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Rapid high-performance liquid chromatographic determination of docetaxel (Taxotere) in plasma using liquid–liquid extraction

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Abstract

A new rapid and sensitive high-performance liquid chromatographic method for analysis of docetaxel (Taxotere) in human plasma was developed and validated. After adding an internal standard (paclitaxel, Taxol), plasma was extracted following a simple liquid–liquid extraction with diethyl ether. Extraction efficiency averaged 95% for docetaxel. Separation was performed using a Nucleosil (C₁₈) 5 μm column, monitored at 227 nm. The isocratic mobile phase consisted of acetonitrile–acetate buffer, pH 5–tetrahydrofuran (45:50:5, v/v) pumped at a flow-rate of 1.8 ml/min. The limit of quantification for docetaxel in plasma was 12.5 ng/ml. Retention times for docetaxel and paclitaxel were 7.7 and 9 min, respectively. Standard curves were linear over a range of 25–1000 ng/ml. This new method is rapid since it does not require time-consuming extraction procedures, or complex chromatographic conditions. This rapidity, along with the lack of chromatographic interferences with various other drugs likely to be administered to the cancer patients (pain killers, corticoids, antiemetics drugs) make this method suitable for daily routine analysis of Taxotere, a major anticancer drug extensively used in clinical oncology. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Docetaxel; Taxotere

1. Introduction

Docetaxel (Taxotere) is an anti-neoplastic agent whose chemical structure is related to paclitaxel

(Taxol) [1]. Whereas paclitaxel is isolated from the bark of *Taxus brevifolia*, docetaxel is a semi-synthetic drug derived from the needles of *Taxus baccata*.

Both Taxol and Taxotere exert their antiproliferative action by inhibiting microtubule depolymerization [2]. Taxotere displays a broad spectrum of action against various solid tumors including breast, non-small cell lung, head and neck and ovarian carcinomas [3,4].

Most of the pharmacokinetic studies carried out in

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human are based upon high-performance liquid chromatography (HPLC) determination of Taxotere in biological fluids by modified Taxol methods [5–7].

Indeed, several HPLC methods for the determination of taxanes in biological fluids have been published (Table 1). Some of the methods described require gradient elution chromatography [8] or specific chromatographic conditions such as the use of column oven or warming bath [9,10], which may not be very convenient for daily routine analysis. Besides, most of these methods need multi-steps sample preparation involving complicated liquid–liquid (LLE) [9] or more often solid-phase (SPE) [8,10–12] extraction procedures. This is time-consuming and does not meet the requirements of multi-samples analysis in human pharmacokinetics studies or routine drug-monitoring programs.

An original method for determination of Taxotere using automated SPE has been described [13] but extraction recovery remained below 80% and its reproducibility may be affected if the samples are prepared manually.

We have therefore developed a specific, rapid and simple isocratic HPLC method for determination of Taxotere in human plasma. This method has been fully validated following the US Food and Drug Administration (FDA) requirements [14] and French clinical GBEA procedures [15], and is suitable for monitoring this drug during pharmacokinetics studies in man.

2. Material and methods

2.1. Chemical and reagents

Acetonitrile (RS grade), ethanol (HPLC grade), diethyl ether (RPE grade) were obtained from Carlo Erba (Milan, Italy). Internal standard paclitaxel and Cremophor EL were purchased from Sigma (St. Quentin Fallavier, France). Docetaxel was a gift from Aventis Pharma (Anthony, France). All other chemicals were HPLC grade. Deionized water was used throughout the study. Pooled human plasma collected on citrate was obtained from the Agence Française du Sang (Marseilles, France). Ready-to-use Titrisol buffer was purchased from Merck s.a (Nogent sur Marne, France), ammonium acetate buffer (35 mM, pH 5) was prepared by adding 2.7 g ammonium acetate and acetic acid to 1 l deionized water.

2.2. HPLC instrumentation and operating conditions

A solvent delivery pump (Waters 600E, system Controller) equipped with a UV detector (Kontron HPLC 432) and coupled to a Waters 717 autosampler and Chromatographic Maxima 820 data integration package were used. Separation was performed using a 250×4.6 mm Nucleosil 5 μm particle C₁₈ column (Macherey–Nagel).

The mobile phase pumped at flow-rate of 1.8

Table 1
Main published HPLC methods for determination of docetaxel in biological fluids

Authors	Extraction	Mean recovery (%)	Column	Temperature (°C)	Internal standard	LOD or LOQ
Vergniol et al. [8]	SPE	88	Spherosil C ₁₈ 5 μm	Ambient	Taxol	LOD=5 ng/ml
Bisset et al. [5]	SPE	n.g.	Spherisorb S5 ODS2	Ambient	Taxol	LOD=15 ng/ml
Burris et al. [18]	SPE	n.g.	Spherisorb C ₁₈ 5 μm	Ambient	Taxol	LOD=15 ng/ml
Rosing et al. [13]	SPE	78	Apex octyl 5 μm	Ambient	2'-Methyl paclitaxel	n.g.
Loos et al. [10]	LLE	84	Inertsil ODS-80A	60	Taxol	LOQ=10 ng/ml
Ardiet et al. [9]	SPE	58	Uptisphere ODSB 5 μm	28	Taxol	LOQ=5 ng/ml
Ceruti et al. [6]	SPE	77	Symmetry C ₁₈	Ambient	n.g.	n.g.

LLE, Liquid–liquid extraction; SPE, solid-phase extraction; n.g., not given.

ml/min consisted of acetonitrile–35 mM ammonium acetate buffer (pH 5)–tetrahydrofuran (45:50:5, v/v) and was filtered (Millipore system, 0.45 μm) under vacuum and degassed. Chromatographic separation was monitored at 227 nm using a Kontron UV detector. All solutions were analyzed at room temperature.

2.3. Stock solutions

Docetaxel stock solutions were prepared by dissolving 5 mg of this drug in 10 ml of ethanol. Exact concentration was determined by UV spectrophotometry after appropriate dilution using the classical Beer–Lambert formula ($\epsilon=818$ at 227 nm). Working standard solutions of docetaxel (100 $\mu\text{g}/\text{ml}$ and 10 $\mu\text{g}/\text{ml}$) were prepared by appropriate dilutions of the stock solutions in ethanol. The docetaxel stock solutions were stored in 200 μl aliquots at -20°C until needed. The lowest standard solution (1 $\mu\text{g}/\text{ml}$) was prepared daily by dilution of the 10 $\mu\text{g}/\text{ml}$ solution. Docetaxel stock solutions were stable for at least 5 months at -20°C . Paclitaxel (internal standard) stock solutions were prepared by dissolving 10 mg of drug in 10 ml of ethanol.

Working standard solution (10 $\mu\text{g}/\text{ml}$) was obtained by several dilutions of the stock solution in ethanol. All solutions were stored at -20°C .

2.4. Preparation of standard and quality control samples

For preparation of the standards used for construction of calibration curve, an appropriate volume of working standard solution was added to 1 ml aliquots of blank human plasma. The calibration samples for docetaxel in drug-free plasma ranged from 25 ng/ml to 1000 ng/ml as following: 25, 50, 100, 250, 500, and 1000 ng/ml. Quality control samples were prepared similarly from a different fresh stock solution at concentrations of 42.5, 425 and 750 ng/ml in blank plasma and stored at -20°C .

2.5. Sample collections

Whole blood samples (5 ml) were collected into

heparinized tubes, then placed at 4°C and centrifuged within 2 h after collection. Resultant plasma samples were stored at -20°C until assayed.

2.6. Sample preparation procedures

A 1-ml volume of standard solution, 1 ml of quality control solution and 1 ml of patient sample were placed into a glass tube. A 50- μl volume of 10 $\mu\text{g}/\text{ml}$ paclitaxel solution, 100 μl of Titrisol buffer (pH 5) and 7 ml of diethyl ether were then successively added. Tubes were mixed by vibration for 45 s and then centrifuged at 3500 g at $+4^\circ\text{C}$ for 5 min. Organic layers were placed in glass tubes and quickly dried under nitrogen at ambient temperature. The residue was next reconstituted with 200 μl of HPLC mobile phase. These samples were mixed for 20 s and centrifuged at 3000 g for 3 min. The solution was transferred to a microvial and the 140 μl was injected onto the system.

2.7. Calibration and quantification

A standard curve was prepared by injecting various known concentrations of Taxotere solutions in plasma. The calibration curves were obtained by weighted $1/x$ least-squares linear regression analysis of known drug concentrations versus peak heights. Plasma samples and quality control samples concentrations were calculated by using the regressed equation of the straight line $y=ax+b$ where y is the peak height, a is the slope, b is the y -intercept and x is the unknown concentration. The limit of detection (LOD) of Taxotere was calculated as three standard deviations from a forced baseline integration in blank plasma. The limit of quantification (LOQ) was defined as the lowest concentration of the drug that could be assayed with a good level of precision and accuracy.

Since during pharmacokinetic studies, sampling strategies often require that late sampling times are assayed from larger volumes of blood, the LOQ was determined from a 2 ml plasma extract with the actual concentration being re-calculated using a 0.5 dilution factor.

2.8. Application to human pharmacokinetics study

To assess the applicability of the method, blood samples were taken from a patient participating in a phase I clinical study in our institute. Fifteen samples were withdrawn 0.5, 0.75, 1, 1.2, 1.4, 1.6, 2, 3, 4, 6, 8, 12, 18, 24 and 36 h after a 1-h infusion of 175 mg/m² of Taxotere and subjected to HPLC analysis as described above. Identification of Taxotere pharmacokinetics parameters was carried out using Apis 4.1 software (Mipps, Marseilles, France).

3. Results

3.1. Chromatographic separation

Chromatographic separation of docetaxel and internal standard was completed within 10 min. The overall run length lasted 20 min. Fig. 1 displays the chromatograms of extracts prepared from a blank plasma, an LOQ standard and a patient receiving Taxotere. Under the conditions described above, the retention times for Taxotere and its internal standard were 7.7 and 9 min, respectively. Both Taxotere and Taxol elute as sharp symmetrical peaks and no significant endogenous peak that could overlap docetaxel or its internal standard were observed after testing several batches of human plasma. Similarly, no chromatographic interference was observed either between docetaxel, Taxotere and more than 50 drugs likely to be administered to cancer patients, including anesthetics, corticoids, antiemetics and other antineoplastic agents (Table 2, Fig. 2).

3.2. Calibration

A standard curve was constructed for docetaxel by plotting calculated peak heights versus standard concentrations. The method demonstrated excellent linearity over the range of 25–1000 ng/ml (Table 3). The typical equation describing the calibration curve in plasma was $y=0.0027x-0.0012$ where y is the height of the peak and x the concentration of the drug, with a mean correlation coefficient of 0.9999.

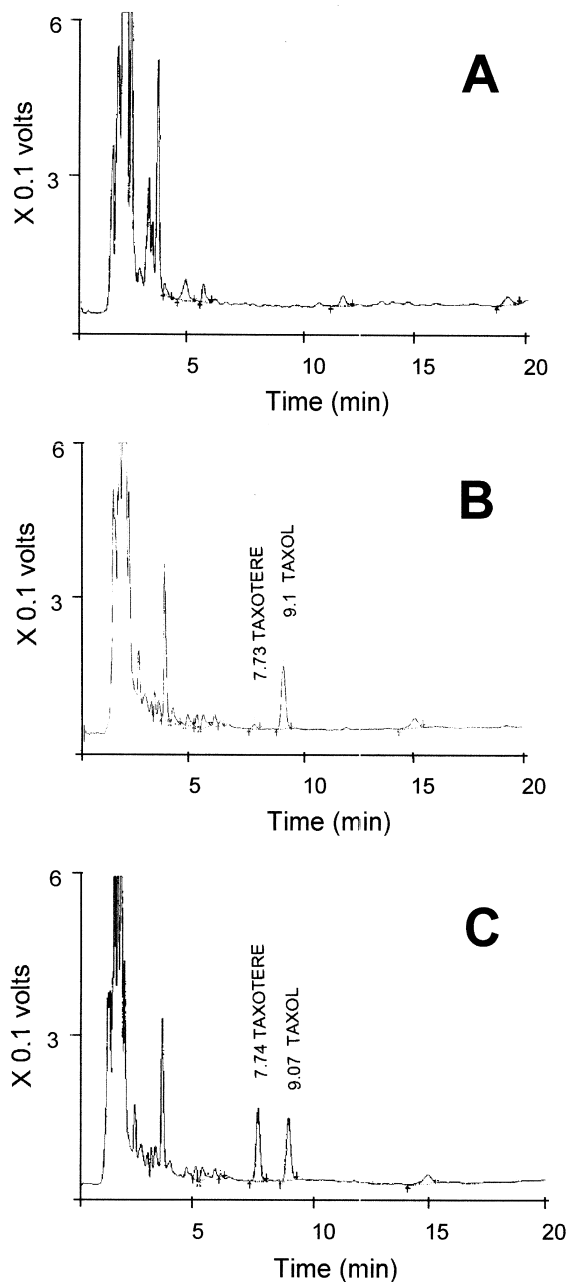


Fig. 1. Taxotere chromatograms from blank plasma (A), LOQ-level standard (B) and 300 ng/ml patient sample (C). Samples were extracted and subjected to HPLC analysis as described in Materials and methods.

Table 2
Retention times (t_R) of drugs tested for interference in docetaxel analysis during a 20 min run

Drug	t_R (min)	Drugs	t_R (min)
Pyopen	1.346	6-OH paclitaxel	5.47
Rocephin	1.384	Vincristine	6.029
Cimetidine	1.5	Nelfinavir	6.621
Vancomycin	1.699	Thiopental	6.745
Acetomorphene	1.8	Vinblastine	6.776
Topotecan	1.9	Diazepam	6.777
Morphine	1.95	Vindesine	6.873
Clonidine	2.25	Docetaxel	7.773
Digoxin	2.375	Paclitaxel	9.033
Chloroquine	2.387	Ritonavir	10.357
Etoposide	2.441	Lopinavir	10.8
Granisetron	2.732	Chlorpromazine	11.8
Quinine	2.865	Paracetamol	n.d.
Phenobarbital	2.94	Amphotericin	n.d.
Cisplatin	2.982	Fluconazole	n.d.
Ifosfamide	2.985	Pefloxacin	n.d.
Carbamazepine	3.257	Peflacin	n.d.
Ondancetron	3.261	Cafeine	n.d.
Dexchloropheniramine	3.8	Ciclophosphamide	n.d.
Bupivacaine	3.8	5-FU	n.d.
Vepeside	3.943	Prednisolone	n.d.
Clonazepam	4.11	Prednisone	n.d.
Hydroxysine	4.15	Furosemide	n.d.
Amprenavir	5.283	Amiodarone	n.d.
Ibuprofen	6.327		

n.d., Not detectable.

3.3. Recovery

Recovery of docetaxel during extraction procedures was calculated by comparing peak height for direct injection versus extracted 100 and 1000 ng/ml plasma samples. The efficiency of extraction from plasma averaged 95%. Similarly, the paclitaxel recovery was found at 87% after LLE as described above.

3.4. Limit of detection and limit of quantification

The limit of detection (LOD) for docetaxel in plasma defined as a minimum signal-to-noise of 3 was 5 ng/ml. The LOQ for Taxotere in 200 μ l of extracted plasma was 12.5 ng/ml (RSD: 2.54%, accuracy: +14%, $n=6$).

3.5. Intra- and inter-day assay variabilities

In plasma spiked with 42.5, 425 and 750 ng/ml of docetaxel, the intra-day assay variabilities expressed as relative standard deviation (RSD) were 3.91, 1.28 and 0.63%, respectively. The inter-day assay variabilities at the same concentrations were 4.03, 0.96 and 2.09%, respectively (Tables 4 and 5).

3.6. Application to human pharmacokinetics study

The described method was next applied to a phase I study of high-dose Taxotere. No endogenous peaks were found interfering with docetaxel or paclitaxel, despite multiple co-medications. Data collected were used to plot a concentration–time curve that was best fitted with a three-compartment model (Fig. 3), which is in agreement with the literature [16].

4. Discussion

Pharmacokinetics studies in human often involve analysis of large batches of samples and therefore require simple, rapid and reliable analytical methods.

Besides, daily analysis of an increasing number of drugs as part of the routine drug monitoring activity of general hospital's clinical pharmacokinetics laboratory urges the need for HPLC methods that can be easily run and rapidly set up. Ideally, the same HPLC system should be used to analyze a wide range of different drugs with only some minor modifications in the chromatographic conditions, which would be cost- and time-effective.

In this respect, we needed to develop a method for docetaxel determination in plasma simple and robust enough to be run on standard HPLC apparatus and using only basic analytical reagents.

The mobile phase was selected to obtain the best resolution of paclitaxel and docetaxel. Using 45% acetonitrile shortened the elution time for the two compounds and, subsequently, overall run length. The addition of 5% tetrahydrofuran to the mobile phase enhanced the separation of the drugs and increased the sensitivity of the method. Indeed, a significant diminution of peak heights was observed without tetrahydrofuran after 1 week of preliminary experiments (data not shown).

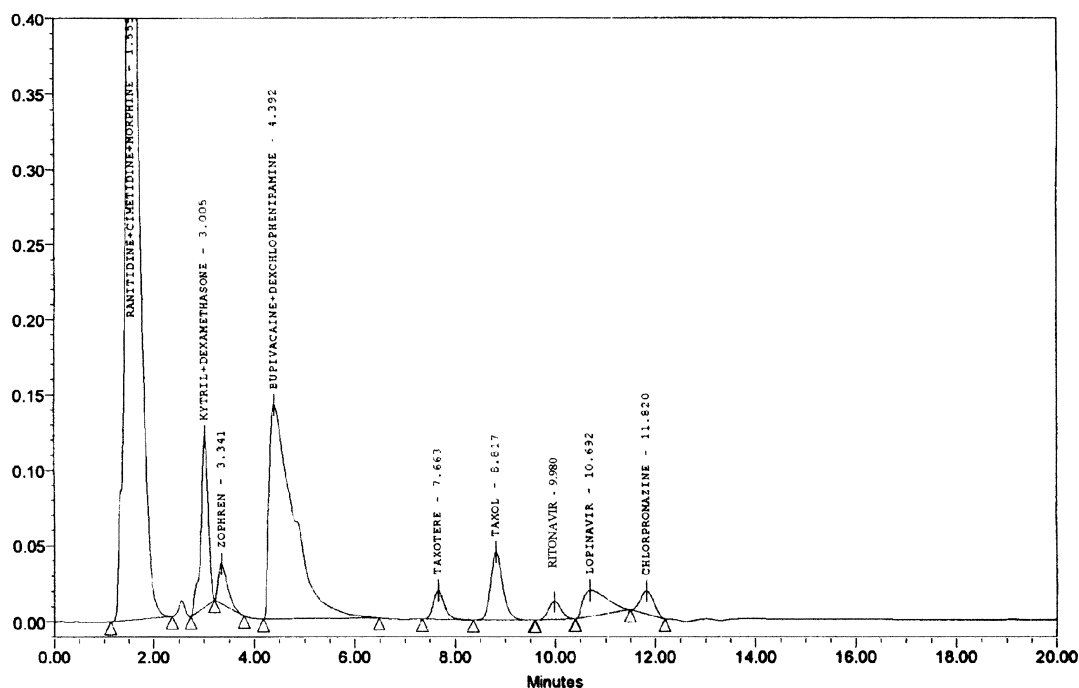


Fig. 2. Absence of chromatographic interferences between Taxol, Taxotere and some representative drugs likely to be administered to cancer patients. Analgesics (morphine, bupivacaine), corticoids (dexamethasone), antiemetics (kytril, zophren), antipsychotics (chlorpromazine), antiviral (ritonavir, lopinavir) antiallergic (dexchlorpheniramine), antiulcerous (ranitidine, cimetidine) drugs did not interfere with the elution of taxanes.

Variation in pH extraction had an effect on the peak height of docetaxel. pH 4, 5 and 7 were therefore tested (data not shown). pH 5 was selected

Table 3
Statistics on parameters of docetaxel standard curves in plasma

Analysis group	Slope	Intercept	Correlation coefficient
1	0.0026	0.00460	0.999
2	0.0029	0.00207	0.9999
3	0.0027	-0.00537	0.9999
4	0.0027	-0.00046	0.9999
5	0.0026	-0.00332	1
6	0.0027	-0.00387	0.9999
7	0.0026	-0.00173	1
Mean	0.0027	-0.0012	0.9999
SD	0.0001	0.0035	-
RSD (%)	3.98	-	-
n	7	7	7

SD=Standard deviation, RSD=relative standard deviation.

because it provided the highest extraction yield at 500 ng/ml.

The internal standard we chose was paclitaxel since its chemical structure and physical properties are similar to docetaxel. Moreover, paclitaxel is commercially available and therefore easy to obtain without batch-to-batch variabilities. Conversely, the

Table 4
Precision and accuracy of docetaxel determination in plasma samples during intra-day assay

	Quality control		
	Low, 42.5 ng/ml	Medium, 425 ng/ml	High, 750 ng/ml
Mean (ng/ml)	46.87	430	732.75
SD (ng/ml)	1.833	5.537	4.62
Precision (RSD, %)	3.91	1.28	0.63
Accuracy (%)	+10.28	+1.2	-2.3
n	6	6	6

Table 5
Precision and accuracy of docetaxel determination in plasma samples during inter-day assay

	Quality control		
	Low, 42.5 ng/ml	Medium, 425 ng/ml	High, 750 ng/ml
Mean (ng/ml)	47.3	420.8	735.2
SD (ng/ml)	1.91	4.04	15.4
Precision (RSD, %)	4.3	0.96	2.09
Accuracy (%)	+12.77	-0.96	-1.96
<i>n</i>	6	6	6

SD=Standard deviation, RSD=relative standard deviation.

other internal standards we could have used such as *N*-cyclohexyl-benzamide, cephalomane or 2'-methyl paclitaxel were hardly available.

Different procedures for docetaxel extractions, whether liquid–liquid or solid–liquid, with various solvents were tested in our laboratory. Some showed interferences of endogenous compounds with Taxotere. Others had an insufficient extraction yield to allow proper pharmacokinetics application (data not shown). Indeed, docetaxel phase I trials involving

sometimes starting doses as low as 5 to 10 mg/m² require high extraction efficiency for accurate monitoring of plasma levels in patients [16–18].

Different solvents were tested (chloroform, ethyl acetate, butyl methyl ether) but only diethyl ether combined both excellent recovery (95%) with a high evaporation profile that greatly shortened sample preparation time.

The use of LLE is particularly advantageous for routine therapeutic drug monitoring since this method is rapid, sensitive, specific and requires a simple material that is already available in most bioanalytical laboratories.

In this respect, the method we present here is more simple and faster than most HPLC determinations of docetaxel based upon complex SPE procedures. Thus, running this method for bioanalysis of Taxotere in patients enrolled in phase I trial permitted the analysis of at least 30 samples/days at low cost. This HPLC method is therefore suitable to plasma level determination of Taxotere during pharmacokinetics studies.

Besides, the chromatographic conditions presented here are now run in our laboratory to analyze as well

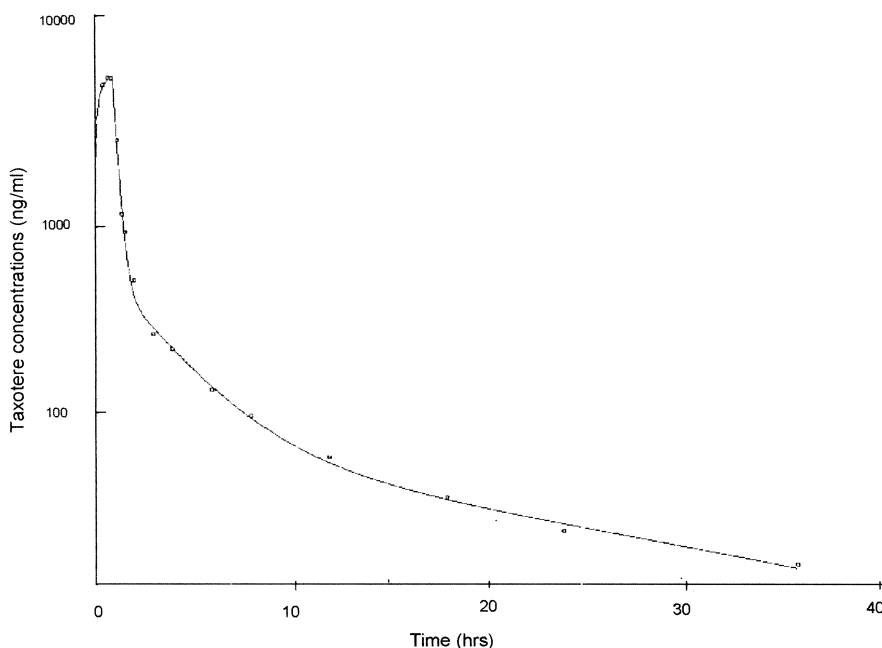


Fig. 3. Plasma concentration–time of Taxotere after administration of 175 mg/m² by 1-h infusion. Experimental concentrations determined by HPLC were fitted by a three-compartment model using Apis Software.

Taxol, VP16, along with anthracyclines such as doxorubicine and epirubicine (provided that the system is equipped with a fluorescence detector), thus greatly improving the management of routine therapeutic monitoring of anticancer drugs.

5. Conclusion

The assay described here has the desired characteristics for the practical application of liquid chromatography to the determination of docetaxel in plasma during clinical pharmacokinetic studies. We have validated a rapid isocratic liquid chromatography method that quantifies 25 up to 1000 ng/ml of docetaxel in human plasma. This assay requires 1 ml of plasma, uses an available internal standard and a simple mobile phase. Although only basic HPLC apparatus and simple LLE procedure are required, this method demonstrated a high degree of accuracy and precision and a good sensitivity.

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