ELSEVIER

Journal of Chromatography B, 664 (1995) 383–391

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Determination of paclitaxel and metabolites in mouse plasma, tissues, urine and faeces by semi-automated reversed-phase high-performance liquid chromatography

Alexander Sparreboom[a,b,*], Olaf van Tellingen[b], Willem J. Nooijen[b], Jos H. Beijnen[a]

[a]*Department of Pharmacy, Slotervaart Hospital, Louwesweg 6, 1066 EC Amsterdam, Netherlands*
[b]*Department of Clinical Chemistry, Antoni van Leeuwenhoek Huis, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, Netherlands*

First received 20 July 1994; revised manuscript received 24 October 1994; accepted 25 October 1994

Abstract

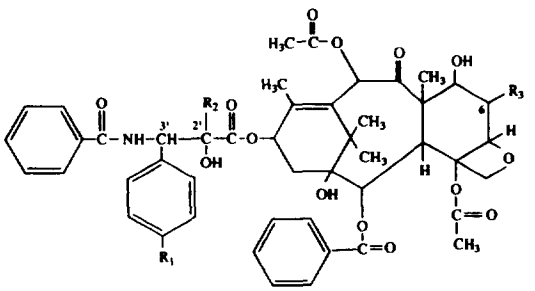
We have developed and validated a sensitive and selective assay for the quantification of paclitaxel and its metabolites 6α,3′-*p*-dihydroxypaclitaxel, 3′-*p*-hydroxypaclitaxel and 6α-hydroxypaclitaxel in plasma, tissue, urine and faeces specimens of mice. Tissue and faeces were homogenized (approximately 0.1–0.2 g/ml) in bovine serum albumin (40 g/l) in water, and urine was diluted (1:5, v/v) in blank human plasma. Sample pretreatment involved liquid–liquid extraction of 200–1000 μl of sample with diethyl ether followed by automated solid-phase extraction using cyano Bond Elut columns. 2′-Methylpaclitaxel was used as internal standard. The overall recovery of the sample pretreatment procedure ranged from 76 to 85%. In plasma, the lower limit of detection (LOD) and the lower limit of quantitation (LLQ) are 15 and 25 ng/ml, respectively, using 200 μl of sample. In tissues, faeces and urine the LLQs are 25–100 ng/g, 125 ng/g and 25 ng/ml, respectively, using 1000 μl (faeces: 200 μl) of homogenized or diluted sample. The concentrations in the various biological matrices, for validation procedures spiked with known amounts of the test compounds, are read from calibration curves constructed in blank human plasma in the range 25–100 000 ng/ml for paclitaxel and 25–500 ng/ml for the metabolites. The accuracy and precision of the assay fall within the generally accepted criteria for bio-analytical assays.

1. Introduction

Paclitaxel [Taxol; 5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one-4,10-diacetate-2-benzoate-13-ester with (2*R*,3*S*)-N-benzoyl-3-phenylisoserine] (Fig. 1) is a taxane diterpene amide, that was first extracted from the stem bark of the western yew, *Taxus brevifolia* [1]. This natural product has proven to be useful in the treatment of a variety of human neoplastic disorders, including platinum-resistant

* Corresponding author. Address for correspondence: Department of Clinical Chemistry, Antoni van Leeuwenhoek Huis, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, Netherlands.

0378-4347/95/$09.50
SSDI 0378-4347(94)00495-1



compound	R_1	R_2	R_3
6α,3'-*p*-dihydroxypaclitaxel	OH	H	OH
3'-*p*-hydroxypaclitaxel	OH	H	H
6α-hydroxypaclitaxel	H	H	OH
paclitaxel	H	H	H
2'-methylpaclitaxel	H	CH_3	H

Fig. 1. Molecular structures of paclitaxel, metabolites and 2'-methylpaclitaxel (internal standard for HPLC).

ovarian cancer [2], breast [3] and non-small cell lung cancer [4], and leukemia [5]. The mode of action of paclitaxel is believed to stem from its unique propensity to promote microtubule formation and inhibit post-mitotic spindle disassembly [6–8]. In spite of the great interest in this drug, which is reflected in the substantial number of clinical reports on paclitaxel (reviewed in Ref. [9]), knowledge about its pharmacokinetic behavior, in particular tissue distribution and metabolism, is scanty. Due to the limitations, for known reasons, in the acquisition of human tissues, systematic tissue distribution studies are mainly performed in animal models. A sensitive assay for the drug and its metabolites is warranted to obtain a clear picture of the pharmacokinetic behavior, since paclitaxel exerts its cytotoxic activity at concentrations as low as 50 n*M* (43 ng/ml) [10]. Although several analytical methods have been described, including a tubulin-binding biochemical assay [11], immunoassays [12,13], a multimodal thin-layer chromatographic method [14] and assays based on high-performance liquid chromatography (HPLC) [15–25], these are not suited for quantitation in tissue specimens.

Recently, a highly sensitive and specific procedure was developed by Willey et al. [26] for the analysis of paclitaxel in plasma and urine of cancer patients. The method involves solid-phase extraction with cyano Bond Elut columns for sample clean-up, and reversed-phase HPLC with UV detection at 227 nm. We have extended the usefulness of this assay and validated it for the determination of paclitaxel and its three major metabolites 6α,3'-*p*-dihydroxypaclitaxel (I), 3'-*p*-hydroxypaclitaxel (II), and 6α-hydroxypaclitaxel (III) in all relevant mouse biological matrices, by the introduction of a liquid–liquid extraction procedure.

2. Experimental

2.1. Chemicals

Paclitaxel (batch 80617492D) and 2'-methylpaclitaxel were used as supplied by the Bristol-Myers Squibb Company (Princeton, NJ, USA). Metabolites 6α,3'-*p*-dihydroxypaclitaxel (I), 3'-*p*-hydroxypaclitaxel (II) and 6α-hydroxypaclitaxel (III) were isolated and purified from human faeces samples, obtained from patients treated with 135 or 175 mg/m^2 paclitaxel in a 3 or 24 h intravenous infusion, in our laboratory according to a method described in detail previously [27]. In brief, the isolation of the mono- and dihydroxylated paclitaxel derivatives was achieved using diethyl ether extraction of homogenized human faeces samples followed by semi-preparative HPLC. On-line photodiode-array detection and fast-atom bombardment mass spectrometry were applied for structure confirmation. Boseral (lyophilized bovine albumin powder) was purchased from Organon Teknika B.V. (Boxtel, Netherlands). Other chemicals and solvents were of analytical or LiChrosolv gradient grade, and were purchased from E. Merck (Darmstadt, Germany). Filtered and deionised water, obtained from a Milli-Q

Plus system (Millipore, Milford, MA, USA), was used throughout the study.

2.2. Mouse samples

Female FVB mice (weight 24–30 g, aged 10–14 weeks), were lightly anaesthetized with diethyl ether, exsanguinated and blood specimens were collected in heparinized tubes. The blank samples were centrifuged at 2100 *g* for 10 min using a Beckman Model J-6B/P centrifuge (Palo Alto, CA, USA) at 0°C, and the plasma fraction was stored at −20°C. Blank tissues, including brains, abdominal fat, muscle, breast, organ fat, colon, appendix, small intestine, stomach, liver, gall-bladder, kidneys, lungs, spleen, heart, ovaries, uterus, thymus and lymph nodes, were rapidly dissected from the animals. Adhering fat and other visceral debris were removed carefully from the organs with surgical tweezers. After weighing in polystyrene tubes the tissues were homogenized in 4% Boseral (w/v in water) using a Biospec Products tissue homogenizer (Bartlesville, OK, USA) at 4°C, and were stored at −20°C until analysis. About 1 ml of Boseral solution was used per 0.1–0.2 g of tissue. Urine and faeces were collected at ambient temperature from FVB mice housed in metabolic cages. Urine was diluted with blank human plasma (1:5, v/v) obtained from healthy volunteers, and was frozen at −20°C. Faeces samples were homogenized in Boseral solution (0.1–0.2 g faeces per ml) similar to the procedures described above for tissues. To demonstrate the applicability of the analytical method, samples of one mouse were collected after administration of 2 mg/kg of Taxol by intravenous bolus injection.

2.3. Sample pretreatment

Frozen samples were thawn in a 4°C waterbath and were homogenized by vortex-mixing. The internal standard 2′-methylpaclitaxel (25 μl of 20 μg/ml in methanol) was added to 200 μl of plasma or faeces suspension, 200–1000 μl of tissue homogenate, or 1000 μl plasma-diluted urine in 10-ml glass tubes supplied with PTFE-covered screw caps. The samples were extracted with 4 ml of diethyl ether. The tubes were mixed vigorously for 5 min, followed by centrifugation at 460 *g* for 5 min. Next, the aqueous layer was frozen in ethanol–solid carbon dioxide and the organic layer was decanted into a clean glass tube. The aqueous layer was thawn, and the extraction procedure was repeated once again. The diethyl ether fractions were combined and evaporated by vacuum concentration in a Speed-Vac Plus SC210A system (Savant, Farmingdale, NY, USA) at 43°C. The residue was reconstituted in 250 μl of blank human plasma by sonication for 3 min and vortex-mixing for 30 s. Next, a modified solid-phase extraction (SPE) procedure, as reported by Willey et al. [26] was used employing 1 ml/100 mg cyano Bond Elut columns (batch 131795; Varian, Harbor City, CA, USA). All steps were performed automatically by an automated sample preparation with extraction columns system (ASPEC; Gilson Medical, Middleton, WI, USA). The SPE columns were conditioned with 2 ml of methanol and 2 ml of 0.01 *M* ammonium acetate buffer (pH 5.0). The samples were mixed with 300 μl of 0.2 *M* ammonium acetate buffer (pH 5.0) and a volume of 500 μl was then loaded on the SPE column and washed with 2 ml of 0.01 *M* ammonium acetate buffer (pH 5.0) and 1 ml of 20% (v/v) methanol in 0.01 *M* ammonium acetate buffer (pH 5.0). Elution was performed in 500 μl of acetonitrile–triethylamine (1000:1, v/v) into 0.7-ml polypropylene microtubes (Eppendorf, Hamburg, Germany). Samples were dried by vacuum concentration, and redissolved in 200 μl of acetonitrile–methanol–water (40:10:50, v/v) by vortex-mixing for 30 s. Samples of 50 μl were injected onto the HPLC system.

2.4. HPLC instrumentation and conditions

The HPLC equipment consisted of a Spectroflow SF400 solvent delivery system (Kratos, Ramsey, NJ, USA), a Model MSI660 autosampler (Kontron, Basel, Switzerland), a Spectoflow SF757 absorbance detector (Kratos), and a Model 345 solvent recycler (Alltech, Deerfield,

IL, USA). Peak detection and integration was performed with a SP4600 DataJet integrator connected to a WINner/286 data station (Spectra Physics, San Jose, CA, USA). A stainless steel (150 × 4.6 mm I.D.) analytical column packed with 5 μm APEX-octyl material (Jones Chromatography, Littleton, CO, USA) was used. Paclitaxel and the metabolites were eluted at ambient temperature with a mobile phase comprised of acetonitrile–methanol–0.2 *M* ammonium acetate buffer (pH 5.0) (40:10:50, v/v) using a flow-rate of 1 ml/min. Ultraviolet (UV) detection was performed at a wavelength of 227 nm, and a rise time of 1 s.

2.5. Calibration

Standard solutions of paclitaxel and the metabolites I, II and III were prepared separately by dissolving about 1 mg powder, accurately weighted, in 1.00 ml of absolute ethanol. A mixture of all four compounds was obtained by dilution of the standard solutions in ethanol, yielding a final concentration of 50 μg/ml. For determination of paclitaxel and metabolite concentrations between 25 and 500 ng/ml, the standard stock solution was diluted in blank human plasma by addition of an appropriate volume of the ethanolic standard, so that the same amount of ethanol was added to each plasma calibration sample. Dilutions were prepared containing 25, 50, 100, 250 and 500 ng/ml. A second calibration curve, used for paclitaxel concentrations between 500 and 100 000 ng/ml, was constructed from the 1 mg/ml paclitaxel stock in human plasma containing concentrations of 300, 1000, 3000, 10 000, 30 000 and 100 000 ng/ml. Calibration curves were computed using the ratio (y) of the peak area of paclitaxel (or metabolite) and the internal standard, and by using weighted ($1/y$) least squares linear regression analysis.

2.6. Validation

A validation run of mouse plasma samples included both calibration curves in duplicate and determination of quality control samples at three concentrations (50, 500, 3000 ng/ml) for paclitaxel and at two concentrations (50, 500 ng/ml) for the metabolites in quadruplicate. This validation procedure was performed at three separate days. Blank samples of the various tissue homogenates, urine, or faeces suspension were spiked at 50 and 500 ng/ml with the mixture of paclitaxel and the metabolites for evaluation of precision and accuracy by analysis over several days with repeated freeze–thaw cycles of the samples. The precision and accuracy of the assay were assessed by the between-day (BDP) and within-day (WDP) precision and percentage deviation (%Dev) from the nominal concentration, respectively. An estimate of the precision was obtained using one-way analysis of variance (ANOVA), by calculating values for the day mean square (DayMS), the error mean square (ErrMS) and the grand mean (GM). The between-day precision was calculated as:

Component of between-day precision

$$= \{((\text{DayMS} - \text{ErrMS})/n)^{0.5}/\text{GM}\} \cdot 100 \quad (1)$$

where n represents the number of replicates within each run. The within-day precision was calculated as:

Component of within-day precision

$$= \{(\text{ErrMS})^{0.5}/\text{GM}\} \cdot 100 \quad (2)$$

The limit of detection (LOD), defined as the lowest concentration that the analytical process can reliably differentiate from background levels, was estimated at a signal-to-noise ratio of three. The concentration of the lowest standard in the analytical run quantitated with a definite level of certainty, i.e. the lower limit of quantitation (LLQ), was accepted when the values for %Dev and within-day precision were less than 20%.

The recoveries of the analytes and the internal standard, measured in three analytical runs were determined by comparing the peak areas obtained from direct injections of standard solutions with those obtained in samples subjected to the complete extraction procedure.

3. Results and discussion

Due to the potency of paclitaxel, there are high demands on the sensitivity of bio-analytical procedures for pharmacokinetic investigations. Based on the results from cytotoxicity tests [10] and from clinical pharmacokinetic studies [22] detection limits below 50 n*M* (43 ng/ml) are required. Because the chromophore of the paclitaxel molecule lacks any useful absorbance at higher wavelengths and the compound is also not equipped with functional groups enabling electrochemical detection, the use of a UV detector operating at low wavelengths (227 nm) is mandatory for detection down to the ng/ml range. However, as many endogenous substances also demonstrate a strong absorbance in this UV region, a very selective sample pretreatment procedure is needed. Many studies reported so far have used a combination of reversed-phase HPLC and liquid–liquid extraction [16] or acetonitrile precipitation [15,17,18,24]. In all cases the LLQ of these assays is insufficient. Recently, Willey et al. [26] have described a very selective sample pretreatment procedure based on SPE with cyano Bond Elut columns offering a LLQ of 10 ng/ml in human plasma.

However, SPE cannot be used for turbid specimens like tissue homogenates, because the extraction column will become blocked by the particles present in these samples. In such cases liquid–liquid extraction is an appropriate alternative. To enable the sensitive and selective determination of paclitaxel in all relevant biological matrices, we have utilized liquid–liquid extraction with diethyl ether for the primary isolation of the compounds. After evaporation of the organic extraction solvent the residue was dissolved in blank human plasma. This enabled us to process the samples as if they were human plasma samples by SPE on cyano columns according to a (slightly modified) procedure reported by Willey et al. [26]. Due to the turbidity, which occurred in extracts originating from organs containing relatively large amounts of fat some time after the addition of the 0.2 *M* ammonium acetate buffer, the buffer solution was mixed with the sample just prior to loading onto the SPE column. Other modifications from the method of Willey et al. [26] included the deletion of the washing step with *n*-hexane and a reduction of the elution volume from 2000 to 500 μl of acetonitrile–triethylamine (1000:1, v/v). The SPE procedure has been automated by using the Gilson ASPEC and can be performed during overnight runs. This sample handling procedure also permits the simultaneous extraction of the three major human metabolites (6α,3′-*p*-dihydroxypaclitaxel, 3′-*p*-hydroxypaclitaxel and 6α-hydroxypaclitaxel) and the related compound 2′-methylpaclitaxel (used as internal standard) with similar recoveries.

To save expenses, SPE columns were re-used for an additional two times without any loss of performance. After each run, the SPE columns were washed with 2 ml of acetonitrile–triethylamine (1000:1, v/v), 2 ml of acetonitrile–water (50:50, v/v) and 2 ml of acetonitrile consecutively, and then allowed to dry.

Linear calibration curves were obtained over the concentration ranges tested, i.e. 25–100 000 ng/ml for paclitaxel and 25–500 ng/ml for each of the metabolites, with regression correlation coefficients ≥ 0.995. By using weighted $(1/y)$ linear regression analysis, deviations of the interpolated concentrations of all standard samples were always within the acceptable 85–115% range. The intercept values of the standard curves for all compounds were not significantly different from zero by Student's t-test ($p > 0.05$).

Chromatograms of a blank and spiked plasma samples containing 500 ng/ml are shown in Fig. 2, while Fig. 3 depicts a blank liver sample and a liver sample obtained after intravenous administration of 2 mg/kg Taxol. In the latter, metabolites II and III were detected. The retention times for metabolites I, II, III, paclitaxel and 2′-methylpaclitaxel are 4.0, 5.1, 7.4, 10.6 and 15.3 min, respectively. The selectivity for all analytes is shown by the sharp resolution of the peaks and no interfering peaks in the drug-free sample. No interferences with the LLQ or quantitation of paclitaxel and metabolites have been observed in any of the biological matrices. However, some organs like colon, contained

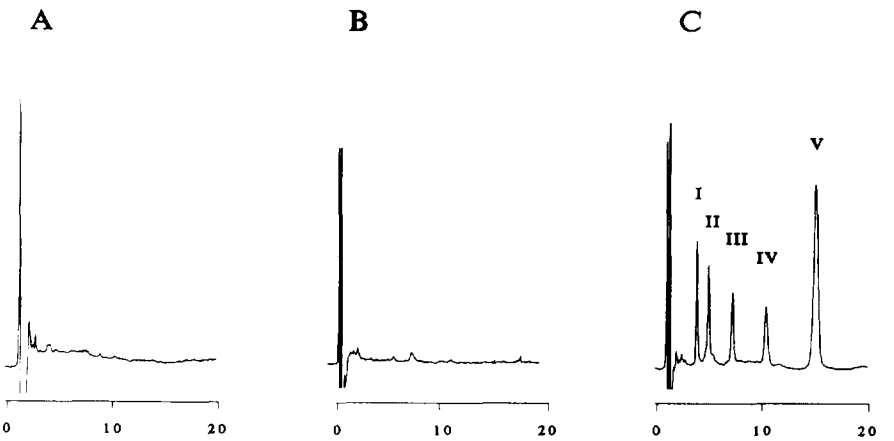


Fig. 2. HPLC chromatograms of blank control plasma (A), blank mouse plasma (B) and spiked (500 ng/ml) mouse plasma (C). Peaks labelled I, II, III, IV and V correspond to metabolites I, II, III, paclitaxel and 2'-methylpaclitaxel, respectively. x-Axis is retention time (min).

endogenous substances that eluted after 2'-methylpaclitaxel. Therefore the total run time is 30 min.

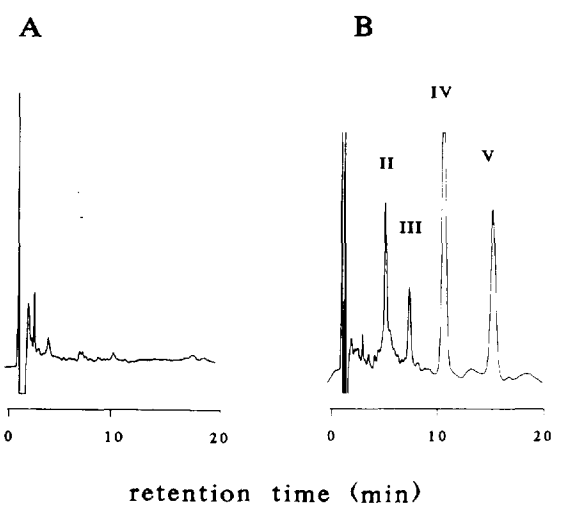


Fig. 3. HPLC chromatograms of a blank liver sample (A) and a liver sample taken 1 h after administration of 2 mg/kg Taxol as an intravenous bolus dose (B). Peaks labelled II, III, IV and V correspond to metabolites II, III, paclitaxel and 2'-methylpaclitaxel, respectively.

The LODs for paclitaxel and metabolites I, II and III were approximately 15 ng/ml for the various biological matrices tested. The LLQ was 25 ng/ml for the four compounds. The higher value of the LLQ for paclitaxel as compared to 10 ng/ml reported for paclitaxel determination in human plasma utilizing a similar clean-up and HPLC procedure [26], was a consequence of the use of a proportionally smaller sample volume (200 μl vs. 500 μl) for the extraction from mouse plasma.

Paclitaxel as well as metabolites I, II and III were found to be stable in all mouse samples investigated for at least three weeks, when homogenized in Boseral solution and stored at −20°C, with repeated freeze–thaw cycles. The between-day and within-day precision for paclitaxel and metabolites varied upto 7.3% for plasma (50, 500 and 3000 ng/ml), upto 14.0% for tissues (50 and 500 ng/ml), and upto 11.0% for faeces and urine (50 and 500 ng/ml) (Table 1). The accuracy for all analytes showed values ranging within ± 14% of the nominal values in the various matrices.

Extraction recoveries were concentration independent and ranged from 76 to 85% for all materials.

Table 1
Accuracy (%Dev), within-day precision [WDP(%)] and between-day precision [BDP(%)] of 6α,3′-*p*-dihydroxypaclitaxel, 3′-*p*-hydroxypaclitaxel, 6α-hydroxypaclitaxel and paclitaxel in mouse samples

Sample	Sample volume (μl)	Concentration in homogenate (ng/ml)	Calculated concentration (ng/g)	6,3′-*p*-Dihydroxypaclitaxel				3′-*p*-Hydroxypaclitaxel				6-Hydroxypaclitaxel				Paclitaxel			
				%Dev	WDP(%)	BDP(%)	*n*	%Dev	WDP(%)	BDP(%)	*n*	%Dev	WDP(%)	BDP(%)	*n*	%Dev	WDP(%)	BDP(%)	*n*
Plasma	200	50	–	+4.9	6.8	7.1	12	+8.1	7.3	6.5	12	+4.4	3.8	3.1	12	−7.7	1.2	3.2	12
	200	500	–	−10.5	5.9	3.6	12	+4.2	1.1	0.5	12	−3.1	6.5	1.9	12	+1.0	1.4	3.1	12
	200	3000	–	N.D.	N.D.	N.D.	–	N.D.	N.D.	N.D.	–	N.D.	N.D.	N.D.	–	+0.4	3.4	1.6	12
Brain	1000	50	50	−3.5	5.4	2.5	6	−2.5	8.6	5.8	6	+2.5	6.5	4.3	6	−9.5	5.7	4.6	6
	200	50	250	−7.1	7.7	8.6	6	−10.1	12.7	10.3	6	−6.3	5.3	4.7	6	−10.3	8.7	9.7	6
	200	500	2500	−4.6	6.2	4.7	6	−4.6	9.7	4.6	6	7.3	7.4	6.9	6	−1.2	1.6	4.1	6
Abdominal fat	1000	50	100	−4.3	5.6	4.6	6	−6.8	8.6	5.1	6	+1.5	6.3	4.8	6	−5.8	6.5	5.7	6
	200	50	500	−10.7	9.8	5.6	6	−9.4	13.5	13.9	6	−8.7	9.6	8.5	6	−9.7	4.4	4.1	6
	200	500	5000	4.1	6.7	1.8	6	−5.1	4.7	0.5	6	−6.7	4.1	5.2	6	+1.2	6.8	3.2	6
Muscle	1000	50	100	−5.6	6.5	4.3	6	−2.1	9.4	8.5	6	+4.6	5.2	1.1	6	−8.6	8.6	7.3	6
	200	50	500	−8.6	5.3	9.6	6	−9.9	7.7	10.4	6	−9.5	7.3	8.5	6	−10.4	10.7	11.4	6
	200	500	5000	−4.3	9.1	2.9	6	−4.1	7.8	4.6	6	−1.2	0.9	3.4	6	−2.8	5.4	3.9	6
Breast	1000	50	100	−1.2	5.6	4.3	6	−2.5	6.8	8.6	6	−1.1	1.9	1.1	6	−5.6	4.2	2.3	6
	200	50	500	−3.6	6.7	5.9	6	−7.7	12.1	12.6	6	+6.4	5.3	4.6	6	+6.3	7.7	8.6	6
	200	500	5000	−0.3	4.9	3.4	6	−3.6	5.1	2.4	6	+0.4	4.4	3.1	6	0.3	4.4	2.4	6
Organ fat	1000	50	100	−0.3	6.5	4.6	6	+3.5	6.8	5.6	6	+1.2	5.2	2.5	6	+5.2	2.1	2.1	6
	200	50	500	−3.3	5.7	8.4	6	−2.6	5.3	8.4	6	−2.1	4.7	6.3	6	−2.7	4.4	7.3	6
	200	500	5000	+2.9	7.8	7.8	6	−0.9	5.7	4.9	6	−2.1	5.5	3.3	6	−6.1	3.8	0.6	6
Colon	1000	50	100	−1.3	3.2	3.1	6	−2.3	3.2	5.2	6	−2.3	5.3	2.4	6	−1.2	2.1	1.1	6
	200	50	500	+4.6	5.6	8.2	6	−8.7	10.7	8.9	6	+4.1	8.6	7.1	6	−7.7	8.3	8.9	6
	200	500	5000	−0.3	3.8	2.6	6	−5.4	7.3	1.9	6	−6.7	6.5	2.7	6	−6.6	1.5	3.2	6
Appendix	1000	50	100	−5.1	2.3	2.5	6	−2.5	9.6	8.5	6	+6.5	5.6	4.3	6	5.6	4.6	7.2	6
	200	50	500	+5.2	6.5	4.3	6	+6.7	9.5	9.6	6	+4.3	6.6	7.2	6	+7.6	4.7	8.6	6
	200	500	5000	+0.4	0.3	0.5	5	−2.4	1.1	4.1	5	−3.9	4.5	2.4	5	−5.1	4.8	2.3	5
Small intestine	1000	50	50	−4.8	5.6	4.2	6	−2.1	5.3	2.3	6	−5.6	7.8	5.9	6	+9.8	8.9	10.2	6
	200	50	250	−3.7	8.7	6.5	6	−4.7	5.2	6.1	6	+5.6	10.7	13.7	6	+11.7	5.6	4.9	6
	200	500	2500	+1.9	4.8	3.1	6	+3.9	1.9	3.5	6	−5.9	2.5	2.9	6	+2.5	3.9	3.2	6
Stomach	1000	50	100	−3.1	9.8	7.8	6	−2.3	5.6	4.2	6	+3.2	6.3	5.3	6	−4.3	2.1	1.3	6
	200	50	500	−10.2	8.7	3.2	6	−8.7	10.2	9.5	6	−11.4	9.6	8.7	6	−9.9	7.4	8.6	6
	200	500	5000	−2.1	4.5	0.9	6	−0.8	5.7	4.9	6	+0.1	7.2	4.4	6	+2.2	3.3	0.7	6
Liver	1000	50	50	−4.5	3.2	0.2	6	+0.5	5.2	3.8	6	+2.6	5.3	4.9	6	−6.9	5.8	5.8	6
	200	50	250	−4.4	8.7	9.1	6	−2.2	5.6	5.9	6	−0.4	7.6	4.3	6	−4.7	7.9	7.7	6
	200	500	2500	−6.5	5.6	0.9	6	−8.8	1.1	2.5	6	−5.7	0.9	3.5	6	+3.1	5.9	1.8	6

Table 1 (*continued*)

Sample	Sample volume (μl)	Concentration in homogenate (ng/ml)	Calculated concentration (ng/g)	6,3′-*p*-Dihydroxypaclitaxel				3′-*p*-Hydroxypaclitaxel				6-Hydroxypaclitaxel				Paclitaxel			
				%Dev	WDP(%)	BDP(%)	*n*	%Dev	WDP(%)	BDP(%)	*n*	%Dev	WDP(%)	BDP(%)	*n*	%Dev	WDP(%)	BDP(%)	*n*
Gall-bladder	1000	50	1000	−5.3	3.4	0.2	6	−0.1	5.9	1.2	6	+9.9	4.5	1.2	6	+2.3	5.6	1.3	6
	200	50	5000	+2.5	6.8	7.6	4	−2.6	2.2	4.1	4	+8.7	7.9	2.3	4	−6.8	1.3	4.3	4
	200	500	50 000	−6.7	2.9	2.4	4	−3.4	4.1	3.9	4	−0.8	0.6	3.1	4	−6.6	3.1	1.9	4
Kidneys	1000	50	100	−6.5	2.5	6.5	6	−2.6	4.5	2.3	6	+6.9	8.9	7.3	6	−2.5	5.3	4.1	6
	200	50	500	−12.1	6.6	5.5	6	−5.9	5.8	8.5	6	−10.7	11.6	7.6	6	−7.3	5.3	1.4	6
	200	500	5000	−9.1	3.9	4.2	6	−2.2	4.7	1.9	6	−0.1	4.2	1.6	6	+2.1	5.7	2.1	6
Lungs	1000	50	100	−5.5	9.8	10.2	6	−1.2	5.6	4.3	6	−6.5	8.4	2.3	6	+3.6	5.3	4.3	6
	200	50	500	+4.6	8.7	7.7	6	−5.6	8.9	7.9	6	−7.6	10.7	4.4	6	−7.9	6.6	5.6	6
	200	500	5000	−3.7	1.9	3.7	6	−7.8	3.6	3.6	6	−5.6	3.4	1.3	6	−1.2	1.9	2.1	6
Spleen	1000	50	100	−3.6	5.6	4.3	6	−5.6	9.8	2.6	6	−4.5	5.6	8.4	6	−5.7	9.8	4.9	6
	200	50	500	−5.1	6.5	7.6	6	−10.7	4.6	5.9	6	−3.2	5.3	1.3	6	−0.7	4.3	4.9	6
	200	500	5000	−1.6	6.1	4.6	6	+0.4	1.9	0.6	6	+1.5	4.9	3.7	6	+1.7	5.6	1.6	6
Heart	1000	50	100	−4.2	4.5	0.3	6	−4.6	5.1	3.2	6	−8.9	4.6	5.3	6	−8.1	5.6	4.7	6
	200	50	500	+1.7	5.5	4.6	6	+8.7	5.9	2.7	6	+2.6	8.6	7.7	6	−2.2	2.4	0.6	6
	200	500	5000	+11.2	3.8	5.7	5	−4.7	5.6	1.9	5	−4.4	10.5	2.5	5	−5.4	4.4	1.7	5
Uterus	1000	50	200	−2.5	8.8	6.39	6	−3.4	2.6	0.3	6	+1.5	5.8	6.1	6	−3.6	6.2	5.8	6
	200	50	1000	−4.7	8.7	9.5	6	−7.8	7.8	5.4	6	−6.6	4.4	3.3	6	−7.4	1.4	0.4	6
	200	500	10 000	+1.3	5.5	3.9	6	−5.8	3.4	2.3	6	−2.7	4.9	3.2	6	−3.5	4.9	0.6	6
Ovary	1000	50	200	−5.1	0.3	0.1	6	+2.6	6.5	4.9	6	−2.3	2.1	5.4	6	−3.5	1.2	1.1	6
	200	50	1000	−8.7	5.7	4.9	6	−7.7	5.1	4.2	6	−5.7	5.3	1.3	6	−7.7	8.3	10.3	6
	200	500	10 000	+1.9	2.7	4.1	6	+1.1	3.1	4.2	6	−1.7	2.5	3.9	6	−1.6	2.9	3.4	6
Thymus	1000	50	100	−1.2	4.2	3.2	6	−1.5	2.5	0.2	6	−1.6	4.4	1.2	6	−2.5	2.5	1.9	5
	200	50	500	−3.2	5.7	8.5	6	−3.6	4.7	5.7	6	+4.1	3.3	5.4	6	−5.4	4.7	5.8	6
	200	500	5000	+0.8	2.2	1.5	6	−3.8	3.8	2.3	6	−1.5	4.4	2.3	6	+1.8	5.5	2.4	6
Lymph nodes	1000	50	100	−1.3	4.4	4.3	6	−8.6	4.6	3.8	6	−0.4	0.9	0.8	6	−9.9	9.9	5.7	6
	200	50	500	+4.2	2.4	3.2	6	−10.7	10.7	12.9	6	−8.7	14.1	8.7	6	−10.1	5.7	4.7	6
	200	500	5000	−4.2	1.6	2.1	6	+3.9	4.3	1.1	6	−2.6	2.1	2.2	6	+2.4	1.7	2.1	6
Urine	1000	50	–	−13.3	6.5	3.9	6	−4.7	2.1	1.7	6	+1.1	2.2	4.4	6	−4.7	3.7	1.7	6
	1000	500	–	−8.3	2.7	1.2	6	−3.8	3.5	2.2	6	−1.8	3.3	0.6	6	−4.5	1.1	1.2	6
Faeces	200	50	250	−8.6	9.5	5.4	6	+3.7	9.4	10.9	6	5.6	10.9	9.7	6	+6.7	9.2	9.9	6
	200	500	2500	−5.3	3.6	0.8	6	−6.9	4.2	3.2	6	−0.1	7.1	1.4	6	−7.4	4.3	2.6	6

N.D. = not determined.

4. Conclusions

A sensitive, selective and reproducible assay for the simultaneous determination of paclitaxel and its metabolites 6α,3′-*p*-dihydroxypaclitaxel, 3′-*p*-hydroxypaclitaxel and 6α-hydroxypaclitaxel in several biological matrices from mice has been described. The method involves a solvent extraction followed by an automated solid-phase extraction using a sample robot, that permits the analysis of large numbers of samples. The presented HPLC procedure will be implemented in future studies on murine metabolism and pharmacokinetics of paclitaxel.

Acknowledgement

Thanks are due to Ton Schrauwers for valuable biotechnical assistance.

References

[1] M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon and A.T. McPhail, *J. Am. Chem. Soc.*, 93 (1971) 2325.
[2] W.P. McGuire, E.K. Rowinsky, N.B. Rosenheim, F.C. Grumbine, D.S. Ettinger, D.K. Armstrong and R.C. Donehower, *Ann. Intern. Med.*, 111 (1989) 273.
[3] F.A. Holmes, R.S. Walters, R.L. Theriault, A.D. Forman, L.K. Newton, M.N. Raber, A.U. Buzdar, D.K. Frye and G.N. Hortobagyi, *J. Natl. Cancer Inst.*, 83 (1991) 1797.
[4] A. Chang, K. Kim, J. Glick, T. Anderson, D. Karp and D. Johnson, *Proc. Am. Soc. Clin. Oncol.*, 11 (1992) 293.
[5] E.K. Rowinsky, P.J. Burke, J.E. Karp, R.W. Tucker, D.S. Ettinger and R.C. Donehower, *Cancer Res.*, 49 (1989) 4640.
[6] P.B. Schiff, J. Fant and S.B. Horwitz, *Nature*, 277 (1979) 665.
[7] S.B. Horwitz, L. Lothstein, J.J. Manfredi, W. Mellado, J. Parness, S.N. Roy, P.B. Schiff, L. Sorbata and R. Zeheb, *Ann. N.Y. Acad. Sci.*, 466 (1986) 733.
[8] E.K. Rowinsky, L.A. Cazenave and R.C. Donehower, *J. Natl. Cancer Inst.*, 82 (1990) 1247.
[9] E.K. Rowinsky and R.C. Donehower, *Sem. Oncol.*, 20 (Suppl. 3) (1993) 16.
[10] J.E. Liebmann, J.A. Cook, C. Lipschultz, D. Teague, J. Fisher and J.B. Mitchell, *Br. J. Cancer*, 68 (1993) 1104.
[11] E. Hamel, C.M. Lin and D.G. Johns, *Cancer Treat. Rep.*, 66 (1982) 1381.
[12] J.-G. Leu, B.-X. Chen, P.B. Schiff and B.F. Erlanger, *Cancer Res.*, 53 (1993) 1388.
[13] P.G. Grothaus, T.J.G. Raybould, G.S. Bignami, G.B. Lazo and J.B. Byrnes, *J. Immunol. Methods*, 158 (1993) 158.
[14] M.W. Stasko, K.M. Witherup, T.J. Ghiorzi, T.G. McCloud, S. Look, G.M. Muschik and H.J. Issaq, *J. Liq. Chromatogr.*, 12 (1989) 2133.
[15] J.L. Grem, K.D. Tutsch, K.J. Simon, D.B. Alberti, J.K.V. Wilson, D.C. Tormey, S. Swaninathan and D.L. Trump, *Cancer Treat. Rep.*, 71 (1987) 1179.
[16] S.M. Longnecker, R.C. Donehower, A.E. Cates, T.-L. Chen, R.B. Brundrett, L.B. Grochow, D.S. Ettinger and M. Colvin, *Cancer Treat. Rep.*, 71 (1987) 53.
[17] P.H. Wiernik, E.L. Schwartz, J.J. Strauman, J.P. Dutcher, R.B. Lipton and E. Paietta, *Cancer Res.*, 47 (1987) 2486.
[18] P.H. Wiernik, E.L. Schwartz, A. Einzig, J.J. Strauman, R.B. Lipton and J.P. Dutcher, *J. Clin. Oncol.*, 5 (1987) 1232.
[19] K.M. Witherup, S.A. Look, M.W. Stasko, T.G. McCloud, H.J. Issaq and G.M. Muschik, *J. Liq. Chromatogr.*, 12 (1989) 2117.
[20] T. Brown, K. Havlin, G. Weiss, J. Cagnola, J. Koeller, J. Kuhn, J. Rizzo, J. Craig, J. Phillips and D. von Hoff, *J. Clin. Oncol.*, 9 (1991) 1261.
[21] M.E Wall, M.C. Wani and M.J. Abreo, *Second National Cancer Institute Workshop on Taxol and Taxus*, Alexandria, VA, 1992 (abstr).
[22] M.T. Huizing, A.C.F. Keung, H. Rosing, V. van der Kuij, W.W. ten Bokkel Huinink, I.M. Mandjes, A.C. Dubbelman, H.M. Pinedo and J.H. Beijnen, *J. Clin. Oncol.*, 11 (1993) 2127.
[23] C.A. Jamis-Dow, R.W. Klecker, G. Sarosy, E. Reed and J.M. Collins, *Cancer Chemother. Pharmacol.*, 33 (1993) 48.
[24] J. Leslie, J.M. Kujawa, N. Eddington, M. Egorin and J. Eiseman, *J. Pharm. Biomed. Anal.*, 11 (1993) 1349.
[25] J. Rizzo, C. Riley, D. von Hoff, J. Kuhn, J. Phillips and T. Brown, *J. Pharm. Biomed. Anal.*, 8 (1990) 159.
[26] T.A. Willey, E.J. Bekos, R.C. Gaver, G.F. Duncan, L.K. Tay, J.H. Beijnen and R.H. Farmen, *J. Chromatogr.*, 621 (1993) 231.
[27] A. Sparreboom, M.T. Huizing, J.J.B. Boesen, W.J. Nooijen, O. van Tellingen and J.H. Beijnen, *Cancer Chemother. Pharmacol.*, (in press).