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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