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Journal of Chromatography B, 696 (1997) 89–98

JOURNAL OF CHROMATOGRAPHY B

Bio-analysis of docetaxel and hydroxylated metabolites in human plasma by high-performance liquid chromatography and automated solid-phase extraction

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Received 3 December 1996; revised 5 March 1997; accepted 24 March 1997

Abstract

A semi-automated reversed-phase high-performance liquid chromatography (HPLC) method has been developed and validated for the quantification of the novel anticancer drug docetaxel in human plasma. The chromatographic system also separated putative hydroxylated metabolites. A limited validation was performed for the assay of the metabolites while these reference compounds were not available in large quantities. The sample pretreatment of the plasma samples involves a solid-phase extraction (SPE) on Cyano end-capped columns. 2′-Methylpaclitaxel was used as internal standard. An APEX-octyl column (150×4.6 mm I.D.; particle size 5 μm) was used with acetonitrile–0.02 *M* ammonium acetate buffer pH 5 mixture as the mobile phase. UV detection was performed at 227 nm. In patient samples hydroxylated docetaxel metabolites were detected and quantified by using the docetaxel calibration curve. The accuracies and precisions of the assay fall within ±15% for all quality control samples and within ±20% for the lower limit of quantitation, which was 10 ng/ml using 1.00 ml of sample for both the parent drug and its metabolites. The overall recovery of the sample pretreatment procedure for docetaxel was 78.0%±5.8% and 84.8%±3.1% for the internal standard 2′-methylpaclitaxel. Docetaxel was found to be stable in human plasma at −30°C for at least 6 months. At ambient temperature docetaxel was stable for at most 15 h in human plasma. © 1997 Elsevier Science B.V.

Keywords: Docetaxel

1. Introduction

Docetaxel (RP 56976, Taxotere®, Fig. 1) is a semisynthetic paclitaxel analogue obtained from a precursor extracted from the needles of the *Taxus baccata L* [1]. The drug belongs to a new group of tubulin promoting and microtubulin stabilizing agents. In clinical studies docetaxel showed activity against several tumor types: breast, lung, head and neck and advanced platinum-refractory ovarian carcinomas [2]. From the analysis of bile it became clear that docetaxel is extensively metabolised by the liver [3,4], however no metabolites could be found in

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PII S0378-4347(97)00209-0

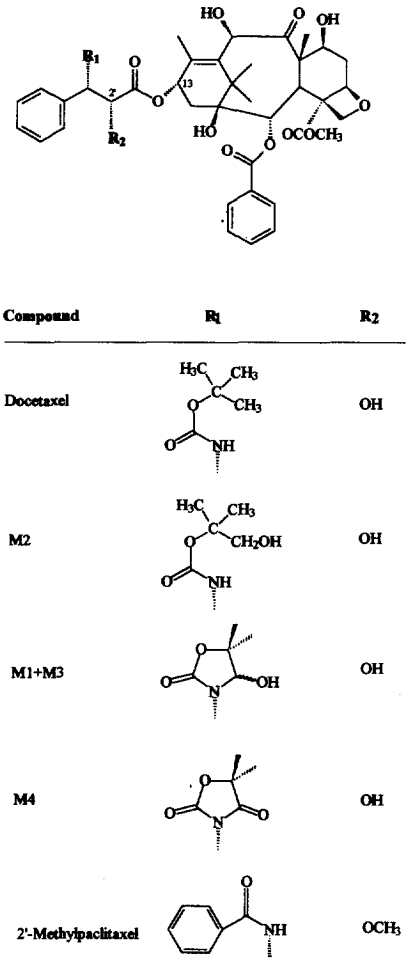

Fig. 1. The structures of docetaxel, its four major human hydroxylated metabolites and the internal standard 2′-methylpaclitaxel.

plasma of patients in early reported pharmacokinetics studies. Several metabolites of docetaxel have been identified in faeces of patients and bile from rats [5,6] as products originating from oxidation reactions of the *tert.*-butyl moiety in the C13-side chain of the parent drug (Fig. 1). The pharmacologic importance of these metabolites is largely unknown. So far, methods for the quantification of these compounds in human plasma have not been described. Only the analysis of the parent drug in human plasma by HPLC with UV detection using C2 solid-phase extraction columns have been reported [7]. However this sorbent is not suitable to retain the hydroxylated metabolites of docetaxel.

For the quantification of docetaxel in human plasma, an HPLC assay, utilizing an automated solid-phase extraction on cyano columns as a sample pretreatment procedure has been developed. 2′-Methylpaclitaxel (Fig. 1) was used as internal standard. This assay has been used in a pharmacokinetics phase II study whereby several metabolic products could be detected in plasma of patients with liver dysfunction. We have isolated and purified the metabolites from human faeces [8] to be used as reference in a limited validation of the HPLC assay. Here the development and validation of a bioanalytical method is described for the quantification of docetaxel in human plasma. Furthermore docetaxel calibration curves have been employed for the quantification of the metabolites in plasma in absence of these compounds as reference. As in early reported pharmacokinetics studies [9–15] no metabolites could be found in plasma of patients, a case study is presented in which hydroxylated metabolites of docetaxel were detected and quantified.

2. Experimental

2.1. Chemicals and reagents

Docetaxel (lot 14PROC 92320; purity 92.5%, Fig. 1) reference was obtained from Rhône-Poulenc Rorer (Antoni Cedex, France). The internal standard, 2′-methylpaclitaxel (lot 183061-01-002; purity 95%, Fig. 1), originated from Bristol Myers Squibb (Syracuse, NY, USA). Docetaxel metabolites (Fig. 1) were isolated and purified from patient faeces samples. The structure of these metabolites was confirmed by on-line photodiode array detection and mass spectrometry [8]. Acetonitrile (HPLC gradient grade) was obtained from Biosolve (Amsterdam, The Netherlands). Methanol (ChromAR®) was obtained from Promochem (Wesel, Germany). Ammonium acetate, glacial acetic acid and triethylamine (all analytical grade) were obtained from Merck (Darmstadt, Germany). Double distilled water was used throughout. Drug free human plasma originated from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands).

2.2. HPLC instrumentation and conditions

The HPLC system was composed of a P1000 pump and a AS3000 autosampler (Thermo Separation Products (TSP), Fremont, CA, USA). Chromatographic separation was conducted on an APEX-Octyl column (150×4.6 mm I.D., particle size 5 μm) (Jones Chromatography, Littleton, CO, USA). The mobile phase consisted of acetonitrile–0.02 *M* ammonium acetate buffer pH 5.0 (36.8:63.2, w/w). The flow-rate of the mobile phase was 1.0 ml/min and the eluent was monitored at 227 nm with a UV1000 detector which was coupled to a PC1000 data system (both TSP).

2.3. Preparation of stock solutions, working solutions and plasma standards

Docetaxel, 2′-methylpaclitaxel and metabolites M1, M2, M3 and M4 stock solutions were prepared separately in methanol at concentrations of 1000 μg/ml, 1000 μg/ml, 225 μg/ml, 220 μg/ml, 275 μg/ml and 680 μg/ml, respectively. The working solution of the internal standard was 10 μg/ml, prepared by a 100-fold dilution of the stock solution in methanol. All solutions were stored at −30°C and were stable for at least 6 months.

For the validation of the docetaxel assay, a plasma standard of 10 000 ng/ml was prepared by adding 40 μl of the docetaxel stock solution to 4000 μl of control human plasma. This plasma standard was further diluted in control human plasma to achieve analyte concentrations of 10, 50, 100, 500 and 1000 ng/ml.

For the validation of the assay for metabolite quantification, two mixtures of three reference compounds were prepared by dilution of the stock solutions in methanol, yielding analyte concentrations of 10 000 ng/ml: mixture 1 containing M1, M3 and docetaxel; mixture 2 containing M2, M4 and docetaxel. Subsequently, these mixtures were diluted separately in control human plasma to obtain analyte concentrations of 10, 50, 100, 250 and 500 ng/ml.

2.4. Sample processing

To 1200 μl plasma, 60 μl of the internal standard working solution (10 μg/ml) was added. The samples were mixed for 20 s and centrifuged at 9500 *g* for 10 min. A 1100 μl sample volume was pipetted into 3.0 ml polypropylene sample tubes. A fully automated solid-phase extraction on 100 mg IST endcapped cyano columns (Sopachem BV, Niewegein, The Netherlands) was performed using an ASPEC XL system (Gilson Medical Electronics, Middleton, WI, USA). The extraction columns were first activated and washed with 1 ml acetonitrile–triethylamine (1000:1, v/v), 2 ml methanol and 2 ml of 0.01 *M* ammonium acetate pH 5.0. A 1000 μl sample volume was loaded onto the columns with a dispense flow of 0.4 ml/min. After successive washings with 2 ml of 0.01 *M* ammonium acetate pH 5.0 and 1 ml of methanol–distilled water (2:8, v/v), elution of docetaxel and the metabolites was performed with 500 μl of acetonitrile–triethylamine (1000:1, v/v). The elution solvent was evaporated to dryness under a nitrogen stream at 40°C. The samples were dissolved in 100 μl of acetonitrile–methanol–distilled water (4:1:5, v/v/v) by mixing for 30 s. A 50 μl volume was injected onto the HPLC column.

2.5. Validation procedures

A full validation (three analytical runs) of the analysis of docetaxel in human plasma was completed. A one-run validation was performed for the quantification of the metabolites as only limited amounts of the reference compounds were available.

2.5.1. Linearity

Five calibration standards were prepared (see Section 2.3) and analyzed in duplicate. The linear regression of the ratio of the areas of the analyte and internal standard peaks versus the concentration were weighted by $1/x$ (reciprocal of the concentration). The *F* test for lack of fit ($\alpha=0.05$) was used to evaluate the linearity of the calibration curves [16].

2.5.2. Accuracy and precision

For the validation of the docetaxel assay, four quality control samples containing 10, 100, 500 and 750 ng/ml were prepared separately in human control plasma as described in Section 2.3. Replicates of each quality control sample were processed and analyzed in three different runs with plasma

calibration standards to determine the docetaxel concentration.

For the metabolite assay, four quality control samples were prepared yielding metabolite concentrations of 10, 100, 250 and 500 ng/ml. Replicates of each quality control sample were processed and analyzed in one analytical run with plasma calibration standards to determine the metabolite concentrations.

The accuracy was calculated at each test concentration: the measured concentration was divided by the nominal concentration and multiplied by 100.

An estimate of the between-day precision for the docetaxel assay was obtained by one-way analysis of variance (ANOVA) for each test concentration using the run day as the classification variable. The day mean square (DayMS), error mean square (ErrMS) and the grand mean (GM) of the observed concentrations across run days were obtained. The between-day precision (pr.) was calculated for each quality-control concentration:

$$\text{Between-day pr.} = \frac{[(\text{DayMS} - \text{ErrMS})/n]^{0.5}}{\text{GM}} \times 100\%$$

n = the number of replicates within each run .

The estimate of the within-day precision was also calculated for each test concentration:

$$\text{Within-day pr.} = \frac{(\text{ErrMS})^{0.5}}{\text{GM}} \times 100\%$$

The within-day precision for the metabolite assay was determined by calculating the relative standard deviation while only one analytical run was executed.

2.5.3. Selectivity and specificity

Six batches control human plasma were processed and analyzed to determine whether endogenous plasma constituents co-eluted with docetaxel and metabolites.

To investigate the potential interference of the co-medication with the analytical method, the tested drugs were added to control plasma in therapeutic concentrations. These samples were processed and analyzed according the described method. The choice of the tested concomitant drugs was based on an inventory of the co-medication given in previous pharmacokinetics phase II studies with docetaxel.

2.5.4. Recovery

The influence of the sample loading flow in the SPE procedure on the recoveries of docetaxel (1000 ng/ml) and 2′-methylpaclitaxel (500 ng/ml) extracted from plasma has been studied at 0.2, 0.4, 0.5, 1.0 and 2.0 ml/min.

The impact of the methanol content in the 1 ml of methanol–distilled water wash solvent on the recoveries of docetaxel (1000 ng/ml) and 2′-methylpaclitaxel (500 ng/ml) extracted from plasma has been investigated. Contents of 0, 20, 40, 60, 80 and 100% methanol were tested.

The overall extraction recoveries were determined by comparing the slopes of the processed human plasma calibration curves to a standard curve prepared in acetonitrile–methanol–distilled water (4:1:5, v/v/v).

To determine the recovery of the internal standard 2′-methylpaclitaxel, 75 μl of working solution (10 μg/ml) was diluted with 75 μl distilled water. This sample was prepared freshly for each run and analyzed in singular.

2.5.5. Stability

The stability of docetaxel in plasma has been studied at a concentration of 500 ng/ml during 6 months at −30°C. Three to four replicates were analysed at 0, 3 and 6 months.

The influence of two freeze–thaw cycles on the stability of docetaxel in plasma has been investigated at a concentration of 500 ng/ml. Samples were analysed in duplicate.

To perform automatic solid-phase extractions overnight, the stability of docetaxel in plasma was studied at a concentration of 500 ng/ml during 48 h at ambient temperature. Samples were analysed in duplicate at 0, 5, 24 and 48 h.

The stability of docetaxel in plasma extracts in elution solvent [acetonitrile–triethylamine = 1000:1 (v/v)] at ambient temperature has been studied at a concentration of 500 ng/ml during 48 h. Duplicates were analyzed at 0, 4, 24 and 48 h.

The stability of docetaxel in reconstituted plasma extracts has been studied at a concentration of 500

ng/ml at ambient temperature during 48 h. Duplicates were analyzed at 0, 6, 24 and 48 h.

2.5.6. Robustness

Several batches cyano SPE-columns (100 mg) from two suppliers (Varian, Harbour City, CA and Sopachem BV, Nieuwegein, The Netherlands) were tested to investigate the influence on the recoveries of docetaxel and 2′-methylpaclitaxel extracted from plasma at analyte concentrations of 1000 ng/ml and 500 ng/ml, respectively.

2.6. Pharmacokinetic case study

A female patient at the age of 51, suffering from breast cancer was treated with docetaxel (Taxotere®) at a dose level of 100 mg/m^2 given as a one hour infusion. The hepatic functions at the time of the treatment were: SGOT 43 U/l, SGPT 56 U/l, AF 205 U/l and billirubin 15 μmol/l. Whole blood samples were collected from the contralateral arm receiving the infusion. Sampling times were prior to start, at 30 min and at the end of the infusion. Post infusion samples were taken at 5, 10, 20, 30 min and 1, 2, 3, 4, 8, 18 and 24 h. The whole blood samples (5 ml) were collected in EDTA tubes and immediately centrifuged (10 min at 2500 *g*). The plasma layer was then removed and stored at −30°C until analysis.

The docetaxel concentration vs. time curve was fitted using the MW\PHARM® software program (Medi\Ware BV, Groningen, The Netherlands) [17]. The docetaxel kinetics were best described by the use of a three compartment model. The Area Under the Curve (AUC) was calculated by the integration of the curve, with extrapolation to infinity. The slope of the terminal phase was obtained by linear regression and was used to calculate the terminal plasma elimination half-life ($t_{1/2}$). Plasma clearance (Cl) was calculated by dividing the delivered dose by the AUC. Volume at the steady-state (V_{ss}) was calculated by the following equation:

$$V_{ss} = \text{dose}/(C_1 + C_2 + C_3) \times (1 + k_{1,2}/k_{2,1} + k_{1,3}/k_{3,1})$$

where C_i is the initial concentration of the i-th component of the curve and $k_{i,j}$ represents the rate constant between the compartments i and j.

3. Results and discussion

3.1. Analytical procedure

The use of two mixtures of reference compounds was necessary while metabolites M1 and M2 were not separated in the HPLC system used.

The assay was linear over a concentration range of 10–1000 ng/ml for docetaxel and over a range of 10–500 ng/ml for the metabolites in human plasma as determined by the F test for lack of fit ($\alpha = 0.05$).

The assay performance data for the quantification of docetaxel and metabolites is presented in Table 1. For docetaxel the within-day and between-day precisions at 10, 100, 500 and 750 ng/ml were less than 6% and thus less than the required 15% [18] for all tested docetaxel concentrations. For the quantification of the metabolites the precisions of the assay also fall within ±15% at 100, 250 and 500 ng/ml and within ±20% for the lower limit of quantitation (10 ng/ml). The accuracies were all within ±10%.

Chromatograms of six batches control human plasma samples contained no endogenous peaks co-eluting with docetaxel and the metabolites.

The retention times and capacity factors of the co-medication which were added to control plasma to investigate potential interferences with the analytical method are listed in Table 2. None of the tested compounds co-eluted with docetaxel, the metabolites or the internal standard.

The influence of the sample loading flow on the recovery has been investigated. Changing the sample loading flow from 0.2 to 1.0 ml/min, the recovery of docetaxel decreased 9.1%. A sample loading flow of 0.4 ml/min was found to be optimal. It is stressed that the flow-rate should be well controlled for routine analysis; recoveries may vary since cation-exchange interactions (secondary interaction on cyano columns) usually occur at a lower rate than polar/non polar interactions. Validation was performed at 0.4 ml/min.

In Fig. 2 the recoveries of docetaxel and 2′-methylpaclitaxel are plotted versus the methanol content in the wash solvent. Using 20% (v/v)

Table 1
Assay performance data

Nominal concentration (ng/ml)	Measured concentration (ng/ml)	Accuracy (%)	Within-day precision (%)	Between-day precision (%)	Number of replicates
Docetaxel					
10	9.34	93.4	5.4	5.6	18
100	108	108	3.6	4.3	18
500	510	102	4.1	3.2	15
750	761	101	1.9	4.5	15
Metabolite M1					
10	10.2	102	3.5	N.D.	6
100	107	107	1.4	N.D.	6
250	267	107	2.0	N.D.	5
500	508	102	3.2	N.D.	6
Metabolite M2					
10	9.05	90.5	8.6	N.D.	5
100	99.4	99.4	2.9	N.D.	6
250	263	105	2.8	N.D.	6
500	512	102	3.0	N.D.	6
Metabolite M3					
10	9.79	97.9	10.4	N.D.	6
100	90.3	90.3	2.6	N.D.	6
250	258	103	1.7	N.D.	5
500	521	104	2.5	N.D.	6
Metabolite M4					
10	9.14	91.4	17.0	N.D.	5
100	98.8	98.8	3.8	N.D.	6
250	245	98.0	7.0	N.D.	6
500	501	100.2	3.1	N.D.	6

N.D., Not determined.

methanol, the chromatograms of control plasma samples contained significantly less endogenous peaks and no loss of the analytes was measured in contrast with higher contents of methanol.

The mean overall extraction recovery of docetaxel from human control plasma was 78.0%±5.8% ($n=3$) and 84.8%±3.1% ($n=3$) for the internal standard 2′-methylpaclitaxel.

For the metabolites recoveries between 72 and 79% were found, not significantly different from docetaxel (two-tailed t-test, $\alpha=0.05$). The molar absortivities at 227 nm, the extraction recoveries and the slopes of the calibration curves were all in the same range of that of docetaxel. Therefore the metabolites can be quantified by the use of the docetaxel calibration curve, when these compounds are not available as references.

Docetaxel was found to be stable in human plasma for at least 6 months, when stored at −30°C, with repeated freeze–thaw cycles. The drug is, however, not stable in plasma at ambient temperature. After 48 h the concentration was 86%±2.7% of the initial concentration (Fig. 3). In elution solvent [acetonitrile–triethylamine=1000:1 (v/v)] at ambient temperature docetaxel was found to be stable for at least 48 h. So, automated solid-phase extractions can be performed overnight whereas the plasma samples are loaded within 15 h (Fig. 3) onto the solid-phase extraction columns. Reconstituted samples were stable in the autosampler at room temperature for at least 48 h.

In Table 3 the recoveries of docetaxel and 2′-methylpaclitaxel extracted from plasma of several batches cyano SPE-columns (100 mg) from two

Table 2
Retention times and capacity factors of the co-medication, analogues and metabolites which were added to control plasma to investigate the potential interference with the analytical method

Compound	Concentration	Retention time (min)	Capacity factor (k')
Docetaxel	500 ng/ml	11.2	9.2
Methylpaclitaxel	500 ng/ml	18.7	16.0
Paclitaxel	500 ng/ml	12.6	10.5
Metabolite M1	50 ng/ml	4.6	3.2
Metabolite M2	50 ng/ml	4.6	3.2
Metabolite M3	50 ng/ml	5.3	3.8
Metabolite M4	50 ng/ml	8.8	7.0
Diphenhydramine	1 μg/ml	N.D.	–
Clemastine	10 ng/ml	N.D.	–
Ranitidine	1 μg/ml	3.7	2.4
Cimetidine	2 μg/ml	2.7	1.5
Prednisolone	10 μg/ml	2.6	1.4
Dexamethasone	1 μg/ml	N.D.	–
Flurazepam	30 ng/ml	N.D.	–
Oxazepam	2 μg/ml	4.0	2.6
Diazepam	2.5 μg/ml	7.8	6.1
Paracetamol	20 μg/ml	N.D.	–
Codeine	0.2 μg/ml	N.D.	–

N.D., Not detected.

suppliers are presented. It is obvious that end-capped cyano columns are to be preferred, probably due to the ability of docetaxel to interact significantly with the isolated silanols resulting in low recoveries. The best results were obtained with the end-capped columns from IST (Sopachem BV, Nieuwegein, The Netherlands).

3.2. Pharmacokinetic case study

Although analytical methods have been published for the analysis of docetaxel in biological matrices, no metabolic products could be detected in human plasma thus far. The described HPLC assay was initially developed for the quantification of only

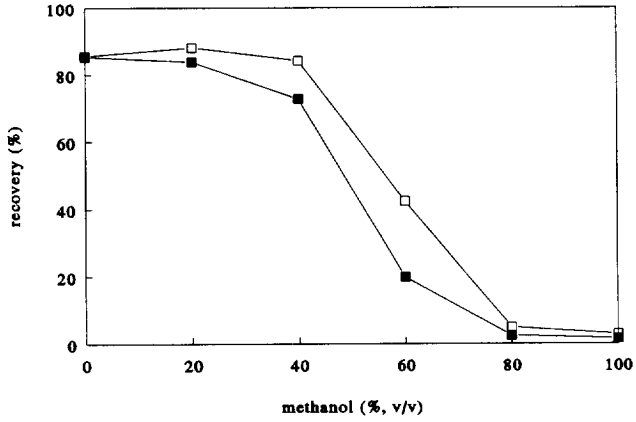

Fig. 2. Influence of the methanol content in the wash solvent on the recoveries of docetaxel (1000 ng/ml, ■) and 2′-methylpaclitaxel (500 ng/ml, □) extracted from plasma.

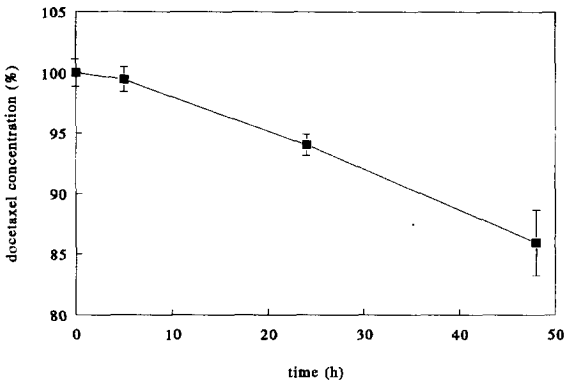

Fig. 3. Stability of docetaxel in plasma at a concentration of 500 ng/ml at ambient temperature. Samples were analysed in duplicate at 0, 5, 24 and 48 h. Percentages of the initial concentration are plotted ±2 times the relative standard deviation.

docetaxel in a phase II study. After measuring samples of 25 patients, in two cases metabolic products were detected (Fig. 4). The quantification of these metabolites has not yet been described, which may be due to (i) the use of a sample pretreatment procedure which is not appropriate for the metabolites [7], (ii) the lack of reference compounds for the development and validation of such an assay and (iii) the low concentration levels of the metabolic products in plasma of patients with normal hepatic functions. For the quantification of the metabolites of docetaxel, a limited validation in human plasma was performed. To show the applicability of the method, the plasma concentration vs. time profiles of docetaxel and metabolites of a patient with liver deficiencies treated with a dose level of 100 mg/m^2 in a 1 h infusion are presented in Fig. 5. Three docetaxel metabolite peaks were detected (Fig. 4) in the plasma samples. Metabolites 1 and 2 were not separated and total concentration levels were determined. Metabolite 4 was found to be the major circulating metabolite of docetaxel. The maximum concentrations (C_{max}) of the metabolites were found at 15 min after the end of the docetaxel administration and decline to undetectable levels (<10 ng/ml) within 9 h after the start of the infusion. At 15 min after the end of the infusion, the C_{max} of M4 was 230 ng/ml plasma (24% of the docetaxel concentration at that time).

The plasma concentration vs. time profiles were triphasic. The relevant pharmacokinetics of docetaxel are given in Table 4 and were compared to published parameters [15]. The C_{max} concentration in the case study is higher due to the short infusion duration. While the interpatient variability in the study of Bruno is relatively high, no significant differences could be demonstrated.

4. Conclusion

For the quantification of docetaxel in human plasma, an accurate, reproducible and selective

Table 3
Recoveries of docetaxel and 2'-methylpaclitaxel (I.S.) extracted from plasma for several batches of cyano SPE-columns (100 mg) originating from two suppliers

	Supplier	End-capped	Batch no.	Docetaxel		I.S.	
				Recovery (%)	Mean±S. (%)	Recovery (%)	Mean±S.D. (%)
1	Varian	no	130247	70.8	63.3±10.1	83.1	83.4±3.9
2			132217	73.0		88.9	
3			132814	55.8		81.1	
4			132837	53.4		80.4	
5	IST	no	4021303 AA	71.7	64.1±6.8	81.0	80.9±1.7
6			4144401 AA	61.9		82.5	
7			5077405 AA	58.6		79.1	
8	IST	yes	3274302 DB	84.2	83.5±1.1	84.8	87.2±2.1
9			4111303 AA	84.1		88.8	
10			5080402 AA	82.3		87.9	

S.D.: Standard deviation.

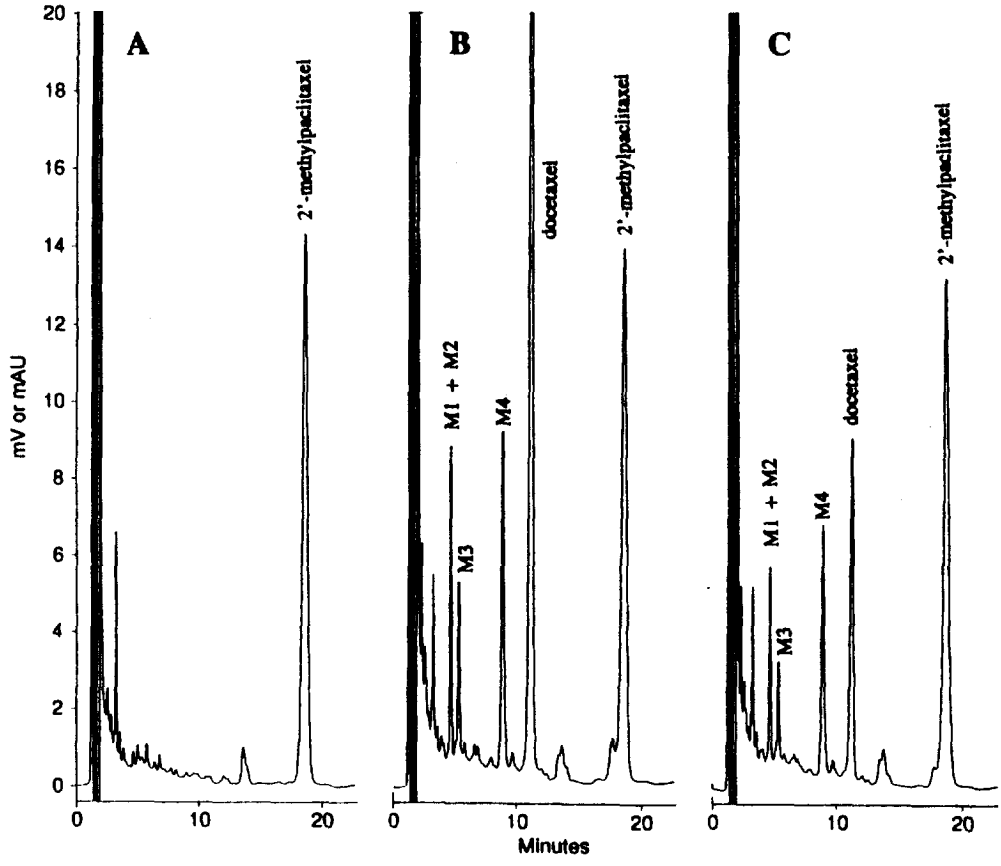

Fig. 4. Chromatograms of samples taken from a patient with liver dysfunction treated with docetaxel at a dose level of 100 mg/m^2 in a 1 h infusion. Sampling times were prior to start (A), and at 14 min (B) and 1 h (C) post infusion.

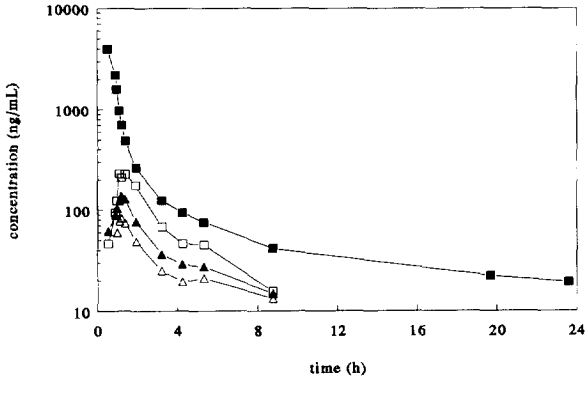

Fig. 5. Plasma concentration vs. time profiles of docetaxel (■) and metabolites M1+M2 (▲), metabolite M3 (△) and metabolite M4 (□) in a patient with liver dysfunction treated with 100 mg/m^2 docetaxel in a 1 h infusion.

HPLC assay, utilizing an automated solid-phase extraction on cyano columns as a sample pretreatment procedure has been developed. The assay quantitates docetaxel concentrations of 10–10 000 ng/ml using a 1000 μl sample volume. The solid-phase extraction procedure is also specific for metabolic products of docetaxel. The concentrations of these metabolites can be quantified, in the absence of reference compounds, using the docetaxel calibration curve. Metabolites 1 and 2 were not separated in the chromatographic system used; total concentrations of the products can be determined.

This assay has been used in a pharmacokinetics phase II study whereby several metabolic products could be detected and quantified in plasma of patients with liver dysfunction.

Table 4
Pharmacokinetic parameters (±S.D.) of docetaxel

	Bruno et al. [15]	Case study
Dose (mg/m^2)	70–115	100
Infusion duration (h)	1.4 (0.5) – 1.8 (0.3)	0.87
C_{max} (μg/ml)	1.91 (0.32)–2.68 (0.93)	3.98
$AUC_{0\rightarrow\infty}$ (h·μg/ml)	2.79 (0.85)–5.19 (0.16)	4.72
Terminal half-life $t_{1/2}$ (h)	11.3 (8.1)	11.2
Clearance (l/m^2/h)	22.2 (6.1)	21.2
Steady state distribution volume V_{ss} (l/m^2)	60.3 (42.3)	94.4

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