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Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 170–176
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Sensitive HPLC method for quantitation of paclitaxel (Genexol®) in biological samples with application to preclinical pharmacokinetics and biodistribution

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Received 7 December 2004; received in revised form 14 February 2005; accepted 18 February 2005
Available online 2 April 2005

Abstract

A sensitive, specific and reproducible HPLC method has been developed and validated for the quantitative determination of paclitaxel in plasma, tissues and tumor of mice. Tissue specimens including liver, kidneys, spleen, lungs, heart and tumor were separately homogenized in bovine serum albumin (BSA, 40 g/l) in water. Plasma or tissue homogenates (0.1 ml) containing paclitaxel and internal standard (dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxy biphenyl-2′,2′ dicarboxylate (DDB), I.S.) were extracted by ethyl acetate (10 ml). A 4.6 mm × 250 mm ODS column was used to separate the components in biological samples with UV detection at 227 nm and gradient system was applied to a quantitation of paclitaxel consisting of acetonitrile-deionized water. The I.S. and paclitaxel were eluted at 13.7 and 18.0 min, respectively, and no interfering peaks were observed. Linear relationships ($r^2 > 0.999$) were obtained between the peak height ratios and the corresponding biological sample concentrations over the range of 0.1–20 μg/ml. The average intra- and inter-day variations (% R.S.D.s and % deviations) of the assay for biological samples were less than 10%. The LOD and LOQ were 5 and 10 ng/ml, respectively, for paclitaxel using a microsample volume (100 μl) of plasma sample. This HPLC method has been successfully applied for the determination of paclitaxel in pharmacokinetic and biodistribution study in after administration of 50 mg equivalent paclitaxel/kg dose of paclitaxel-loaded polymeric micelle and 20 mg equivalent paclitaxel/kg dose of Taxol® to female SPF C57BL/6 mice.

Keywords: Paclitaxel; HPLC; Pharmacokinetics; Biodistribution; Polymeric micelle

1. Introduction

Paclitaxel is the most effective antitumor agent developed in the past three decades (Fig. 1). It has been used for effective treatment of a variety of cancers including refractory ovarian cancer, breast cancer, non-small cell lung cancer, AIDS-related Kaposi's sarcoma, head and neck carcinoma and other cancers [1–6]. Paclitaxel is a member of the taxane family of diterpenes, all having unique tri- or tetracyclic 20 carbon skeletons. Only paclitaxel, structurally most complex in its family, and its congener cephalomannine, have antitumor activities. Paclitaxel inhibits cell proliferation by inducing a sustained mitotic block at the metaphase/anaphase boundary, formation of an incomplete metaphase plate of chromosomes and an abnormal organization of spindle microtubules, as a result of the stabilization of microtubule dynamics [7].

Since paclitaxel exerts cytotoxic activity at concentrations as low as 50 nM [8], sensitive analytical methods need to be developed to study the pharmacokinetic and biodistribution of paclitaxel which is applicable to use in preclinical and clinical pharmacokinetic analysis of paclitaxel at later time points after administration of the drug when the concentration of paclitaxel in plasma and tissue is quite

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doi:10.1016/j.jpba.2005.02.023

low. HPLC (high performance liquid chromatography)–UV [9–13], HPLC–MS [14–16] and immunoassays [17,18] have been used to analyze paclitaxel with a limit of detection (LOD) of 10, 0.2 and 0.3 ng/ml, respectively. Although immunoassays have higher sensitivity, they lack sufficient specificity [19]. The high cost of mass spectrometry prevents it from routine analytical use. Therefore, HPLC–UV has become the most popular analytical tool for paclitaxel due to its reproducibility, simplicity, efficiency and low cost.

To recover paclitaxel from biological samples such as plasma and tissues, most of the analytical methods involved multiple steps of liquid–liquid extraction, solid-phase extraction or protein precipitation, followed by evaporation to prepare and concentrate the sample prior to injection to an HPLC system. The pretreatment of the biological samples are laborious and time-consuming. Due to the multiple steps of sample pretreatment, a relatively large volume of biological sample (0.5–2 ml) is usually needed for these methods to maximize the sensitivity of the assays. Recently, Wang et al. and Lee et al. developed an HPLC method for paclitaxel analysis that minimized the sample volume and also achieved a higher sensitivity with a LOD of 5 ng/ml. They used a small volume of plasma sample (100 μl) from a single rat and mouse; the recovery rate was below 90% and over 90%; and the intra- and inter-day variances were within 15 and 8%, respectively [19,20].

In the present study, we report an HPLC method for analysis of paclitaxel using DDB [21] (Fig. 1) as a new internal standard in biological samples that outperforms in both sensitivity and reproducibility. This method has been validated and also successfully applied in the pharmacokinetic and biodistribution studies of paclitaxel to female SPF C57BL/6 mice.

2. Experimental

2.1. Materials

Paclitaxel (Genexol®) was obtained from Samyang Genex Co. (Seoul, Korea). Acetonitrile, toluene, dichloromethane and ethyl acetate (all HPLC grades) were purchased from Fisher Scientific (NJ, USA). Taxol® was purchased from Bristol-Myers Squibb (NJ, USA) via a local pharmacy in Korea. DDB was obtained from Daehwa Pharm. Co. (Seoul, Korea). Purified deionized water was prepared by the Milli-Q plus system from Millipore Co. (MA, USA). Female SPF C57BL/6 mice were obtained from Charles River Japan Inc. (Kanagawa, Japan).

2.2. Analysis of paclitaxel in plasma and tissue samples by HPLC

Blood was obtained from more than 10 mice under diethyl ether anesthesia from the venae cava inferior. The blood was collected in 1.5 ml polypropylene microtubes (Eppendorf, Hamburg, Germany) containing 7 USP units sodium heparin and the tubes were centrifuged immediately (2500 rpm) for 5 min. Tissue samples were homogenized in 5 or 10 volumes of 40 g/l BSA in deionized water using a PowerGen homogenizer (Model 1800D, Fisher Scientific, NJ, USA). Plasma or tissue homogenates samples (100 μl) were mixed with 10 ml ethyl acetate that contained 100 μl of 7.5 μg/ml of DDB as an internal standard and were then centrifuged at 2500 rpm for 5 min. After that the organic layer was transferred to a clean tube and evaporated to dryness under pressured gas blowing concentrator (Model MGS-2100, EYELA, Tokyo, Japan) at 40 °C.

The extraction residue was reconstituted in 100 μl of 40% acetonitrile solution, and 75 μl aliquots were injected into an HPLC system. The HPLC system consisted of a HP1100 series (Agilent Technologies, Palo Alto, USA): G1322A online degasser, G1312A binary pump, G1313A autosampler, G1316A thermostated column compartment and G1315A diode-array detector. Data was acquired and processed with a HP Chemstation® (LC Rev. A.06.03 [509]) chromatography manager software from Agilent Technologies.

Chromatographic separations were achieved using a 218MR54 column (4.6 mm × 250 mm, 5 μm particle size, C_{18}, Vydac, USA) at 25 °C. The mobile phase consisted of

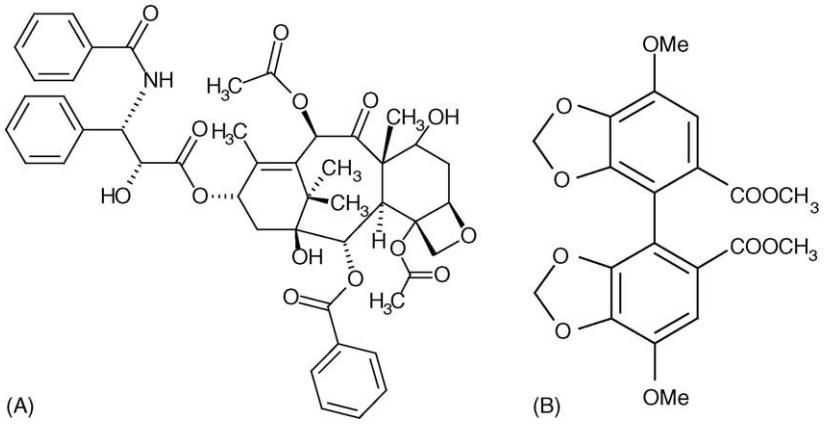

Fig. 1. Molecular structures of paclitaxel (A) and internal standard dimethyl 4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxy biphenyl-2′,2′ dicarboxylate (DDB) (B).

deionized water and HPLC grade acetonitrile, with 34% acetonitrile in deionized water held for 5 min, then linear gradients to 58% acetonitrile in 16 min, then linear gradients to 70% acetonitrile in 2 min, then linear gradients to 34% acetonitrile in 4 min and 34% acetonitrile held for 5 min and was delivered at a flow rate of 1.0 ml/min. UV detection was performed at 227 nm.

2.3. Linearity, limit of detection (LOD) and limit of quantitation (LOQ)

Standard calibration samples were prepared by spiking 100 μl of blank mouse plasma at concentrations ranging from 0.01 to 20 μg/ml with the internal standard at fixed concentrations of 7.5 μg/ml. Calibration curves of paclitaxel were constructed using the ratio of peak height of paclitaxel and internal standard by weighted ($1/y$) linear regression analysis.

The limit of detection was defined as the lowest concentration of paclitaxel resulting in a peak height greater or equal to three times from background noise ($S/N \geq 3$). The LOQ was investigated in plasma samples from five different days. For the determination of LOQ, the percentage deviation and %R.S.D. are to be less than 20%.

2.4. Evaluation of precision and accuracy for HPLC analysis of paclitaxel

The precision and accuracy were determined by analyzing spiked plasma and tissue samples at eight different concentrations ranging from 0.1 to 20 μg/ml. The precision of an HPLC method was determined as the coefficient of variation (%R.S.D.) of intra- and inter-day. The intra-day precision was determined by analyzing the spiked plasma and tissue samples prepared within a day. The inter-day precision was determined by analyzing the spiked plasma and tissue samples prepared on three different days. After concentrations were calculated by re-fitting peak response ratios obtained with different standard solutions into a derived regression equation from the set of these standard solutions, %R.S.D. was determined at each concentration of the standard solutions from their average value and S.D.

The accuracy of the HPLC method was demonstrated by percentage deviation. The calculated concentrations (or conc. found) were obtained by re-fitting peak response ratios from standard solutions of known concentrations (or conc. added) into a derived regression equation. The conc. found and conc. added was then used to determine the absolute percentage deviation at each concentration of the standard solutions.

2.5. Pharmacokinetic and biodistribution studies of paclitaxel

Female SPF C57BL/6 mice (8 weeks, 20 ± 2 g) were inoculated subcutaneously into the right flank with murine B16 melanoma cells (5×10^5 cells). When tumors were 50–400 mm^3 in size, the mice were injected intravenously through the tail vein with 20 mg equivalent paclitaxel/kg of Taxol® and 50 mg equivalent paclitaxel/kg of paclitaxel-loaded polymeric micelle as previously determined dose at maximum tolerated dose (MTD) studies in a volume of 20 ml/kg. Four mice were sacrificed at 3 min, 30 min, 1, 2, 3, 4, 8, 12 and 24 h after drug administration. Samples of plasma, liver, kidneys, spleen, lungs, heart and tumor were harvested and stored at −50 °C until analyzed for paclitaxel. The paclitaxel plasma concentration–time data were analyzed by noncompartmental analysis with the aid of the program WinNonLin® (version 4.1, Pharsight Co., Mountain view, CA). The peak plasma concentration (C_{max}) was obtained from experimental observations. Other pharmacokinetic parameters were calculated by standard methods [22].

3. Results and discussion

3.1. Determination of paclitaxel by reverse phase HPLC

To facilitate pharmacokinetics study of paclitaxel, a sensitive, specific and reproducible HPLC method has been developed and validated for quantitative determination of paclitaxel in plasma, tissues and tumor of mice. After the pretreatment with a rapid single liquid–liquid extraction step, the biological specimens containing paclitaxel and internal standard DDB were separated by reverse HPLC with UV detection at 227 nm. Cephalomannine or 2-methyl paclitaxel, members of the taxane family, is usually used as the internal standard for paclitaxel analysis. The present study is the first report to establish DDB as an internal standard for HPLC analysis of paclitaxel.

The representative chromatograms of paclitaxel and I.S. spiked in mice plasma (A) and the blank mice plasma (B) are shown Fig. 2. The retention times of I.S. and paclitaxel were 13.7 and 18.0 min, respectively. The paclitaxel and I.S. peaks were sharp. There was good baseline separation of paclitaxel, I.S. and the major components from mice plasma and tissue samples. No endogenous or extraneous peaks were observed interfering with the separation and quantitation of paclitaxel. Paclitaxel was stable during the time of analysis and no degradation products were found using the acetonitrile/water mobile phase.

3.2. Linearity, limit of detection (LOD) and limit of quantitation (LOQ)

Table 1 shows the concentration of the paclitaxel standards and 7.5 μg/ml of I.S. spiked in mouse plasma and their corresponding chromatographic heights and height ratios. The calibration curves for paclitaxel in mouse plasma and tumor were linear in the range of 0.1–20 μg/ml. For paclitaxel in mouse plasma, the regression equation of the calibration curve was calculated as $y = 0.134x + 0.005$ (correlation coefficient, $r = 0.999$), where y is the peak height ratio of paclitaxel over I.S. and x is the concentration of paclitaxel. Similarly,

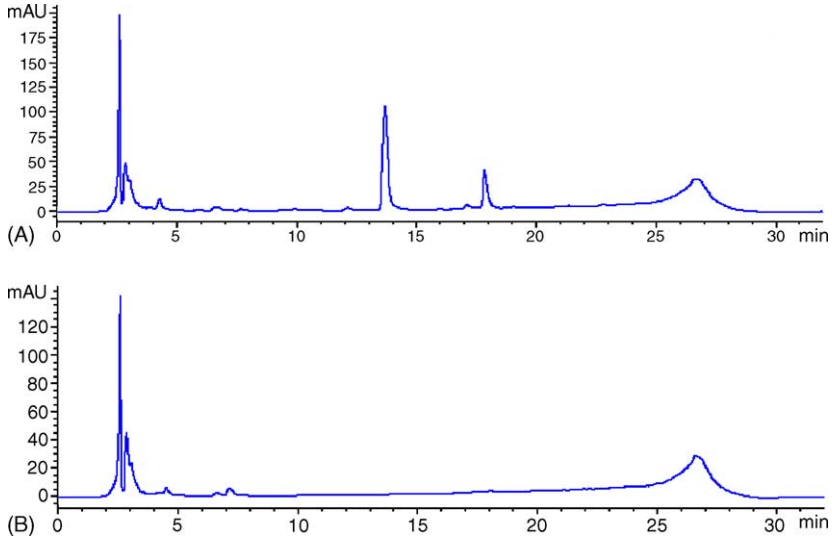

Fig. 2. Representative chromatograms of paclitaxel and I.S. (A) Chromatogram of paclitaxel in mouse plasma. (B) Chromatogram of blank mouse plasma.

calibration curve of paclitaxel in tumor was also obtained with the correlation coefficient over 0.999. These results demonstrated a good linearity between the peak height ratios versus concentrations. The limit of detection (LOD) and limit of quantitation (LOQ) was 5 ng/ml ($S/N \geq 3$) and 10 ng/ml using a 100 μl plasma sample. The sensitivity of this method was comparable with most of the HPLC–UV methods developed for paclitaxel where LOD is usually around 10 ng/ml [19,20,23]. The extraction recovery of paclitaxel was concentration dependent with recovery rates of 93.7 and 96.9% at 10 μg/ml of paclitaxel and at 7.5 μg/ml of I.S., respectively. Thus, the sensitivity and recovery rates of the assay were comparable with previous methods [19,20]. Furthermore, unlike the traditional HPLC methods that require at least 500 μl of plasma sample for the experiment and complicated extraction method like as liquid–liquid extraction (LLE), solid-phase extraction (SPE) and combination of LLE plus SPE with multiple extraction step [10,23], this method used only a 100 μl sample volume and a rapid single-step extraction. These results provide the possibilities to determine paclitaxel at nanomolar level using HPLC with a rapid single liquid–liquid extraction of a microsample.

Table 1
Chromatographic heights and height ratios for paclitaxel and internal standards in mouse plasma

Spiked conc. (μg/ml)	Paclitaxel height (mAU)	I.S. height (mAU)	Height ratio
0.10	1.35	78.37	0.02
0.20	2.38	80.10	0.03
0.51	5.79	78.56	0.07
1.01	11.12	79.31	0.14
2.02	22.63	79.51	0.28
5.06	55.75	80.45	0.69
10.12	105.37	78.48	1.34
20.24	220.78	80.81	2.73
Slope			0.134
Intercept			0.005
Correlation (r)			0.999

3.3. Precision and accuracy

The precision of the assay method was validated by the determination of the intra- and inter-day coefficient of variation (%R.S.D.) and percentage deviation. Table 2 shows the intra-day precision data in mouse plasma over the concentration range of 0.1–20 μg/ml for paclitaxel. All %R.S.D.s were less than 10% and the average %R.S.D. was 5.47% for paclitaxel. Table 2 also shows the inter-day precision data in mouse plasma over the concentration range of 0.1–20 μg/ml for paclitaxel. All %R.S.D.s were less than 10% and the average %R.S.D. was 2.78% for paclitaxel. The relatively higher %R.S.D. at the lowest concentrations is typical (%R.S.D. is 9.35% at 0.1 μg/ml), which is due to the imprecision in measurement of peak responses at these concentrations. The assay of paclitaxel in tumor and other tissues was also readily reproducible. The intra- and inter-day %R.S.D.s for paclitaxel in tumor were shown in Table 3. Over the range of 0.1–20 μg/ml for paclitaxel, the entire %R.S.D.s was well below 4%, even for the lowest concentrations. The average precision rate for all the biological specimens, including plasma, tumor, liver, spleen, kidneys, lungs and heart are listed in Table 4. Over the range of the paclitaxel tested, the average inter- and intra-day precision of paclitaxel in these biological samples were within 5.5%, indicating a highly reproducible and robust assay.

The intra- and inter-day accuracy data expressed as percentage deviation of paclitaxel assay in mouse plasma is included in Table 2. The accuracy rates for tumor tissues are listed in Table 3. It seemed that the assay for paclitaxel in tumor was more accurate than in plasma. Most of the percentage deviation rates in tumor sample were well below 4% except for one intra-day percentage deviation at the highest

Table 2
Intra-day and inter-day precision and accuracy of paclitaxel assay in mouse plasma

Spiked conc. (μg/ml)	Recovered conc. (μg/ml)		|%Deviation|		%R.S.D.	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.10	0.10[a]	0.09[d]	8.64	6.69	9.35	1.75
	0.11[b]	0.09[e]	7.74	9.87		
	0.11[c]	0.09[f]	8.13	8.65		
0.20	0.19	0.18	5.44	7.81	7.38	1.27
	0.20	0.19	2.61	6.45		
	0.22	0.19	8.55	5.44		
0.50	0.48	0.47	3.57	5.82	1.42	1.46
	0.49	0.48	1.31	3.27		
	0.48	0.48	3.83	3.57		
1.00	0.94	1.08	6.54	8.06	7.91	8.33
	0.96	0.94	4.20	5.78		
	1.08	0.94	8.06	6.54		
2.00	1.97	1.95	1.83	2.90	0.78	0.55
	1.98	1.95	1.41	2.51		
	1.95	1.97	2.90	1.83		
5.01	5.28	5.14	5.35	2.68	4.57	1.82
	4.91	5.33	2.08	6.38		
	5.34	5.28	6.68	5.35		
10.02	10.57	9.84	5.52	1.78	4.75	3.78
	9.68	10.01	3.40	0.10		
	9.84	10.57	1.78	5.52		
20.04	21.73	20.36	8.45	1.59	7.63	3.27
	18.65	21.16	6.94	5.60		
	20.36	21.73	1.59	8.44		
Mean			4.86	5.11	5.47	2.78

[a] 1st analysis: conc. = 0.08 × peak height + 0.02, correlation = 0.999.
[b] 2nd analysis: conc. = 0.08 × peak height − 0.00, Correlation = 0.999.
[c] 3rd analysis: conc. = 0.08 × peak height + 0.01, correlation = 0.999.
[d] Day I analysis: conc. = 0.08 × peak height + 0.01, correlation = 0.999.
[e] Day II analysis: conc. = 0.07 × peak height + 0.02, correlation = 0.999.
[f] Day III analysis: conc. = 0.03 × peak height + 0.01, correlation = 0.999.

concentration where the percentage deviation was 4.87%. The intra- and inter-day accuracy for plasma samples were scattered from 0.1 to 10%. The average inter- and intra-day precision rates for all biological samples were below 6% as shown in Table 4.

3.4. Pharmacokinetic and biodistribution studies of paclitaxel

The applicability of the assay method was demonstrated in a pharmacokinetic study of paclitaxel-loaded polymeric micelle and Taxol® in female SPF C57BL/6 mice. After the administration of 50 mg equivalent paclitaxel/kg dose of paclitaxel-loaded polymeric micelle, the peak plasma concentration (C_{max}) obtained was 83.83 μg/ml, while after the administration of 20 mg equivalent paclitaxel/kg dose of Taxol®, the peak plasma concentration (C_{max}) was 94.08 μg/ml (Fig. 3A). The terminal half life ($t_{1/2\beta}$), the area under the plasma concentration–time curve (AUC), volume of distribution at steady state (V_{ss}), total body clearance (CL), mean residence time (MRT) and the area under the moment curve (AUMC) were 2.50 ± 0.11 h, 75.58 ± 3.60 μg h/ml, 0.83 ± 0.07 l/kg, 0.66 ± 0.03 l/h/kg, 1.25 ± 0.09 h, 94.33 ± 8.76 μg h^2/ml and 2.72 ± 0.66 h, 82.75 ± 5.95 μg h/ml, 0.34 ± 0.04 l/kg, 0.24 ± 0.02 l/h/kg, 1.40 ± 0.23 h, 116.39 ± 25.55 μg h^2/ml for 50 mg equivalent paclitaxel/kg dose of paclitaxel-loaded polymeric micelle and 20 mg/kg dose of Taxol®, respectively.

Paclitaxel concentrations investigated in tumor, after i.v. administration of 50 mg equivalent paclitaxel/kg dose of paclitaxel-loaded polymeric micelle and 20 mg equivalent paclitaxel/kg dose of Taxol® are presented in Fig. 3B. After i.v. administration, paclitaxel was widely distributed into most tissues. The highest paclitaxel concentration was found in the tumor. In tumor, paclitaxel concentrations were maximal at 1–2 h after drug administration and declined progressively with the time thereafter. The tissue distribution of paclitaxel after i.v. administration of paclitaxel-loaded polymeric micelle was altered markedly from that of Taxol®. Despite the higher plasma levels after administration of

Table 3
Intra- and inter-day accuracy and precision of paclitaxel assay in tumor

Spiked conc. (μg/g)	Recovered conc. (μg/g)		|%Deviation|		%R.S.D.	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.10	0.10[a]	0.10[d]	3.01	1.26	0.14	2.30
	0.10[b]	0.11[e]	3.01	1.72		
	0.10[c]	0.10[f]	0.03	0.03		
0.21	0.23	0.21	3.61	0.67	2.21	1.98
	0.21	0.20	0.84	1.93		
	0.21	0.21	0.02	0.02		
0.52	0.52	0.53	0.31	2.16	1.92	3.29
	0.52	0.51	0.00	2.80		
	0.54	0.54	0.04	0.04		
1.04	1.06	1.03	2.20	0.94	0.83	1.57
	1.07	1.06	2.82	2.18		
	1.05	1.05	0.01	0.01		
2.08	2.09	2.11	0.56	1.46	3.18	1.09
	2.00	2.16	3.66	3.70		
	2.13	2.13	0.03	0.03		
5.20	5.39	5.23	3.74	0.68	3.31	0.86
	5.06	5.17	2.66	0.59		
	5.15	5.15	0.01	0.01		
10.40	10.24	10.40	1.50	0.44	0.61	1.05
	10.31	10.60	0.80	1.79		
	10.36	10.36	0.00	0.00		
20.79	19.78	20.49	4.87	1.47	1.98	1.51
	20.51	20.15	1.35	3.11		
	19.88	19.88	0.04	0.04		
Mean		1.46	1.13	1.77	1.71	

[a] 1st analysis: conc. = 0.1011 × peak height + 0.01777, correlation = 0.9997.
[b] 2nd analysis: conc. = 0.0945 × peak height + 0.0049, correlation = 0.9999.
[c] 3rd analysis: conc. = 0.0990 × peak height + 0.0140, correlation = 0.9998.
[d] Day I analysis: conc. = 0.0907 × peak height + 0.0097, correlation = 0.9999.
[e] Day II analysis: conc. = 0.0932 × peak height + 0.0114, correlation = 0.9997.
[f] Day III analysis: conc. = 0.0990 × peak height + 0.0140, correlation = 0.9998.

20 mg equivalent paclitaxel/kg dose of Taxol®, the levels observed in all of the tissues were higher at all time points with 50 mg equivalent paclitaxel/kg dose of paclitaxel-loaded polymeric micelle. Paclitaxel levels measured in various tissues after administration of 50 mg equivalent paclitaxel/kg dose of paclitaxel-loaded polymeric micelle were 2–3-fold higher as compared to those measured after administration of 20 mg equivalent paclitaxel/kg dose of Taxol® (data not shown). These findings were thought to be consistent with the literature that an overproportional increase in plasma level of paclitaxel was found upon dosage escalation with doses of Taxol® increasing from 2 to 10 mg/ kg, but the paclitaxel levels in tissues increased proportionally [24].

Table 4
Intra- and inter-day accuracy and precision of paclitaxel assay in biological specimens

Biological specimens	Intra-day accuracy (%)	Intra-day precision (%)	Inter-day accuracy (%)	Inter-day precision (%)
Plasma	2.17	4.76	3.28	2.67
Liver	1.05	1.45	1.84	2.95
Spleen	2.08	1.94	2.32	1.51
Kidneys	3.27	1.78	1.52	1.99
Lungs	3.65	3.49	4.11	3.07
Heart	2.73	3.25	2.04	4.17
Tumor	1.46	1.77	1.13	1.71

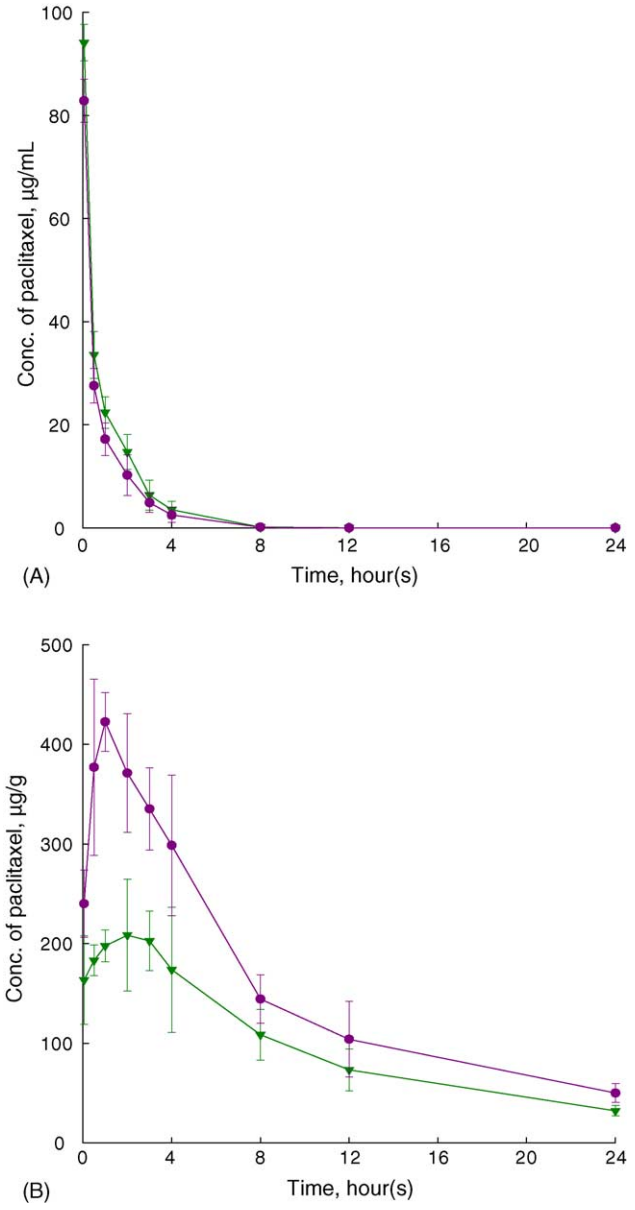

Fig. 3. Time courses of paclitaxel levels in mice plasma (A) and in tumor of murine B16 melanoma-induced mice (B), after i.v. administration of 50 mg equivalent paclitaxel/kg dose of paclitaxel-loaded polymeric micelle (●) and 20 mg equivalent paclitaxel/kg dose of Taxol® (▼). Each point represents the mean ± S.D. of four mice per time point.

4. Conclusion

A sensitive, specific and reproducible assay to determine paclitaxel in microsize biological specimens including plasma, tumor, liver, spleen, kidneys, lungs and heart has been developed. The pretreatment of the biological specimens involved only a rapid single liquid–liquid extraction step. DDB was used the first time as an internal standard for paclitaxel analysis and successfully applied to a quantitation of paclitaxel in plasma and tissues with a best resolution. DDB was chosen as the new internal standard because of the similarities in structure and physical properties of paclitaxel and cost, availability in the market than other possible internal standards. This HPLC method using a new internal standard has comparable specificity, linearity, precision and accuracy and sensitivity with previously published HPLC methods [11,19,20]. This assay method has been successfully applied to a pharmacokinetic and biodistribution study of paclitaxel-loaded polymeric micelle and Taxol® in female SPF C57BL/6 mice.

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