (min)	MRT (min)	AUC(0–t) (μg min mL^{-1})	AUC(0–infinity) (μg min mL^{-1})	F (%)
Test preparation	4.60 ± 0.21	10.0 ± 0.0	194.50 ± 12.19	267.56 ± 35.33	767.51 ± 77.21	770.90 ± 76.76	
Reference preparation	4.51 ± 0.24	10.0 ± 0.0	203.57 ± 7.58	254.12 ± 13.06	683.71 ± 70.79	687.35 ± 71.18	113.1 ± 14.0

C = concentration; T = time; MRT = mean residence time; AUC = area under the curve; $F = AUC_{test\ preparation}/AUC_{reference\ preparation} \times 100$.

3.7. Characterization of the test preparation

DTX–SBE-β-CD inclusion compound freeze-dried powder prepared in this study was white powder. The mean DTX encapsulation efficiency was determined as 88.7% ($n = 6$).

3.8. Relative bioavailability study

After a single i.v. administration of test preparation and reference preparation of 5 mg/kg in rabbits, Fig. 3 reports the mean plasma concentration–time profiles of DTX, and the main pharmacokinetic parameters, such as AUC(0–infinity), AUC(0–t), C_{max}, T_{max}, $t_{1/2}$ and MRT were calculated from plasma concentrations for both preparations and summarized in Table 3. The parametric 90% confidence intervals of the geometric mean values of the T/R-ratios were 97.2–127.4% for AUC(0–infinity), 110.3–114.4% for AUC(0–t), and 97.2–107.4% for C_{max}. The mean relative bioavailability between test and reference preparation was 113.1%. T_{max} was same between the two preparations, and $t_{1/2}$ and MRT values of the test preparation were longer than the reference preparation. From the results of the present study, it is concluded that the test preparation has satisfactory relative bioavailability compared with the reference preparation and therefore it can be considered to be a good candidate for further preclinical and clinical development.

4. Conclusion

A novel solid-phase extraction (SPE) procedure based on nylon6 electrospun nanofibers mat as sorbents, coupled with HPLC-UV was developed and validated for determination of DTX in rabbit plasma samples. The proposed sample preparation method was certified simple and fast, and above all, 2.5 mg nanofibers mat was sufficient for the extraction, so 100 μL of desorption solvent was needed accordingly. The analytical method established and validated in this study is simple, selective, sensitive, accurate and precise, which meets the requirements for the analysis of biological specimen. This validated assay method has been successfully applied to the relative bioavailability study of an injectable DTX–SBE-β-CD inclusion complex freeze-dried powder to DTX injection (Taxotere®) in rabbits. The studies showed that the novel DTX preparation had favorable pharmacokinetics and might be good candidate for further preclinical and clinical development.

Acknowledgments

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