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Assay of paclitaxel (Taxol) in plasma and urine by high-performance liquid chromatography

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Abstract

A new, rapid and sensitive high-performance liquid chromatographic method for the analysis of paclitaxel (Taxol) in human plasma and urine was developed and validated. After addition of an internal standard, paclitaxel was extracted from plasma or urine by a liquid–liquid extraction using diethyl ether. Extraction efficiency averaged 90%. Chromatography was performed isocratically on a reversed-phase column monitored at 227 nm. Retention times were 7.7 and 6.7 min for paclitaxel and docetaxel, respectively, and the assay was linear in the range 25–1000 ng/ml. The limits of quantification for paclitaxel were 25 and 40 ng/ml in plasma and urine, respectively. The assay was shown to be suitable for pharmacokinetic studies of children involved in a phase I clinical trial.

Keywords: Paclitaxel; Taxol

1. Introduction

Paclitaxel (Taxol) tax-11-en-9-one,5β,20 epoxy-1,2α,4,7β,10β,13α-hexahydroxy-4,10-diacetate-2-benzoate-13-(α-phenylhippurate) is an antineoplastic agent which belongs to a new group of cytotoxic agents, the taxanes (Fig. 1). Their main mechanism of action is mediated by the stabilization of cellular microtubules. Investigators have now demonstrated taxol activity against adult epithelial ovarian cancer, breast cancer, lung cancer and melanoma [1].

In clinical trials in adults, Taxol was used as single 3, 6, 24, 72 or 96 h infusions, ranging from 15 to 390 mg/m^2 [2–7]. In a phase I study carried out

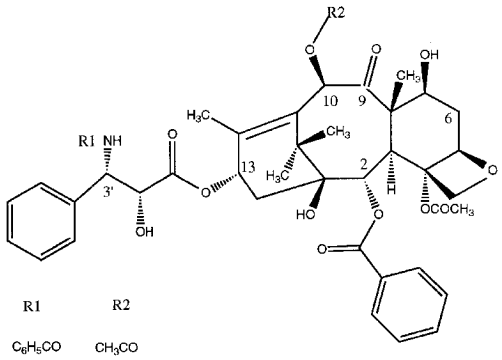

Fig. 1. Structures of paclitaxel and docetaxel (internal standard).

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Table 1
Published HPLC methods for determination of paclitaxel in plasma and urine

Author	Extraction	Column	Elution	Internal standard	LOD or LOQ of paclitaxel
Longnecker et al. (87) [4]	LLE ethylacetate	Waters C_{18} Radial Pak, 10 μm	Gradient	N-cyclohexylbenzamide	LOD^{un}=50 nM (42.7 ng/m)
Wiernik et al. (87) [10]	LLE acetonitrile	Whatman Partisil 10 μm ODS-3 column	Gradient	No	
Grem et al. (87) [11]	LLE+SPE	Varian C_{18} reversed-phase, 10 μm column	Isocratic	No	
Rizzo et al. (90) [12]	LLE+SPE	C_8 column MOS hypersil, 5 μm	Isocratic	No	LOD^a=100 nM (80 mg/ml)
Willey et al. (93) [13]	SPE	APEX octyl, 5 μm	Isocratic	No	LOQ^a=11.7 nM (10 ng/ml)
Sonnischen et al. (94) [9]	LLE+SPE	C_8 column hypersil	Isocratic	No	
Song and Au (95) [14]	LLE+SPE Nova pack C_{18} Waters (column switching)	Reversed-phase Backerbond octadecyl C_{18}, 5 μm	Isocratic	Cephalomannine	LOD^a=5.85 nM (5 ng/ml)
Gianni et al. (95) [5]	SPE (isocratic)	Superspher C_{18} column	Isocratic	Cephalomannine	
Sparreboom et al. (95) [15]	LLE+SPE	APEX octyl, 5 μm	Isocratic	2′-Methyl paclitaxel	LOD^a=17.5 nM (15 ng/ml) LOQ^a=29 nM (25 ng/ml)
Huizing et al. (95) [16]	SPE or LLE	APEX octyl, 5 μm	Isocratic	No	LOD^a=9.4 (8 ng/ml) LOQ^a=11.7 nM (10 ng/ml)

LLE, liquid–liquid extraction; SPE, solid-phase extraction.
LOD, limit of detection (un=undefined), [a]=defined as the concentration of compound giving a signal-to-noise ratio greater than 3:1.
LOQ, limit of quantification, [a]=defined as the lowest concentration that can be measured with accuracy and precision ≤20% [17].

in children, Taxol doses (200–420 mg/m^2) were delivered as single 24-h infusions [8,9].

Several publications have appeared in which paclitaxel is assayed by HPLC. Characteristics of the analytical methods are given in Table 1 [4,5,9–16]. Some assays employ solid-phase extraction (SPE), others use liquid–liquid extraction (LLE) and a few use a combination of liquid and solid extraction. Some assays do not use an internal standard or a complicated extraction procedure. Some techniques show inadequate sensitivity or were not validated according to current requirements.

This paper describes a rapid, simple and robust HPLC method (with internal standard), validated according to FDA requirements [17], for the determination of paclitaxel in human plasma and urine. The assay is suitable for clinical pharmacokinetic studies.

2. Experimental

2.1. Materials

Acetonitrile (RS grade), ethanol (HPLC grade) and diethyl ether (RPE grade) were obtained from Carlo Erba (Milan, Italy). Paclitaxel (Taxol) and Cremophor EL were purchased from Sigma (St. Louis, MO, USA). Docetaxel used as internal standard (I.S.) was a gift from Rhône-Poulenc-Rorer (France). All other chemicals were of analytical grade. Deionized water was used throughout the study. Pooled human plasma (collected on citrate) was obtained from the Centre de Transfusion Sanguine (Marseilles, France). The buffer, 35 mM ammonium acetate (pH 5.0) was prepared by adding 2.7 g ammonium acetate and acetic acid to 1 l deionized water.

2.2. HPLC instrumentation and conditions

A solvent delivery pump (Waters 600E, system controller) was used with a UV detector (Kontron HPLC 432). Data output was monitored using a NEC advanced personal computer (software: MAXIMA 820). Samples were injected with an a Waters 717 chromatographic autosampler (ambient temperature). Separation was performed at ambient temperature on a 250×4.6 mm Nucleosil 5 μm particle C_{18} column (Macherey Nagel). The mobile phase consisted of acetonitrile–35 mM ammonium acetate buffer (pH 5)–tetrahydrofuran (45:50:5, v/v/v) and was filtered (system Millipore, 0.45 μm) under vacuum and degassed. Chromatographic separation was monitored at 227 nm using a flow-rate of 1.8 ml/min.

2.3. Preparation of stock solutions

Paclitaxel stock solutions were prepared by dissolving 5 mg of paclitaxel in 10 ml of ethanol. The exact concentration was determined by UV spectrophotometry after appropriate dilution (ϵ=29 800 at 227 nm). The paclitaxel stock solutions were stored in 200-μl aliquots at −20°C until needed. Paclitaxel stock solutions were stable for at least 5 months. Working standard solutions of paclitaxel (10 ng/μl and 1 ng/μl) were prepared by appropriate dilutions of the stock solutions in ethanol. These solutions were made daily. Docetaxel stock solutions (internal standard) were made by dissolving 10 mg of docetaxel in 10 ml of ethanol. Working standard solutions (10 μg/ml) were obtained by dilution of the stock solution in ethanol, and all solutions were stored at −20°C.

2.4. Preparation of standard and quality control solutions

For preparation of the calibration curves, an appropriate volume of working standard solution was added to 1-ml aliquots of blank human plasma or blank urine. The plasma standards ranged from 25 ng/ml to 1000 ng/ml (25, 50, 100, 250, 500, 1000 ng/ml). Urine was stabilized with Cremophor EL–ethanol (1:1, w/v) according to the method of Huizing et al. [16]. Urine standards ranged from 40 to 1000 ng/ml (40, 100, 250, 500, 1000 ng/ml).

Quality control solutions (QC) were prepared from plasma or urine in the same manner using a different fresh stock solution at concentrations of 44, 440 and 750 ng/ml and stored at −20°C for at least 2 months.

2.5. Clinical samples

Blood samples (5 ml) of patients were collected into heparinized tubes, stored at 4°C and centrifuged (900 g) within 2 h of collection. The resultant plasma samples were stored at −20°C until assayed.

Fresh urine samples, stabilized by adding 0.5 ml of Cremophor EL–ethanol (1:1, w/v) to 9.5 ml urine [16], were also stored at −20°C until assayed.

2.6. Liquid–liquid extraction

For analysis, 1 ml of standard, quality control solution or 50 μl–1 ml of plasma or urine sample were placed in glass tubes. A 50-μl volume of internal standard, 100 μl of 35 mM ammonium acetate buffer (pH 5) and 7 ml of diethyl ether were added successively. The tubes were vortexed for 1–2 s, shaken for 15 min on a Rotmix RK Heto (35 r.p m.) and then centrifuged at 2500 g for 5 min at +4°C to separate aqueous and organic layers. The organic layers were placed in glass tubes and dried under nitrogen at room temperature. The residues were reconstituted in 200-μl aliquots of HPLC mobile phase. These samples were mixed for 20 s and centrifuged at 10 000 g for 3 min. The solution was transferred to a microvial and the autosampler programmed to inject 140 μl into the chromatograph.

2.7. Quantification

Using MAXIMA 820 software (Millipore), the peak height ratios of paclitaxel to internal standard were used to construct the calibration curve. The calibration curves were analysed by weighted 1/x least squares linear regression analysis. The concentration of each unknown substance was calculated from this calibration curve.

2.8. Stability study

The stability of paclitaxel in plasma was assessed from spiked samples (44 ng/ml and 750 ng/ml) after storage of aliquots at −20°C for 10 weeks. The samples were brought to room temperature, well vortexed, and analysed immediately. Each determination was performed in duplicate.

3. Results

3.1. Chromatography

Fig. 2 shows the chromatographic separation of extracts prepared from blank plasma spiked with paclitaxel, from blank plasma, and from plasma of a patient receiving paclitaxel. The approximate retention times for the internal standard and taxol were

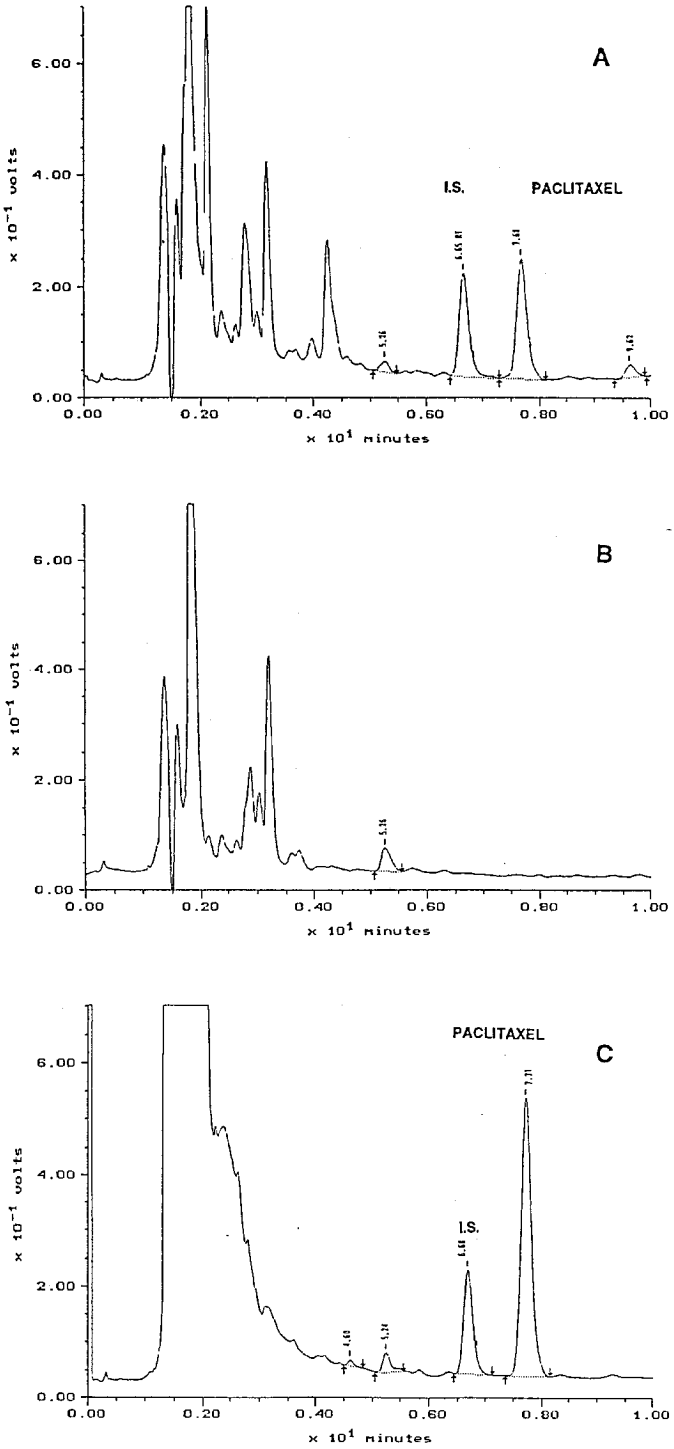

Fig. 2. Chromatograms of (A) an extracted blank plasma standard (QC) spiked with 500 ng/ml paclitaxel and 500 ng/ml docetaxel, (B) an extracted blank plasma sample and (C) an extracted plasma sample from a patient, 10 min after the end of a 350 mg/m^2 3-h infusion ([C]=19.9 μg/ml, sample=50 μl).

6.7 and 7.7 min. Taxol and the internal standard eluted as sharp symmetrical peaks. No significant endogenous peaks that could interfere with the measurement of paclitaxel or internal standard were observed.

3.2. Recovery

The recovery of paclitaxel from plasma or urine was calculated by comparison of peak heights in extracted samples with those in corresponding standard solutions. The efficiency of extraction from plasma was 82% and 92% at concentrations of 100 ng/ml and 1000 ng/ml respectively (n=2). The values for extraction from urine were 87% (n=2) and 97% (n=3) at concentrations of 100 and 500 ng/ml respectively.

3.3. Limit of detection and limit of quantification

The limit of detection (LOD) for paclitaxel in plasma (signal-to-noise of 3) was 10 ng/ml (11.5 nM). The limit of quantification (LOQ) for paclitaxel in plasma was 25 ng/ml (29 nM) (n=6; mean=24.63 ng/ml; S.D.=1.90 ng/ml; C.V.=7.72%). The LOQ is the lowest concentration that can be measured with accuracy and precision ≤20%.

In urine, the LOD was 15 ng/ml (18 nM) and the LOQ was 37 ng/ml (43 nM) (n=6; mean=37.21 ng/ml, S.D.=4.39 ng/ml, C.V.=11.80%).

3.4. Linearity

In plasma, calibration curves were linear over the concentration range 25–1000 ng/ml (29–1171 nM). The typical equation describing the standard line was: y=398R−5.22 An average correlation coefficient of r=0.9993 (n=8) was obtained (Table 2).

In urine, calibration curves were linear over the concentration range 40–1000 ng/ml (59–1171 nM): y=353R−25.7, r=0.9993 (n=8).

3.5. Intra- and inter-assay variabilities

In plasma spiked with 44, 440 and 750 ng/ml, the intra-assay variabilities (precision) were 6.34%, 2.84% and 1.54%, respectively. The inter-assay variabilities at the same concentrations (n=6) were 11.18%, 2.97% and 3.02%, respectively (Table 3).

The intra-assay variabilities of stabilized urine, spiked with 44, 440 and 750 ng/ml were 8.98%, 3.08% and 2.86%, respectively. The inter-assay variabilities of urine under the same conditions were 13.9%, 2.04% and 2.9% at concentrations of 44, 440 and 750 ng/ml respectively (Table 4). Intra-assay and inter-assay accuracies are reported in Tables 3 and 4.

3.6. Stability

After extraction from spiked plasma, the stability of paclitaxel (500 ng/ml) and docetaxel in the mobile phase at room temperature was studied. The peak height ratio of paclitaxel vs. docetaxel remained constant for at least 48 h. This is in agreement with the observations of Song and Au [14] who showed that taxol was stable in 40% acetonitrile for 24 h. Paclitaxel in QC solutions (low control 44 ng/ml and high control 750 ng/ml) stored in glass tubes at −20°C was stable for at least 2 months.

3.7. Interfering drugs

Patients participating in a Phase I clinical trials were receiving several therapeutic compounds which we investigated for possible chromatographic interference. The retention characteristics of these compounds are given in Table 5. No interference from these drugs with the peaks of paclitaxel and docetaxel was observed.

4. Discussion

The assay described here is suitable for the determination of paclitaxel in plasma and urine for clinical pharmacokinetic studies. The mobile phase was optimised to obtain the best resolution of paclitaxel and the internal standard, docetaxel. The quantity of acetonitrile (45%) gave good resolution of the two compounds in a short run time. The addition of tetrahydrofuran (5%) produced sharp symmetrical peaks and enhanced the stability of the two compounds. Storage for 1 week in the mobile phase without tetrahydrofuran caused a decreased in

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