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Determination of paclitaxel in mouse plasma and brain tissue by liquid chromatography–mass spectrometry

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Abstract

Paclitaxel is an anticancer agent extracted from the bark of the yew tree and is widely used in chemotherapy for solid tumors, including non-small cell lung cancer and ovarian carcinoma. Most assays to measure paclitaxel in plasma require a large amount of sample (0.4–1 ml) to achieve the necessary sensitivity, and are not suitable when only small sample sizes are available. To circumvent this latter limitation, we developed a sensitive liquid chromatography–mass spectrometry (LC–MS) method for the determination of paclitaxel in plasma based on the use of small sample volumes (50 μl plasma). A solid phase extraction procedure was employed that enabled the eluent to be directly injected onto a reversed phase chromatographic HPLC system using positive electrospray ionization followed by mass spectrometric detection. The extraction recoveries of paclitaxel were 98 and 83% from plasma and brain tissues, respectively. The mobile phase consisted of 50% acetonitrile in 0.1% formic acid that was pumped at 0.2 ml/min to yield a retention time for paclitaxel of 6.2 and 5.4 min for cephalomannine, the internal standard. The method has been validated at paclitaxel plasma concentrations from 0.036 to 9.9 μg/ml, and from 0.054 to 1.96 μg/ml in brain homogenates. A sensitive and specific assay for paclitaxel has been developed that has the advantages of using small sample sizes, and a single extraction step without solvent evaporation.

Keyword: Paclitaxel

1. Introduction

Paclitaxel is a natural component extracted from the bark of the yew tree and is a mainstay of numerous chemotherapeutic regimens, particularly in solid tumors such as non-small cell lung cancer and ovarian cancer [1,2]. In addition to the widespread clinical use of paclitaxel, it is the subject of numerous preclinical investigations pertaining to its interesting pharmacological properties [3–6]. One area of interest is based on the fact that paclitaxel is a substrate for P-glycoprotein, a membrane transporter that serves as a drug efflux pump that can alter paclitaxel pharmacokinetics and sensitivity to tumor cells. To explore the role of P-glycoprotein in the distribution of paclitaxel in preclinical tumor models, a liquid chromatography–mass spectrometry (LC–MS) method was developed for small sample volumes that afforded the desired sensitivity.

There have been several HPLC methods, using either UV or MS detection, reported for the determination of paclitaxel pharmacokinetic properties in biological samples. Since paclitaxel lacks strong UV absorption, detection at the more permissive wavelength of 227 nm required the use of large volumes (0.4–1 ml) of plasma or serum to achieve low quantitation limits of approximately 10 ng/ml [7–12]. These methods that require relatively large volumes of plasma or serum may be suitable for human pharmacokinetic studies but not for preclinical PK studies in rodents since the collection of multiple large volume blood samples from each animal would be prohibited.

Some efforts have been made to use small sample volumes (50–200 μl) coupled with liquid–liquid extractions, either alone or combined with solid phase extraction with UV detection that have achieved sensitivity limits of paclitaxel of between 130 and 25 ng/ml [13,14]. Recently, a quantitation limit of 10 ng/ml was achieved [15] with 100 μl of mouse plasma using *tert*-butyl methyl ether for liquid–liquid extraction, yet a time-consuming evaporation step was necessary to concentrate the samples.

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Although a number of mass spectrophotometric methods have been developed for paclitaxel, only a few are for the determination of paclitaxel in plasma [16–18]. An LC–MS/MS method employing 100 μl of human serum and a column switching procedure reported a sensitivity of 5 ng/ml that could be extended to 2 ng/ml with 200–300 μl of serum [16]. Another report also achieved a sensitivity limit of 5 ng/ml with solid phase extraction, yet 500 μl of human plasma was required [17]. Another recent LC–MS method reported a limit of quantitation of 1 nM (0.9 ng/ml) using 1 ml of human plasma and solid phase extraction followed by an evaporation step [18]. There has yet to be developed a LC–MS assay for small sample sizes that could be applied to conducting comprehensive pharmacokinetic studies in rodents. In addition, the prevailing methods have not been applied to the determination of paclitaxel in brain tissue, which is a necessary extension to address questions pertaining paclitaxel disposition in brain and brain tumors. This is particularly pertinent for drugs, such as paclitaxel, that serve as substrates for P-glycoprotein, a drug efflux pump known to be expressed on the blood–brain barrier [19,20]. Given the above concerns of developing a robust assay for paclitaxel in small sample volumes of mouse plasma and brain tissue, we developed a new and sensitive LC–MS method using solid phase extraction without an evaporation step that could routinely be applied to pharmacokinetic investigations.

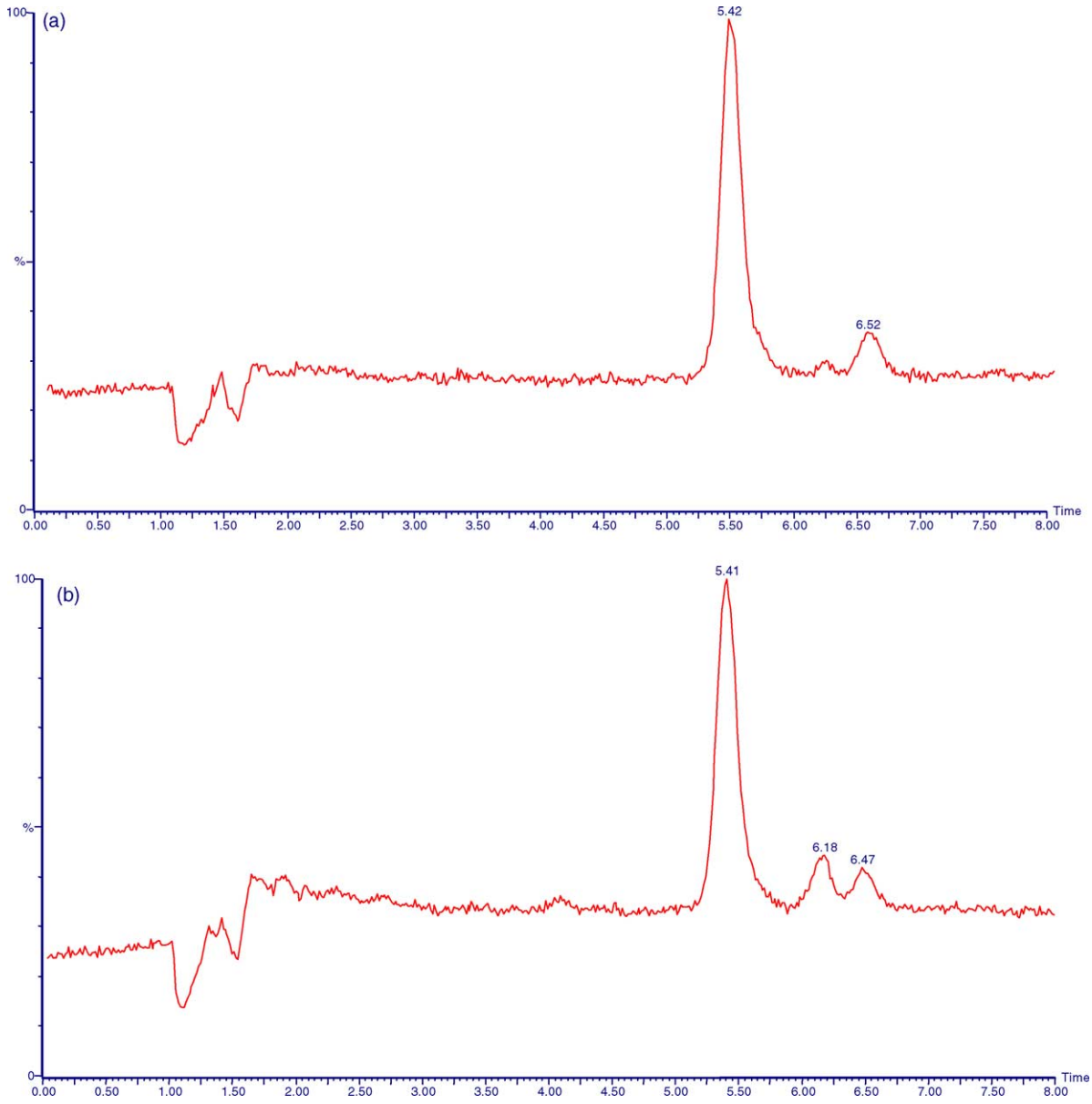

Fig. 1. Chromatograms of paclitaxel in mouse plasma. (a) Blank, (b) 36 ng/ml, (c) 599 ng/ml, (d) mouse plasma obtained at 2 h after administration of 10 mg/kg paclitaxel, (e) mouse plasma obtained at 8 h after administration of 10 mg/kg paclitaxel. The retention times of paclitaxel and the internal standard are 6.2 and 5.4 min, respectively.

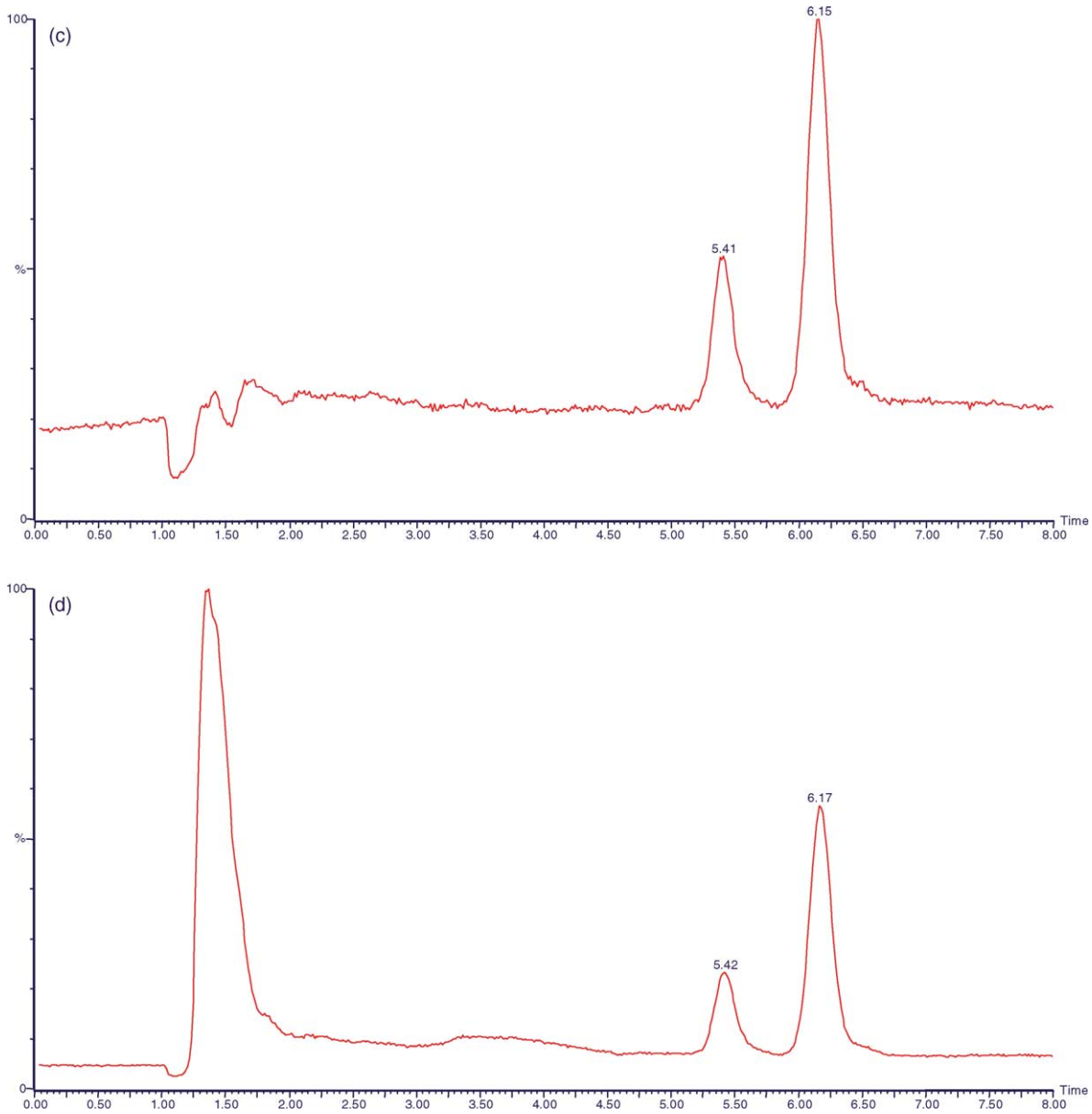

Fig. 1. (*Continued*).

2. Experimental

2.1. Chemicals and reagents

Paclitaxel was purchased from Hande Tech USA Inc. (Houston, TX, USA). Cephalomannine was obtained from the National Cancer Institute. Formic acid was purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile and methanol were purchased from Fisher Chemicals. Purified water (Nanopure deionization system, Barnstead/Thermolyne, Dubuque, IA, USA) was used for all aqueous solutions. Drug-free mouse plasma was purchased from Lampire Biological Laboratories (Pipersville, PA, USA). Bond Elut C2 cartridges (100 mg/1 ml) were purchased from Varian Incorporated (Harbor City, CA, USA).

2.2. Preparation of stock solutions, calibration standards and quality control samples

Standard stock solutions of paclitaxel were prepared in methanol at a concentration of approximately 400 μg/ml and stored at 4 °C. Working solutions were prepared fresh daily by diluting standard stock solutions to concentrations of paclitaxel ranging from 100 to 0.1 μg/ml with methanol. Solutions of the internal standard (IS), cephalomannine, were prepared at 0.25 μg/ml in methanol and stored at 4 °C.

Calibration standards of approximately 10.0, 2.50, 0.600, 0.150, 0.040, and 0.010 μg/ml in mouse plasma were prepared by adding appropriate aliquots of the working solution to plasma. Calibration curves were obtained by least-squares linear regression, weighted by the reciprocal

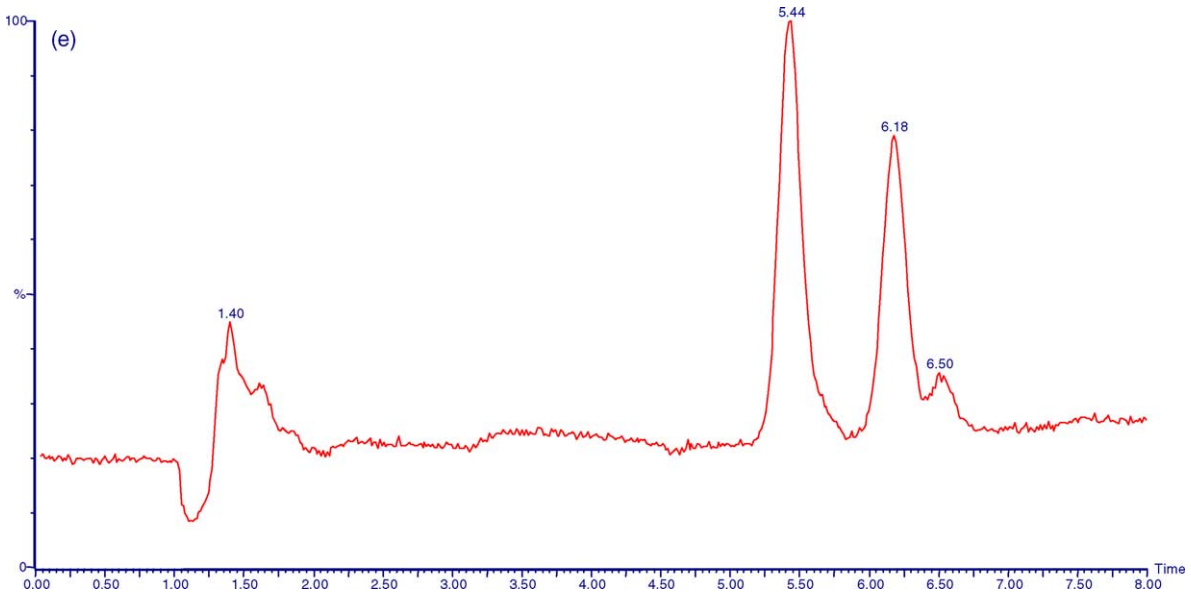

Fig. 1. (*Continued*).

of the concentration, using the peak height ratio of drug to IS. Quality control samples were prepared in a similar way at concentrations within the range of the calibration standards and were assayed in triplicate on each day that samples were analyzed.

Brain tissue homogenates were prepared by adding purified water to brain tissue at ratio of 9:1 (ml:g) and then homogenized. Calibration standards of approximately 2.20, 0.600, 0.200, 0.050 and 0.015 μg/ml were prepared by adding appropriate aliquots of the working solutions to the homogenate. Calibration curves were obtained by least-squares linear regression, weighted by the reciprocal of the concentration, using the peak height ratio of drug to IS. Quality control samples in tumor homogenate were prepared in the same manner at concentrations within the range of the calibration standards and were assayed in triplicate on each day that samples were analyzed.

2.3. Solid phase extraction

For plasma, 50 μl of plasma was mixed with 150 μl of methanol and 50 μl of 0.25 μg/ml of internal standard solution by vortex for 1 min followed by centrifugation at 18,000 × *g* for 5 min. The supernatant was added to 750 μl of water. Under vacuum of about 0.1 bar, the resultant supernatant was applied to a C2 cartridge that was preconditioned with 1 ml methanol and 2 ml of water. The cartridge was then washed with 3 ml of water followed by slow elution of the desired components with 250 μl of acetonitrile. A final mixture of 100 μl of eluent and 100 μl of water was prepared with aliquots of 10 μl injected onto the LC–MS system.

For brain samples, 200 μl of homogenate was mixed with 600 μl of methanol and 50 μl of 0.25 μg/ml of internal standard solution by vortex for 1 min, and then centrifuged at 18,000 × *g* for 5 min. The resultant supernatant was mixed with 3 ml of water and then applied to a preconditioned C2 cartridge and processed as for plasma.

2.4. Method validation

The precision and accuracy of the assay was based on analyses of plasma and brain tissue samples. Plasma and brain homogenate quality control samples were included in all calibration curves and processed in triplicate. The intraday and interday means, standard deviations, and percentage coefficients of variations (%CVs) were calculated by standard methods. The limit of quantitation (LOQ) in plasma and brain tissue homogenates was defined as the lowest concentration at which the signal-to-background noise ratio was greater than 6:1, and both intraday and interday %CVs and percentage biases were less than 20%. The specificity of the assay for paclitaxel in the presence of endogenous components of mouse was evaluated using plasma obtained from different batches of commercial rat plasma as well as from plasma collected from the FVB mouse strain.

2.5. Liquid chromatography–MS (LC–MS)

The HPLC system consisted of a HP model 1100 system coupled to a Finnigan Navigator Quadrupole MS. The nebulizer temperature was maintained at 150 °C and nitrogen was used as both the nebulizer gas and drying gas with flow rate of 310 l/h. A cone voltage of 21 V and capillary voltage 3.43 V were used. The high mass and low mass resolution were 2.6 and 12.1, respectively. Paclitaxel and cephalomannine were detected at *m*/*z* values of 854.3 and 832.4, respectively, with a dwell time of 0.4 s in selected-ion recording (SIR) mode with the multiplier voltage set at 600 V.

The analytical column (Hewlett-Packard, Hypersil, 5 μm particle size, 100×, 2.1 mm) consisted of an ODS stationary phase, and was maintained at ambient temperature. The mobile phase was prepared by mixing 500 ml of solution of 0.1% formic acid and 500 ml of acetonitrile. The flow rate was 0.2 ml/min.

3. Results and discussion

3.1. Mass spectrometry and chromatography

The mass to charge ratios at *m*/*z* 854.3 and 832.4 were chosen for monitoring paclitaxel and cephalomannine, respectively, in two channel mode that can avoid cross interference. The mobile phase provided a separation of paclitaxel and the internal standard with retention times of 6.2 and 5.4 min, respectively. A total run time of 8 min was allowed between successive injections to avoid endogenous interfering peaks.

3.2. Extraction of paclitaxel from biological matrices

Paclitaxel is a lipophilic compound and is strongly retained on C18 solid phase extraction columns. However, this type of affinity causes the retention of numerous endogenous components from biological samples that lead to strong background interferences and reduced drug

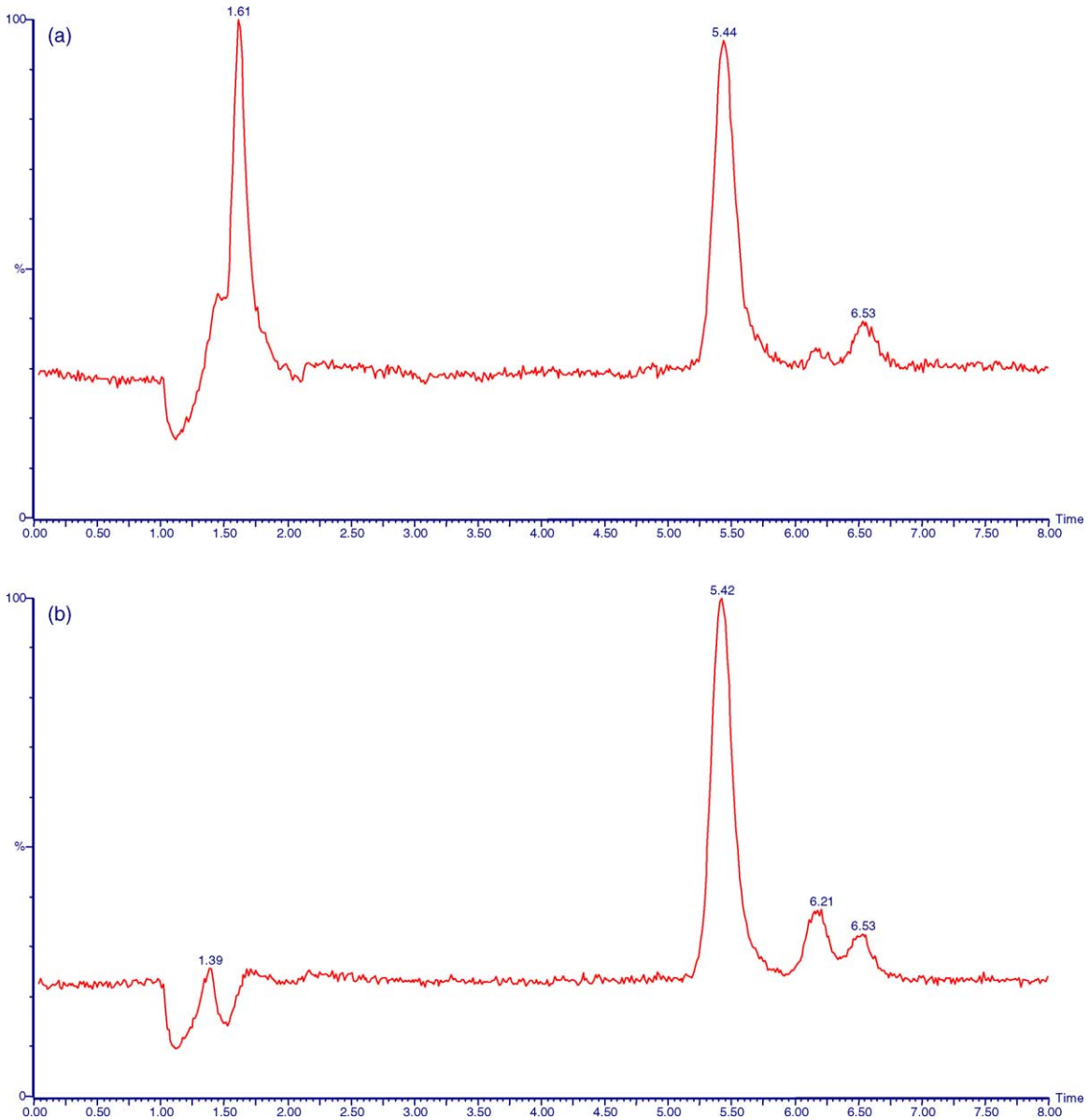

Fig. 2. Chromatograms of paclitaxel in brain tissue homogenate. (a) Blank, (b) 15 ng/ml, (c) 180 ng/ml, (d) mouse brain obtained at 3 h after administration of paclitaxel with a steady state dose regimen. The retention times of paclitaxel and the internal standard are 6.2 and 5.4 min, respectively.

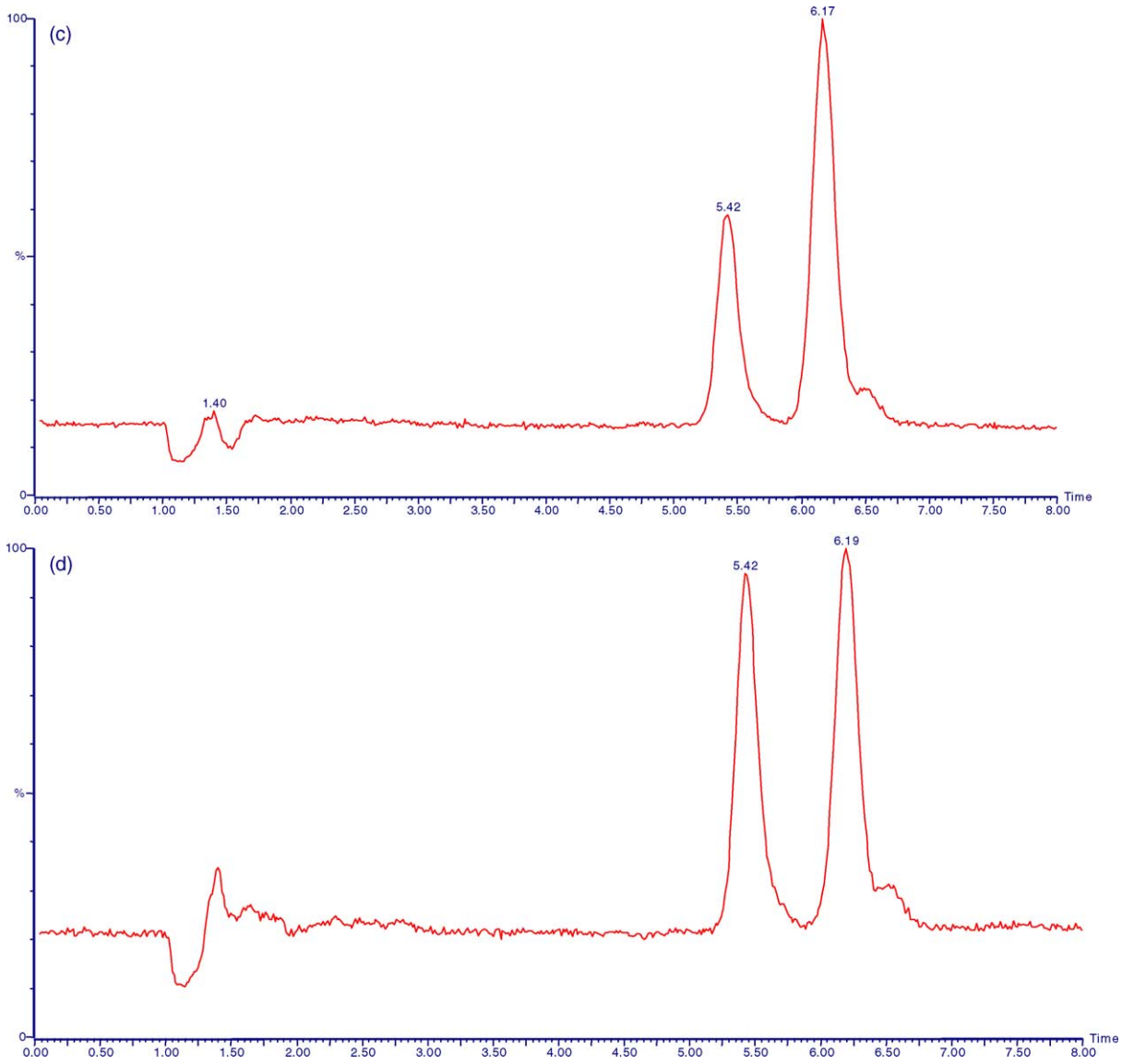

Fig. 2. (*Continued*).

sensitivity. Another disadvantage of using C18 solid phase extraction columns for paclitaxel is that large volumes of organic solvent are needed to overcome the strong retention of paclitaxel, and thus, make an evaporation step inevitable. We found that a C2 column can retain paclitaxel adequately without appreciable retention of many of the interfering endogenous components in plasma and brain. Furthermore, only a small volume (250 μl) of acetonitrile was required to elute paclitaxel efficiently, which enabled elimination of a solvent evaporation step and facilitated a low limit of quantitation. Successful application of the C2 solid phase extraction for paclitaxel required pretreatment of plasma with methanol to disrupt paclitaxel from plasma protein binding sites. The net result of the methanol and C2 solid phase extraction steps yielded high recoveries from mouse plasma with a mean recovery of 98% for paclitaxel (range 95–100%). Analogous advantages of the extraction procedure were also realized in brain homogenate samples in which the mean recovery was 83% (range 79–87%). Following extraction, sample solutions of paclitaxel were stable for at least 12 h at room temperature.

3.3. Method validation

The method was validated for paclitaxel in mouse plasma and in brain tissue homogenates over concentration ranges of 0.036–9.9 μg/ml in plasma and from 0.054 to 1.96 μg/ml in brain homogenate. Calibration curves prepared over these concentration ranges were linear with average correlation coefficients greater than 0.999 in both plasma and in brain homogenates. The method yielded mean intraday and interday precision and accuracy values of less than ±15% (Tables 1 and 2). The LOQs were 0.036 μg/ml for plasma and 0.054 μg/ml for brain homogenate using a 6:1

Table 1
Intraday precision and accuracy of paclitaxel in plasma and brain tissue homogenate ($n = 3$)

	Plasma (μg/ml)					Brain tissue (μg/ml)		
Concentration added	0.0360	0.1470	0.599	2.443	9.96	0.0543	0.3260	1.956
Concentration measured	0.0338	0.1548	0.626	2.411	8.86	0.0510	0.3198	1.802
S.D.	0.0016	0.0055	0.012	0.027	0.38	0.0010	0.0046	0.013
CV (%)	4.7	3.6	1.9	1.1	4.3	2.0	1.4	0.7
Bias (%)	−6.1	5.3	4.5	−1.3	−11	−6.1	−1.9	−7.9

Table 2
Interday precision and accuracy of paclitaxel in plasma and brain tissue homogenate

	Plasma (μg/ml)[a]					Brain tissue (μg/ml)[b]		
Concentration added	0.0360	0.147	0.599	2.44	9.96	0.0543	0.326	1.956
Concentration measured	0.0317	0.152	0.640	2.44	8.99	0.0533	0.325	1.794
S.D.	0.0027	0.017	0.014	0.12	0.53	0.0024	0.011	0.010
CV (%)	8.5	11.2	2.2	4.9	5.9	4.5	3.4	0.6
Bias (%)	−11.9	3.4	6.8	−0.0	−9.7	−1.8	−0.30	8.3

[a] $n = 5$.
[b] $n = 3$.

signal-to-noise ratio. No significant interference peaks were observed for blank plasma and blank brain homogenate tissues samples.

3.4. Method application

The analytical method was subsequently used to analyze samples for pharmacokinetic studies in mice bearing intracerebral tumors. Briefly, FVB and mdr1a/b −/− mice bearing an intracerebral B-16 melanoma were given paclitaxel at a dose of 10 mg/kg by intravenous bolus administration. Serial blood samples (40–80 μl) were normally collected from 2 min to 660 min following drug administration, from which plasma (20–50 μl) was harvested by centrifugation and then stored at −80 °C until analysis. For brain distribution studies, the animals were sacrificed at 180 min following administration of steady state paclitaxel dosing regimens, in which a constant infusion following a fast infusion of paclitaxel was given to reach the steady state at concentration of 5 μg/ml plasma, and the brain tissues were removed and stored at −80 °C prior to analysis.

Representative chromatograms of paclitaxel in plasma and brain tissue homogenate are shown in Figs. 1 and 2. The retention time of paclitaxel was 6.2 min with a total run time of 8 min required to avoid interfering peaks. There were no interfering peaks at the retention time of paclitaxel and internal standard in blank plasma and blank brain tissue samples.

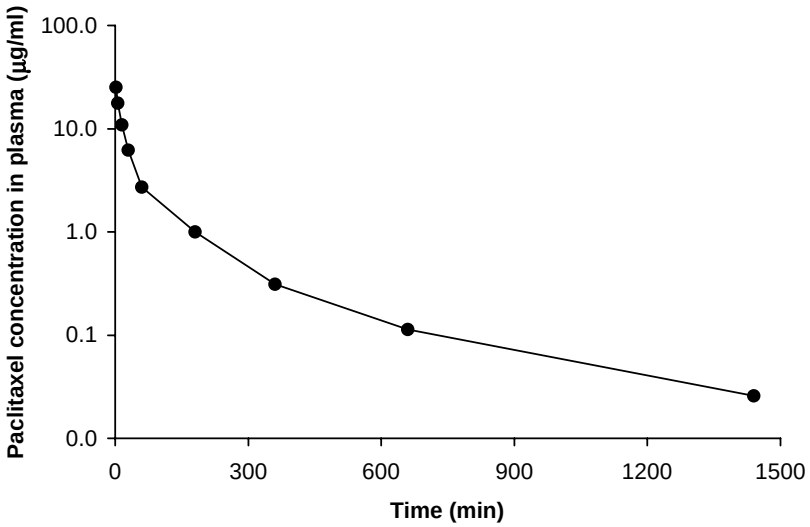

Fig. 3. Plasma concentration–time profile of paclitaxel in a mouse after intravenous administration of 10 mg/kg paclitaxel.

A typical concentration–time profile of paclitaxel in mouse plasma after intravenous administration of 10 mg/kg paclitaxel is shown in Fig. 3.

4. Conclusion

A validated and sensitive LC–MS method for the quantitation of paclitaxel in mouse plasma and brain tissue is presented. The unique features of the assay include the use of low sample volumes and a relatively rapid extraction procedure that did not require solvent evaporation. The assay percentage biases and CVs were less than 15% and readily reached quantitation limits of 36 ng/ml of paclitaxel for plasma and 54 ng/ml for brain tissue homogenate (equivalent to 0.54 μg/g brain tissues), respectively. The method was successfully applied in pharmacokinetic and brain tissue distribution studies in mice bearing brain tumors, a preclinical model that can be used to assess the role of P-glycoprotein on the brain tumor uptake of anticancer drugs. The assay is convenient and should be suitable for measuring paclitaxel in preclinical pharmacokinetics studies.

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