Determination of Paclitaxel in Rat Plasma by LC–MS–MS

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Abstract

A simple, rapid, and sensitive liquid chromatography–mass spectrometry (MS)–MS method for quantitating paclitaxel in rat plasma is developed. Liquid–liquid extraction with tert-butyl methyl ether is used for sample preparation, and docetaxel is used as the internal standard. Paclitaxel and docetaxel are separated on a C_{18} column and quantitated using a triple-quadrupole MS operating in positive ion electrospray selective reaction monitoring mode with a total run time of 6.0 min. The peak area of the *m/z* 876.3 → 307.9 transition of paclitaxel is measured versus that of the *m/z* 830.3 → 549.1 transition of docetaxel to generate the standard curve. The standard curve is linear over the concentration range of 0.2008–1004 ng/mL for rat plasma. The method has high extraction recovery (> 90%) and accuracy (> 90%), with the intra- and interday precision < 15%. Frozen stability, freeze-and-thaw stability, extracted stability, and room temperature solution stability are also examined. This assay is used to support a pharmacokinetic study of paclitaxel self-assembled nanoliposome in rats.

Introduction

Paclitaxel (Figure 1) is a natural product that was first isolated from the bark of the Pacific Yew tree (Taxus brevifolia) by Wani et al. (1). It is a novel antineoplastic agent that exhibits a unique mechanism of action against advanced ovarian, breast, and non-small-cell lung cancers (2). The tumoricidal activity of paclitaxel is attributed to its ability to induce irreversible aggregation of microtubules (1,3).

Several analysis methods for paclitaxel have been published previously. Most of them have used high-performance liquid chromatography (HPLC) with a UV detector, which suffers from low sensitivity because of the weak UV absorbance of paclitaxel (227 nm) (4–6). S.H. Lee et al. (7) described an HPLC method to determine paclitaxel in mouse plasma, which had a lower limit of quantitation (LLOQ) of approximately 10 ng/mL using 1-mL plasma samples. The LLOQ described in sparreboom's assay was 25 ng/mL in human plasma by HPLC (8). Liquid chromatography (LC)–mass spectrometry (MS)–MS methods to quantitate paclitaxel in human, dog, or mouse plasma or tissues have also been published in recent years (9–13). No application of LC–MS–MS on the pharmacokinetic study of paclitaxel in rats can be found.

This work includes the development of an LC–tandem triple-quadrupole MS method to support the pharmacokinetic study of paclitaxel self-assembled nanoliposome in rats, which is approximately 50 times more sensitive than HPLC–UV (7,8). The assay described here used a single-step liquid–liquid extraction with tert-butyl methyl ether (TBME) for rat plasma. Sample extracts are injected onto a C_{18} HPLC column and quantitated using a MS operating in positive ion electrospray (ESI^+) selective reaction monitoring (SRM) mode with a total run time of 6.0 min. Docetaxel (Figure 1) is used as an internal standard. The method has an LLOQ of 0.2 ng/mL for rat plasma. Rat plasma was selected as the biological matrices and validated the methodology.

This method is shown to be suitable for the quantitation of paclitaxel in rat plasma containing a paclitaxel self-assembled nanoliposome (PSANL) formulation or taxol. The method was

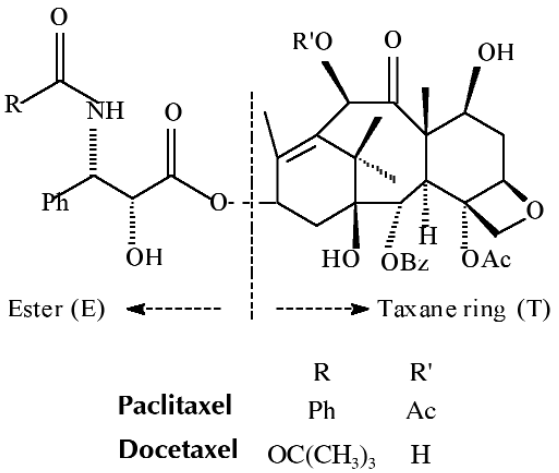

Figure 1. Chemical structures of paclitaxel and docetaxel.

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used for paclitaxel quantitation to support a pharmacokinetic study of PSANL in rats.

Experimental

Chemicals and reagents

Paclitaxel was purchased from Chongqing Meilian pharmaceuticals (Chongqing, China). Docetaxel was purchased from Shanghai Sunve pharmaceuticals (Shanghai, China). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Shanghai Lingfeng Chemistry (Shanghai, China). TBME was purchased from Tedia (Fairfield, OH). Taxol injection was obtained from Beijing Fourring pharmaceutical (Beijing, China). PSANL was prepared in our lab, and all other reagents were obtained from commercial sources.

HPLC–MS–MS system

The HPLC system consisted of a Finnigan Surveyor Autosampler, Finnigan photodiode array detector, and Finnigan LC pump (Thermo Electron San Jose, CA). Chromatographic separation was achieved with a Hypersil C_{18} column (5 µm, 200- × 4.6-mm, Hanbon Science & Technology Co., Jiangsu, China). The mobile phase was methanol and 0.1% formic acid (80:20, v/v) with a total run time of 6 min. The flow rate was 1.0 mL/min, and the column temperature was 30°C.

A Finnigan TSQ Quantum Ultra AM triple-quadrupole MS, operating in ESI$^+$-SRM mode, was used for all analysis. High-purity nitrogen was used as the nebulizing and curtain gases, and argon was collision gas. For quantitation of paclitaxel in plasma samples, the following parameters were used: the ion spray voltage, 5000 V; capillary temperature, 350°C; shealth gas pressure, 35 psi; collision energy, 30 V; source collision induced disassociation, 12 V; and auxiliary gas pressure, 2 psi. Full scan spectra were acquired over the m/z range of 200–1000. SRM mode was used for analyte quantitation with the following parameters: 876.3 → 307.9 for paclitxel Na^+ adduct and the 830.3 → 549.1 for docetaxel adduct, with a dwell time of 0.5 s. The system was operated with Thermo Finnigan Xcalibur 1.4 SR1 software. Peak-area ratios of paclitaxel to internal standard (IS) were plotted versus nominal concentration, and a least-squares linear regression was applied to generate a calibration curve, weighted by the reciprocal of the concentration. All data processing was controlled using Microsoft Excel, version 2003 (Redmond, WA).

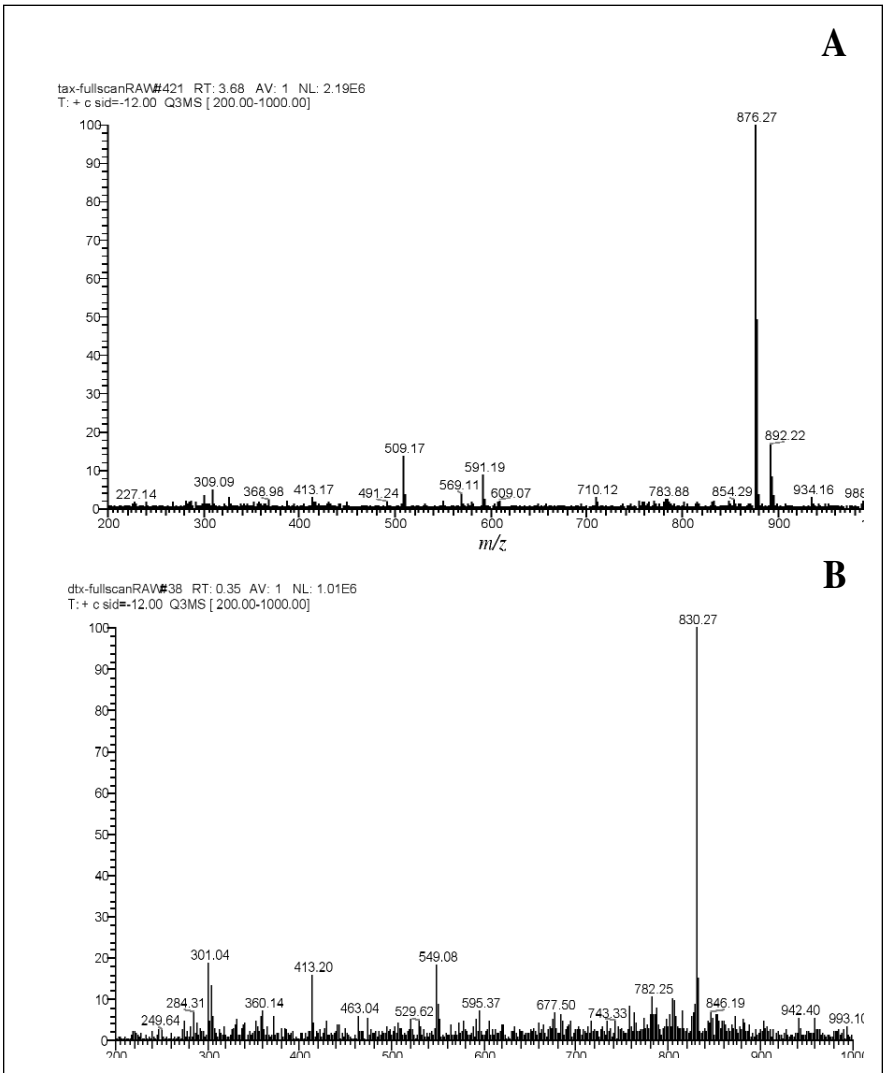

Figure 2. Mass spectra of parent ions of for paclitaxel (A) for docetaxel (B) by ESI$^+$ modes with a concentration of 1 µg/mL in methanol for each of them.

Sample preparation

Preparation of stock solution

Stock solutions of paclitaxel (50.2 µg/mL) and docetaxel (200 µg/mL) were prepared in methanol and stored at –20°C. Working standard solutions of paclitaxel were prepared in methanol at concentrations from 0.4016 ng/mL to 2.008 µg/mL. Working IS was 1.6 µg/mL by dilution from stock solution of docetaxel.

Preparation of calibration standards and QC samples

Rat plasma samples for the construction of standard curves were prepared by spiking 50 µL working solutions of paclitaxel and 40 µL working solution of IS into 10-mL centrifuge tubes. After evaporation of the solvent under a stream of nitrogen, 100 µL of blank plasma was added to each tube. The tubes were briefly vortex mixed to allow dissolution of paclitaxel and doctaxel in plasma. The resultant plasma solutions (containing paclitaxel at 0.2008, 1.004, 5.02, 25.1, 50.2, 100.4, 251, 502, and 1004 ng/mL and IS docetaxel at 640 ng/mL) were used to evaluate the linearity of rat plasma.

Quality control samples in rat plasma samples were prepared in the same way at concentrations of 5.02, 50.2, and 251 ng/mL within the range of calibration standards and were assayed in triplicate on each day when samples were analyzed.

Liquid–liquid extraction

Plasma samples containing paclitaxel and docetaxel were extracted by liquid–liquid extraction with TBME. Working solutions of paclitaxel (50 µL) and working solution of IS (40 µL) were spiked into 10-mL centrifuge tubes. After evaporation of the solvent under a stream of nitrogen, 100 µL of blank rat plasma was spiked into the tubes. The tubes were vortex mixed briefly and mixed with 2 mL TBME. After being vortex mixed for approximately 3 min and centrifugated at 3000 × g for 10 min, the 1.5 mL upper organic layer was transferred to a new glass tube and evaporated to dryness under a stream of nitrogen in a water bath at 35°C. Each dried residue was reconstituted with 100 µL of methanol–water (90:10, v/v) and vortex mixed for 30 s. After centrifugation at 15000 × g for 5 min, the supernatants were transferred to HPLC autosampler vials. A 20-µL aliquot of each sample was injected into the LC–MS–MS system for analysis.

Method validation

The method was evaluated for specificity, precision, accuracy, stability, and extraction efficiency. Plasma quality control samples were included in all calibration curves and processed in triplicate. Percentage relative standard deviation (%RSD) was used as the measure of precision, and the percentage accuracy [%accuracy = (found concentration/added concentration) × 100%] was calculated. Frozen stability, freeze-and-thaw stability, extracted stability, solution stability under ambient temperature, and extraction efficiency were determined.

Results and Discussion

MS and chromatography

Paclitaxel and docetaxel could be ionized under either ESI^+ or negative ESI (ESI^-) conditions because of the amide and hydroxyl groups in both of the structures. However, ESI^+ has higher sensitivity than ESI^-. The first full scan spectra revealed that the Na^+ adduct was more sensitive than the H^+ adduct for paclitaxel (Figure 2A) and docetaxel (Figure 2B), even if it was in an acidic condition that was favorable to the formation of the H^+ adduct. Therefore, the ESI^+ mode was used for analyte quantitation.

$[Paclitaxel+Na]^+$ at m/z 876.3 and $[docetaxel+Na]^+$ at m/z 830.3 were chosen as parent ions for acquisition of daughter fragments (Figure 2). Because paclitaxel and docetaxel are ester derivtatives of 10-deacetylbaccatin (14), the $[ENa]^+$ ion at m/z 307.9 and $[TNa]^+$ ion at m/z 549.1 were the major daughter ions for paclitaxel (Figure 3A) and docetaxel (Figure 3B) from the second full scan spectra, which were chosen as the quantitation ions in subsequent SRM mode detection.

Assay specificity

Assay specificity was evaluated in rat plasma. Chromatograms obtained from blank plasma at the transitions of paclitaxel and docetaxel are shown in Figure 4. The retention times were 4.0 and 4.3 min for paclitaxel and docetaxel, respectively. No siginificant endogenous interfering peaks were observed at or near the retention times of paclitaxel and IS in the chromatograms of rat plasma.

Standard curve

The standard curve was established by plotting peak-area ratios of paclitaxel to IS versus nominal concentration. A least-squares linear regression weighted by the reciprocal of the concentration was applied to generate a calibration curve with correlation coefficients greater than 0.999. Standard curves

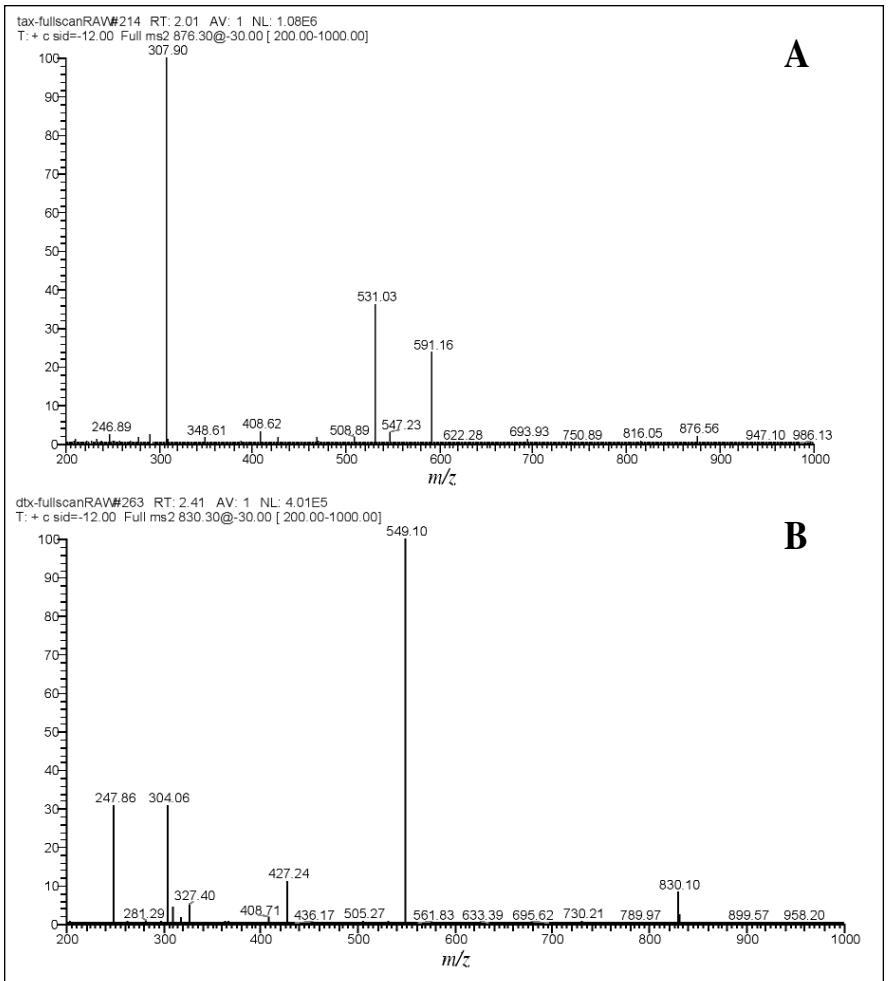

Figure 3. Mass spectra of daughter ions for paclitaxel (A) and docetaxel (B) by ESI^+ modes.

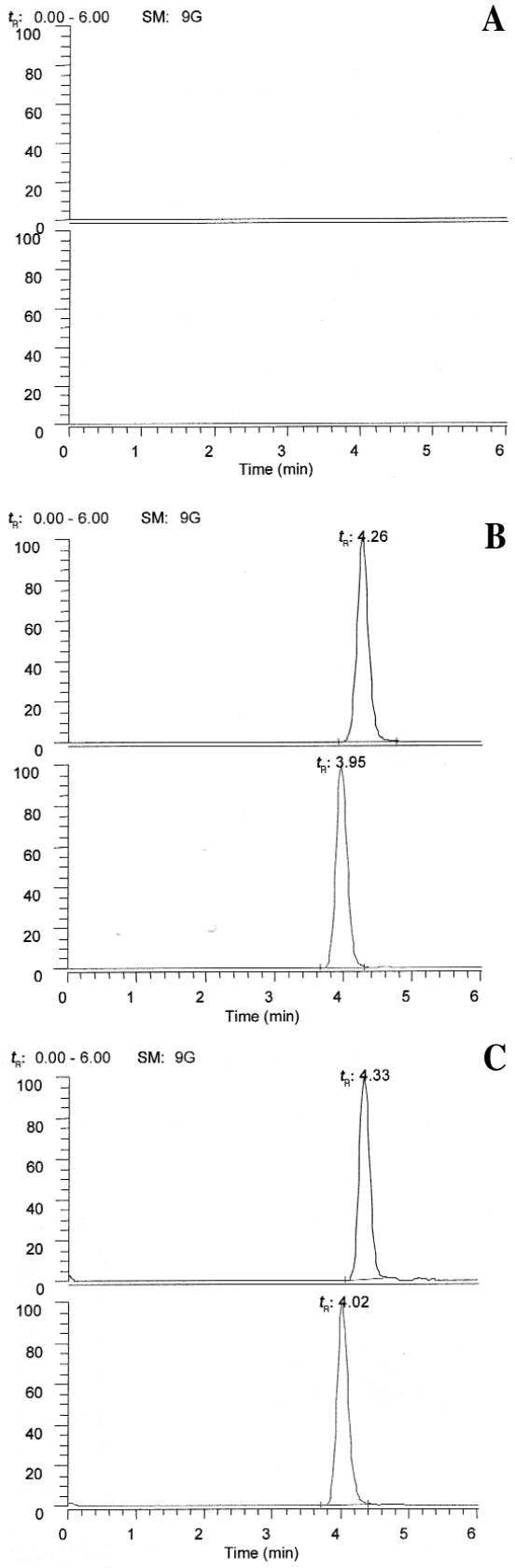

Figure 4. Typical chromatograms of paclitaxel in rat plasma: blank plasma (A), plasma with paclitaxel (50.2 ng/mL) and docetaxel (640 ng/mL) added (B), and plasma with docetaxel (640 ng/mL) added 15 min after being administered with a single dose of taxol and paxlitaxel (5 mg/kg) (C).

generated acceptable data over the concentration range of 0.2008–1004 ng/mL for paclitaxel in rat plasma.

LLOQ

The LLOQ was 0.2 ng/mL in rat plasma with accuracy ranging from 84% to 112% and RSD from 8.24% to 14.2%.

Precision and accuracy

The precision and accuracy of the assay for rat plasma were within acceptable limits as defined in the State Food and Drug Administration (SFDA) guidelines (15). The mean intra- and interday precisions were less than 15%, and accuracy values were larger than 90% (Table I).

Stability and extraction efficiency

Sample stability was tested as the following: frozen stability, freeze-and-thaw stability, extracted stability, and solution stability under ambient temperature.

Plasma samples were frozen at –20°C until extraction on day 1, 3, 5, 10, 20, and 30 and then compared with the thawed samples to measure the frozen stability. The results showed that the plasma samples could be stored at –20°C for at least 10 days without any indication of degradation.

Freeze-and-thaw stability was measured with plasma samples containing paclitaxel under five freeze-and-thaw cycles until analysis was performed. The results showed that paclitaxel was unstable in plasma samples through five freeze-and-thaw cycles. Thirty percent of paclitaxel in rat plasma was degraded after five cycles. The results gave proof to avoid freezing and thawing plasma samples five times during sample preparation.

Plasma samples, after being evaporated, were frozen at –20°C for 20 days to be analyzed by LC–MS–MS for the measurement of extracted stability, with results that were stable within 20 days. No drug degradation was observed after solution samples were left out at ambient temperature for 24 h.

The extraction recovery was measured in rat plasma as the ratio between the peak areas of extracted plasma samples and unextracted samples with the results also shown in Table I. The extraction recovery of plasma samples were lager than 90% with an RSD < 15%, which is within acceptable limits as defined in the SFDA guidelines (15).

Method application

The method described here was successfully employed to quantitate paclitaxel in rat plasma from a pharmacokinetic study of paclitaxel self-assembled nanoliposome in rats. Briefly, Sprague-Dawley rats purchased from the Feeding Center of Qinglong mountain (Jiangsu, China) had ad libitum access to water and to a standard laboratory diet. Rats were randomized according to the randomization method described in the literature (16), grouped six rats per formulation, and administered a single 5-mg/kg intravenous dose of PSANL and taxol injection as the control. At each time point (5, 10, 15, and 30 min, and 1, 2, 4, 6, 8, 12, and 24 h) blood samples were collected by terminal retro-orbital bleeding into prelabled microtubes containing heparin as an anticoagulant. Immediately after collection, each blood sample was gently

Table I. Assay Precision, Accuracy, and Extraction Recovery of LC–MS–MS to Determine Paclitaxel in Rat Plasma (*n* = 5)

	Intraday* precision and accuracy		Interday† precision and accuracy		Extraction recovery	
Spiked concentration (ng/mL)	Average ± SD‡ (%)*	RSD (%)	Average ± SD (%)	RSD (%)	Average ± SD (%)	RSD (%)
5.02	92.47 ± 7.93	8.57	93.00 ± 13.14	14.13	92.19 ± 13.41	14.43
50.2	95.01 ± 12.81	13.49	91.84 ± 10.13	11.03	99.80 ± 13.44	13.47
251	100.37 ± 8.24	8.21	90.16 ± 10.69	11.86	103.1 ± 5.61	5.19

* Five separately spiked samples at each of three concentrations.
† Measured/spiked * 100%.
‡ SD = standard deviation.

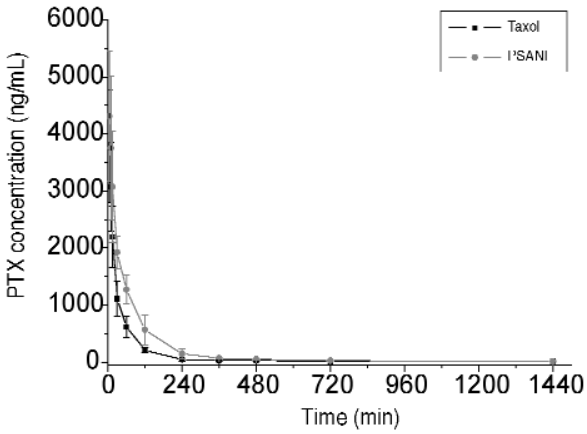

Figure 5. Profiles of the pharmacokinetics of PSANL and taxol. The control group was administered a single dose of PSANL and taxol (5 mg/kg) by tail vein in rats. Data of each formulation at each time point represents the mean ± standard error (*n* = 6).

inverted several times to ensure complete mixing with the anticoagulant. The blood samples were centrifuged for 5 min (3000 × g) at 4°C to separate plasma. All the plasma samples were stored at –20°C prior to analysis. The profiles of pharmacokinetic of paclitaxel following PSANL administration at different time points are presented in Figure 5.

Conclusion

A rapid, simple, and sensitive LC–MS–MS method for the quantitation of paclitaxel in rat plasma has been developed and validated. The assay percentage biases and RSDs were less than 15% and had a high sensitivity with the LLOQ as low as 0.2 ng/mL. The method described in this article was successfully used to quantitate paclitaxel in rat plasma for the pharmacokinetic study of paclitaxel self-assembled nanoliposome in rats. This method was also suitable for the two compound's clinical pharmacology and animal pharmacokinetics because of the high sensitivity and specification. The 6-min run time is suitable for throughput analysis of biological samples, and the LLOQ is approximately 50 times more sensitive than HPLC–UV detector assays of paclitaxel (7,8).

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