Determination of Paclitaxel in Human Plasma by UPLC–MS–MS

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Abstract

A sensitive and specific ultra-performance liquid chromatography–tandam mass spectrometry method for the quantitation of paclitaxel is established. A 200 µL human plasma sample is spiked with $^{13}C_6$-labeled paclitaxel as an internal standard and extracted with 1.3 mL of *tert*-butyl methyl ether. The chromatographic separation is achieved within 2 min on a C_{18} column with methanol–0.1% aqueous formic acid (75:25, v/v) as a mobile phase at a flow rate of 0.4 mL/min. Multiple reaction monitoring mass transitions of m/z 876.2 to 307.9 and 882.2 to 313.9 are measured for paclitaxel and the internal standard, respectively. For paclitaxel at the concentrations of 1.021, 5.105, and 10.21 µg/mL in human plasma, the extraction recoveries are 105.87%, 103.91%, and 100.39%, respectively. The linear quantitation range of the method is 0.1021–20.42 µg/mL in human plasma with a correlation coefficient greater than 0.999. The intra- and inter-day accuracy for paclitaxel at 1.021, 5.105, and 10.21 µg/mL levels in human plasma fell in the ranges of 95.01–99.23% and 95.29–101.28%, and the intra- and inter-day precision were in the ranges of 6.37–10.88% and 7.21–9.05%, respectively. This method is successfully applied to the determination of the half-life of paclitaxel in human plasma.

Introduction

Paclitaxel (Figure 1) is a natural product derived from the bark of the pacific yew tree Taxus brevifola (1). It inhibits mitosis through stabilization of the polymerization and depolymerization of microtubules. Paclitaxel is currently recognized as the most important available drug for the treatment of solid tumors, especially advanced ovarian, breast, and lung nonsmall cell cancers (2). Monitoring of paclitaxel concentrations in human plasma is important because investigations have indicated that the toxicity and efficacy of paclitaxel are correlated to drug exposure (3,4).

Several assay methods have been published for the determination of paclitaxel in biological fluids, including capillary electrophoresis (5), immunoassays (6), high-performance liquid chromatography (7,8), and liquid chromatography–mass spectrometry (LC–MS) (9–14). Of these, the LC–MS methods have been most frequently used in the quantitation of paclitaxel in biological fluids due to their sensitivity and selectivity. These LC–MS methods utilized either solid-phase extraction (9) or liquid–liquid extraction (10–14) as a pretreatment to biological samples. Recently, ultra-performance liquid chromatography (UPLC) has been developed to improve the chromatographic performance. According to the van Deemter equation, as the particle size decreases to less than 2.5 µm, significant efficiency is gained and but it does not diminish at increased flow rates or linear velocities. Therefore, UPLC uses columns with smaller particle size to shorter analysis time, resulting in a gain in separation efficiency, resolution and sensitivity, and minimize sample matrix effects, such as ion suppression, that often can be observed in LC–MS data, particularly when analyzing biological samples (15). Due to recent introduction of UPLC, no applications in the determination of paclitaxel have been reported until now, but an increase in the near future is predictable, because of its advantages over conventional HPLC.

The aim of this investigation was to develop and validate a fast

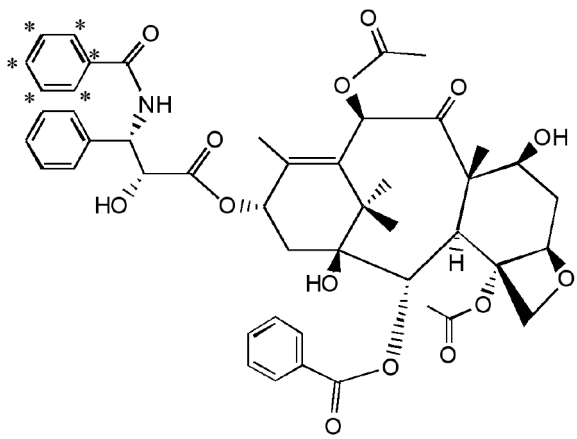

Figure 1 Chemical structures of paclitaxel. The asterisks represent the ^{13}C atoms in the $^{13}C_6$-paclitaxel as an internal standard.

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and sensitive method using liquid–liquid as a pretreatment step followed by UPLC–MS–MS detection for the analysis of paclitaxel in human plasma. Finally, this method was successfully applied to the stability study of paclitaxel in human plasma.

Materials and methods

Materials

Paclitaxel with exact mass of 853.3 and $^{13}C_6$-paclitaxel with exact mass of 859.3 were provided by Sihuan Pharmaceutical Co. (Beijing, China). HPLC-grade methanol and water, formic acid, and *tert*-butyl methyl ether (TBME) were obtained from Fisher (Fair Lawn, NJ). Human plasma was purchased from Valley biomedical incorporation (Winchester, VA).

Preparation of stock and working solutions

Two stock solutions of paclitaxel were prepared by dissolving accurately weighed amounts of paclitaxel in methanol. The stock solution was further serially diluted with methanol to obtain working solutions of calibration standards with final concentrations of 1.021, 5.105, 10.21, 20.42, 51.05, 102.1, and 204.2 µg/mL. The stock solution of $^{13}C_6$-paclitaxel was prepared in methanol at a concentration of 80.7 µg/mL as an internal standard. All solutions were stored at –20°C.

Preparation of calibration standards and quality control samples

Human plasma samples for calibration curve were prepared by adding 20 µL of a series of working solutions of paclitaxel and 20 µL of stock solution of $^{13}C_6$-paclitaxel to 180 µL of human plasma, resulting in final concentrations of paclitaxel being at 0.1021, 0.5105, 1.021, 2.042, 5.105, 10.21, and 20.42 µg/mL in human plasma. QC samples were similarly prepared using a separate weighing of paclitaxel to give concentrations of 1.021, 5.105, and 10.21 µg/mL within the range of calibration standards.

Sample preparation

Twenty microliters (20 µL) of the internal standard solution were added to 200 µL of human plasma sample containing paclitaxel in a micro-centrifuge tube. Following the addition of 1.3 mL of cold TBME, the tube was vortex-mixed for approximately 2 min and then centrifuged at 10,000 × *g* for 10 min (Fisher Micro-centrifuge 235C). One milliliter (1 mL) of resultant supernatant was transferred to another tube and dried under a stream of nitrogen gas at 35°C. The dried residue was reconstituted with 200 µL of methanol–water containing 20mM sodium chloride (80:20, v/v) and vortex-mixed for 0.5 min. After centrifugation at 10,000 × *g* for 10 min, the supernatant was transferred to an HPLC auto sampler vial, and a 10 µL aliquot of sample were injected into UPLC–MS–MS.

UPLC–MS–MS conditions

Chromatographic separation was carried out using an Acquity ultra-performance liquid chromatograph (Waters Corporation, Milford, MA) consisting of a binary pump, auto sampler, degasser, and column oven. A mobile phase of methanol–0.1% aqueous formic acid (75:25, v/v) was pumped through an Acquity UPLC BEH C_{18} column (particle size 1.7 µm, 50 × 2.1 mm i.d., Waters Corporation) at a flow rate of 0.4 mL/min. A run time of 2.0 min at 25°C was used. The LC elute was connected directly to a Quattro Micro triple-quadrupole mass spectrometer (Micromass, Waters) equipped with an electrospray ionization (ESI) ion source Z-spray. The positive multiple reaction monitoring (MRM^+) mode was used for the quantitation using MassLynx version 4.1 software. Mass transitions of *m/z* 876.2 to 307.9 and 882.2 to 313.9 were optimized for paclitaxel Na^+ adduct and $^{13}C_6$-paclitaxel adduct, respectively, and with a dwell time of 0.5 s and inter-scan delay of 0.02 s. The electrospray source parameters were fixed as follows: capillary voltage, 3.5 kV; cone voltage, 50 V; source temperature, 100°C; and desolvation temperature, 250°C. Nitrogen was used as nebulizing gas (30 L/h) and desolvation gas (300 L/h). Argon was employed as collision gas at 3.00×10^{-3} mbar and collision energy was 31 eV. The multiplier voltage was set at 650 V.

Method validation

Linearity

A calibration curve was prepared by injecting the extracts from seven known concentrations of paclitaxel in human plasma with the internal standard. The calibration curve was constructed by weighted reciprocal of the concentration ($1/x$) least-squares linear regression analysis of known drug concentration versus peak area ratios of paclitaxel to the internal standard. The limit of detection (LOD) was defined as the quantity of paclitaxel in the plasma after the sample clean-up corresponding to three times the baseline noise. The lowest calibration standard with a signal to noise ratio of 10 was to be considered as the lower limit of quantitation (LLOQ). In addition, the analyte peak in LLOQ sample should be identifiable, discrete, and reproducible with a precision of 20% and accuracy within 80–120%.

Extraction recovery

The extraction recovery of paclitaxel from human plasma was determined by comparing peak areas obtained after whole extraction of low, medium, and high concentration to those obtained from direct injection of the standard solutions of equivalent concentration. The recovery of the analyte need not be 100%, but the extent of recovery of the analyte should be consistent, precise, and reproducible.

Accuracy and precision

The accuracy and precision were evaluated by analysis of QC samples at levels of 1.021, 5.105, and 10.21 µg/mL using five determinations per concentration on five consecutive days. The accuracy was expressed as percentage value (% accuracy = [measured concentration/nominal concentration] × 100%). The precision was presented as percentage relative standard deviation (%RSD). The evaluation of precision was based on the criteria that RSD for each concentration should not be more than ± 15% except for LLOQ, for which it should not be more than ± 20%. Similarly, for accuracy, the mean value should not deviate by ± 15% of the actual concentration, except for the LLOQ, where it should not deviate by more than ± 20% of the actual concentration.

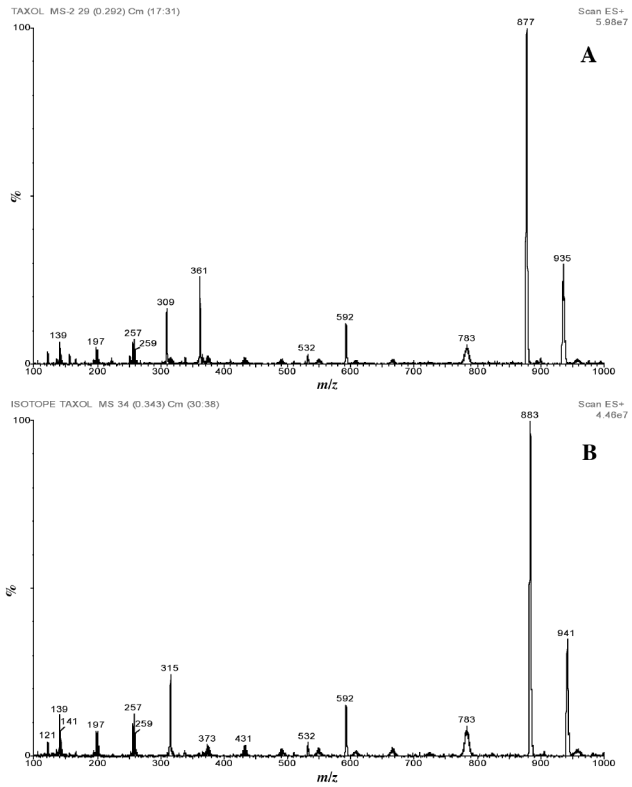

Figure 2. Mass spectra of precursor ions of paclitaxel (A) and $^{13}C_6$-paclitaxel (B) in positive electrospray ionization mode.

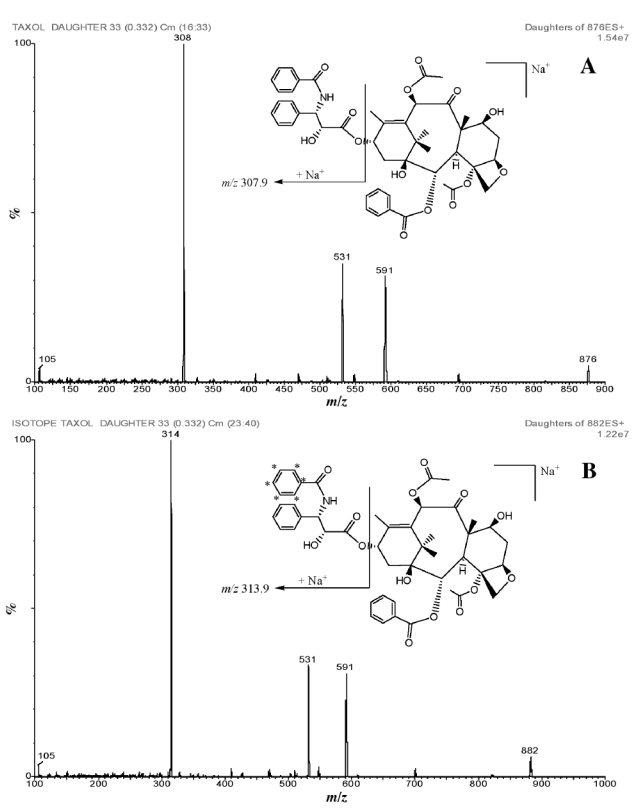

Figure 3. Mass spectra of product ions of paclitaxel (A) and $^{13}C_6$-paclitaxel (B) in positive electrospray ionization mode.

Stability

Stability in plasma. For establishing the long-term stability of paclitaxel in human plasma, five aliquot each of low and high QC samples were kept at –20°C for 21 days. Similarly, short-term stability was determined by keeping five aliquots each of the low and high unprocessed QC samples at ambient temperature for 24 h. Thereafter, the samples were processed and analyzed. The concentrations thus obtained were compared with those obtained for freshly prepared QC samples, and the samples were considered stable if the deviation from the nominal concentration was not more than ± 15%.

Auto sampler stability. Five aliquots each of low and high-processed QC samples were stored at 25°C in auto sampler for 24 h. Thereafter, samples were analyzed and concentrations were compared with those obtained for freshly prepared QC samples, and a deviation of more than ± 15% from the actual value was undesirable.

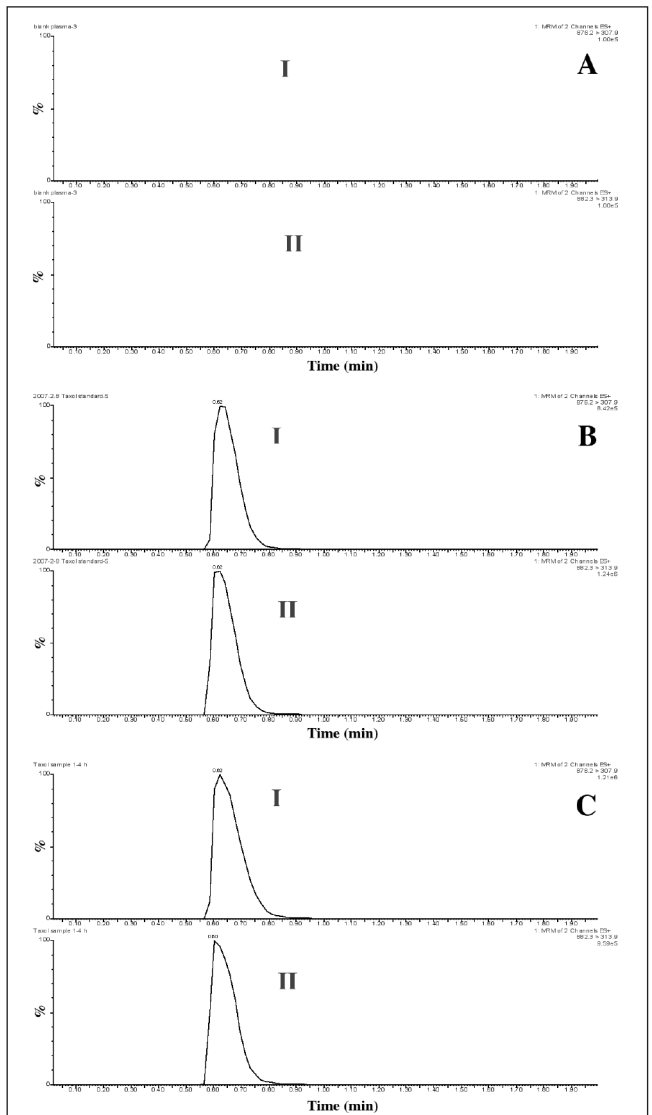

Figure 4. The representative chromatograms of paclitaxel (I) internal standard (II) in human plasma: blank plasma (A), plasma spiked with paclitaxel (5.105 µg/mL) and internal standard $^{13}C_6$-paclitaxel (8.07 µg/mL) (B), plasma sample with $^{13}C_6$-paclitaxel (8.07 µg/mL) 4 h after paclitaxel in human plasma incubated at 37°C (C).

Freeze and thaw stability. Five aliquots each of low and high-unprocessed QC samples were subjected to three freeze and thaw cycles. After the completion of the third cycle, the samples were analyzed and the concentrations thus obtained were compared with those obtained for freshly prepared QC samples. The samples qualified the test if the deviation was within ± 15%.

Determination of the half-life of paclitaxel in human plasma

A 0.2 mL aliquot from 204.2 µg/mL stock solution of paclitaxel was spiked into 3.8 mL of human plasma preincubated for 15 min (37°C) and incubated in a reciprocating water bath at 37°C. At the different time periods (0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 30 h), 200 µL aliquot of samples were taken and immediately added to 1.3 mL of cold TBME, and, subsequently, 20 µL of the internal standard stocking solution (80.7 µg/mL in methanol) was added, and then the described extraction procedure was followed.

Results and Discussion

Mass spectrometry

The first full-scan spectra revealed that the Na^+ adduct was more sensitive than the H^+ adduct for paclitaxel (Figure 2A) and $^{13}C_6$-paclitaxel (Figure 2B). As shown in Figure 2, under ESI^+ mode, the prominent quasi-molecular ions for paclitaxel and $^{13}C_6$-paclitaxel were $[Paclitaxel+Na]^+$ at *m/z* 876.2 and $[^{13}C_6$-paclitaxel$+Na]^+$ at *m/z* 882.2, respectively. Therefore, these quasi-molecular ions were chosen as precursor ions for acquisition of product ion fragments (Figure 3). The Na^+ adducts, *m/z* of 307.9 and 313.9, were the major product ions for paclitaxel (Figure 3A) and $^{13}C_6$-paclitaxel (Figure 3B), respectively, from the second full-scan spectra, which were chosen as the quantitation ions in subsequent MRM mode detection.

Specificity

To serve as blanks, plasma samples were obtained from six different sources and assayed to evaluate the selectivity of the method and the detection of interference. The representative chromatograms of paclitaxel and the internal standard spiked in human plasma and blank human plasma are shown Figure 4. The retention times of paclitaxel and the internal standard were 0.62 min. There was a good baseline separation of paclitaxel and the internal standard from human plasma. No endogenous or extraneous peaks were observed interfering with the separation and quantitation of paclitaxel. Because chromatographic conditions may cause coelution of a number of endogenous compounds that are undetected by the MS–MS but may affect the ionization efficiency, the effect of matrix on the response of the analyte was also evaluated. To determine the possible influence of the matrix on the analysis, the response of six extracted spike matrix blank samples were compared to those of six analytical standards. No matrix effect was detected in the study.

Table I. Method Accuracy, Precision, and Extraction Recovery of Paclitaxel in Human Plasma*

Concentration	Intraday accuracy		Interday accuracy		Extraction recovery	
(µg/mL)	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD
1.021	97.31 ± 7.24	7.44	95.29 ± 6.87	7.21	105.87 ± 5.62	5.31
5.105	95.01 ± 10.34	10.88	97.43 ± 8.54	8.76	103.91 ± 5.21	5.01
10.21	99.23 ± 6.32	6.37	101.28 ± 9.17	9.05	100.39 ± 1.85	3.85

Table II. Summary of the Stability of Paclitaxel From QC Samples of Human Plasma Under Various Storage Conditions (*n* = 5)

Stability conditions	Known concentration (µg/mL)	Accuracy (%)	Precision (%)
Long-term (21 days, –20°C)	1.021	89.25	9.96
	10.21	91.47	4.83
Short-term (24 h, room temperature)	1.021	93.14	10.85
	10.21	95.27	8.37
Auto sampler (24 h, 25°C)	1.021	89.02	8.44
	10.21	90.21	7.23
Three freeze-thaw cycles (–20°C)	1.021	94.63	7.40
	10.21	95.56	3.68

Calibration curve

Using the peak area ratios of paclitaxel to internal standard as a quantitation signal, the seven-point calibration curve (0.1021, 0.5105, 1.021, 2.042, 5.105, 10.21, and 20.42 µg/mL) was linear with correlation coefficients greater than 0.999, indicating a good linearity. By using the 200 mL of plasma sample volume, the LOD of paclitaxel was 2 ng/mL, and the LLOQ was found to be 5 ng/mL with a precision of less than 20%.

Extraction recovery

The extraction recovery was measured in QC plasma samples at the concentrations of 1.021, 5.105, and 10.21 µg/mL. The extraction recoveries of paclitaxel at the concentrations of 1.021, 5.105, and 10.21 µg/mL were 105.87 ± 5.62%, 103.91 ± 5.21%, and 100.39 ± 1.85%, respectively, indicating a good extraction efficiency (Table I).

Accuracy and precision

The intra- and inter-day accuracy for paclitaxel at 1.021, 5.105, and 10.21 µg/mL levels in human plasma fell in the ranges of 95.01–99.23% and 95.29–101.28%, and the intra- and inter-day precision were in the ranges of 6.37–10.88% and 7.21–9.05%, respectively (Table I). The results

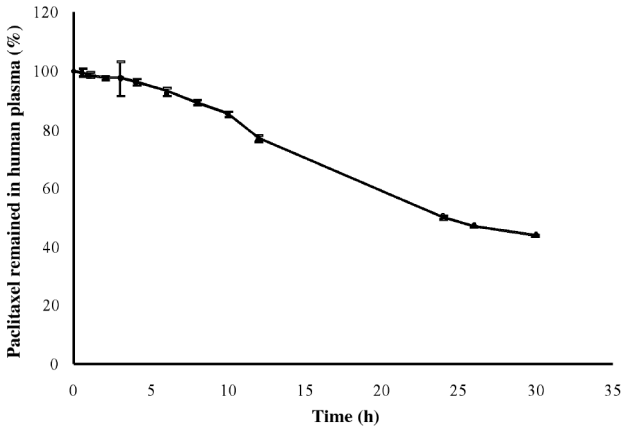

Figure 5. The concentration-time profile of paclitaxel incubated in human plasma at 37°C for 30 h (n = 6).

satisfactorily met the acceptance criteria: mean accuracy within 85–115% and mean precision less than 15%.

Stability

Paclitaxel was stable for 21 days at –20°C and for 24 h at ambient temperature in human plasma. In the auto sampler maintained at 25°C, the plasma samples of paclitaxel were stable for 24 h. Plasma samples containing paclitaxel remained unaffected even after being subjected to three freeze thaw cycles, as shown in Table II.

Determination of half-life of paclitaxel in human plasma

The method was applied to investigate the half-life of paclitaxel at a concentration of 10.21 µg/mL in human plasma. The concentration-time profile of paclitaxel in human plasma was shown in Figure 5. The results showed that in plasma, paclitaxel underwent rapid ester bond hydrolysis and degradation, which were reflected in the disappearance of approximately 33% and 50% of paclitaxel in 12 h and 24 h, respectively, maybe due to the hydrolysis of the ester bond by both chemical and enzymatic mechanisms in plasma. The half-life of paclitaxel in human plasma was 25.2 h.

Conclusion

A sensitive, specific, and reproducible UPLC–MS–MS method for the quantitative determination of paclitaxel in human plasma has been developed and validated. The pretreatment of biological specimens involves only a rapid single liquid–liquid extraction method. The method was successfully used for the quantitation of paclitaxel in human plasma to study the half-life of this drug in human plasma.

References

1. M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, and A.T. McPhail. Plant antitumor agents. VI: the isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J. Am. Chem. Soc.* **93:** 2325–27 (1971).
2. E.K. Rowinsky and R.C. Donehower. Paclitaxel (taxol). *N. Engl. J. Med.* **332:** 1004–14 (1995).
3. M.T. Huizing, G. Giaccone, L.J. van Warmerdam, H. Rosing, P.J. Bakker, J.B. Vermorken, P.E. Postmus, N. van Zandwijk, M.G. Koolen, W.W. ten Bokkel Huinink, W.J. van der Vijgh, F.J. Bierhorst, A. Lai, O. Dalesio, H.M. Pinedo, C.H. Veenhof, and J.H. Beijnen. Pharmacokinetics of paclitaxel and carboplatin in a dose-escalating and dose-sequencing study in patients with non-small-cell lung cancer. *J. Clin. Oncol.* **15:** 317–29 (1997).
4. T. Ohtsu, Y. Sasaki, T. Tamura, Y. Miyata, H. Nakanomyo, Y. Nishiwaki, and N. Saijo. Clinical pharmacokinetics and pharmacodynamics of paclitaxel: a 3-hour infusion versus a 24-hour infusion. *Clin. Cancer Res.* **1:** 599–606 (1995).
5. G. Hempel, D. Lehmkuhl, S. Krumpelmann, G. Blaschke, and J. Boos. Determination of paclitaxel in biological fluids by micellar electrokinetic chromatography. *J. Chromatogr. A.* **745:** 173–79 (1996).
6. P.G. Grothaus, T.J. Raybould, G.S. Bignami, C.B. Lazo, and J.B. Byrnes. An enzyme immunoassay for the determination of taxol and taxanes in Taxus sp. tissues and human plasma. *J. Immunol. Methods.* **158:** 5–15 (1993).
7. D. Fraier, V. Cenacchi, and E. Frigerio. Determination of a new polymer-bound paclitaxel derivative (PNU 166945), free paclitaxel and 7-epipaclitaxel in dog plasma and urine by reversed-phase high-performance liquid chromatography with UV detection. *J Chromatogr. A.* **797:** 295–303 (1998).
8. A. Sparreboom, P. de Bruijn, K. Nooter, W.J. Loos, G. Stoter, and J. Verweij. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B.* **705:** 159–64 (1998).
9. R.A. Parise, R.K. Ramanathan, W.C. Zamboni, and M.J. Egorin. Sensitive liquid chromatography-mass spectrometry assay for quantitation of docetaxel and paclitaxel in human plasma. *J. Chromatogr. B.* **783:** 231–236 (2003).
10. E. Stokvis, M. Ouwehand, L.G. Nan, E.M. Kemper, O. van Tellingen, H. Rosing, and J.H. Beijnen. A simple and sensitive assay for the quantitative analysis of paclitaxel in human and mouse plasma and brain tumor tissue using coupled liquid chromatography and tandem mass spectrometry. *J. Mass Spectrom.* **39:** 1506–12 (2004).
11. W. Guo, J.L. Johnson, S. Khan, A. Ahmad, and I. Ahmad. Paclitaxel quantification in mouse plasma and tissues containing liposome-entrapped paclitaxel by liquid chromatography-tandem mass spectrometry: application to a pharmacokinetics study. *Anal. Biochem.* **336:** 213–20 (2005).
12. M.S. Alexander, M.M. Kiser, T. Culley, J.R. Kern, J.W. Dolan, J.D. McChesney, J. Zygmunt, and S.J. Bannister. Measurement of paclitaxel in biological matrices: high-throughput liquid chromatographic-tandem mass spectrometric quantification of paclitaxel and metabolites in human and dog plasma. *J. Chromatogr. B.* **785:** 253–61 (2003).
13. L.D. Vainchtein, B. Thijssen, E. Stokvis, H. Rosing, J.H. Schellens, and J.H. Beijnen. A simple and sensitive assay for the quantitative analysis of paclitaxel and metabolites in human plasma using liquid chromatography/tandem mass spectrometry. *Biomed. Chromatogr.* **20:** 139–48 (2006).
14. X. Tong, J. Zhou, and Y. Tan. Determination of paclitaxel in rat plasma by LC-MS-MS. *J. Chromatogr. Sci.* **44:** 266–71 (2006).
15. S. Souverain, S. Rudaz, and J.L. Veuthey. Matrix effect in LC-ESI-MS and LC-APCI-MS with off-line and on-line extraction procedures. *J. Chromatogr. A.* **1058:** 61–66 (2004).

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