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Short Communication

Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method

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ABSTRACT

A rapid, selective and reproducible high-performance liquid chromatographic (HPLC) method with ultraviolet detection was developed for the determination of the anti-cancer agent Taxotere in biological fluids. The method involves a solid-phase extraction step (C_2 ethyl microcolumns) using a Varian Advanced Automated Sample Processor (AASP) followed by reversed-phase HPLC. The validated quantitation range of the method is 10–2500 ng/ml in plasma with coefficients of variation $\leq 11\%$. The method is also suitable for the determination of Taxotere in urine samples under the same conditions. The method was applied in a phase I tolerance study of Taxotere in cancer patients, allowing the pharmacokinetic profile of Taxotere to be established.

INTRODUCTION

Taxotere (RP 56976, I) [4-acetoxy-2α-benzoyloxy-5β,20-epoxy-1,7β,10β-trihydroxy-9-oxotax-11-ene-13α-yl-(2*R*,3*S*)-3-*tert.*-butoxycarbonylamino-2-hydroxy-3-phenylpropionate] is a new anti-cancer agent structurally related to taxol (Fig. 1) [1,2]. It is a semi-synthetic compound obtained from a precursor extracted from the needles of the European yew (*Taxus baccata* L.). I, similarly to taxol, is an inhibitor of microtubule depolymerization [2] with a broad spectrum of anti-tumour activity in experimental models [3]. I is currently undergoing phase I clinical evaluation and we have developed a rapid and sensitive high-performance liquid chromatographic (HPLC) method for its determination in biological fluids in order to allow the pharmacokinetics of I in cancer patients to be defined. This semi-automated method involves a solid-phase extraction step with C_2 microcolumns using a Varian Advanced Automated Sample Processor (AASP) followed by HPLC with UV detection. Taxol is used as the internal standard. This method is less time-consuming and more sensitive than those previously described for taxol [4–7].

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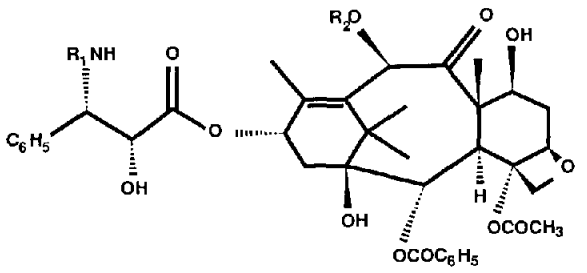

Taxotere : R_1 = -COOC(CH$_3$)$_3$; R_2 = H
Taxol : R_1 = -COC$_6$H$_5$; R_2 = -COCH$_3$

Fig. 1. Structures of Taxotere (I) and of the internal standard (taxol).

EXPERIMENTAL

Chemicals and reagents

Stock standard solutions (100 μg/ml) of I and of the internal standard taxol (RP 48725) were prepared in methanol, kept in the dark and stored at 4°C. Working standard solutions were prepared daily by dilution of the stock standard solutions in HPLC-grade water.

HPLC-grade solvents (acetonitrile and methanol) and analytical-reagent-grade orthophosphoric acid were purchased from Prolabo (Paris, France). HPLC-grade water was obtained from an Elgastat Ultra High Quality (UHQ) water purification system (Elga, UK). AASP C_2 ethyl microcolumns containing 50 mg of sorbent were purchased from Varian (Les Ulis, France).

Sample preparation

Frozen plasma samples obtained from cancer patients were first thawed at ambient temperature and then, after homogenization, centrifuged for 10 min at 1000 *g* (Centra 8, IEC, Needham, MA, USA) to remove fibrous materials and to avoid clogging the extraction column.

AASP C_2 microcolumns were activated by passing through them first 1 ml of methanol and then 1 ml of water using a 140 kPa pressure of nitrogen.

Plasma (0.5–1 ml) was mixed with 50 μl of internal standard working standard solution (3.0 μg/ml in UHQ water) and 1 ml of water–acetonitrile (70:30, v/v). After vortex-mixing, the sample was transferred to the top of the microcolumn. Nitrogen was applied until the reservoir was empty. Water (1 ml) was used to wash off plasma retained in the cartridge bed and highly polar materials adsorbed on the column. A subsequent wash was performed with 1 ml of methanol–water (50:50, v/v). A cassette of ten microcolumns was then loaded in the AASP device for further elution and injection into the analytical column.

Calibration

Plasma standards of I were prepared by spiking 1 ml of blank plasma with 50 μl of the appropriate aqueous working solution of I to achieve a final concentration range of 10–2500 ng/ml. A 50-μl volume of internal standard working standard solution (3.0 μg/ml in UHQ water) was then added and the samples were treated as described above.

Accuracy and reproducibility

The within-day accuracy and reproducibility of the method were determined by analysing six to eight aliquots of eight different concentrations on the same day. The inter-day accuracy and reproducibility were determined by assaying fourteen samples of two different concentrations over a period of six months during the analysis of patients' samples.

The HPLC system consisted of a Jasco (Tokyo, Japan) 880 PU pump, AASP device (Varian) and a Model 875 UV spectrometer (Jasco). The analytical column was a stainless-steel tube (250 mm × 4.6 mm I.D.) packed with 5-μm C_{18} Spherosil (Prolabo). The mobile phase consisted of methanol–0.3% orthophosphoric acid (67.5:32.5, v/v) and was pumped at 1 ml/min.

The sample extract was eluted and then injected into the system by the AASP device. The elution–injection step was performed on-line by passing the mobile phase through the microcolumn for 0.9 min. Prior to and after the injection step, the microcolumn was flushed with methanol–0.2% orthophosphoric acid (50:50, v/v) in volumes of 125 and 375 μl, respectively. The eluent was monitored at 225 nm and the signal

was fed into an HP 1000/900A computer (Hewlett-Packard, Sunnyvale, CA, USA) using an analogue-to-digital converter (Hewlett-Packard). Peak heights were measured by the data acquisition system (Laboratory Automation System, Hewlett-Packard), peak-height ratios were calculated and the calibration graph was determined by unweighted least-squares linear regression of peak-height ratio (I/taxol) *versus* concentration.

RESULTS AND DISCUSSION

Selectivity and specificity

Fig. 2 shows typical chromatograms of patients' plasma before and after intravenous infusion of I (85 mg/m^2). Approximate retention times of taxol and I were 10 and 11 min, respectively. No interfering endogenous peaks were detected. A linear gradient was run over 1 h starting at 70% A [methanol–0.3% orthophosphoric acid (20:80)–30% B [methanol–0.3% phosphoric acid (90:10)] and finishing at 0% A- 100% B with extracts of patients' plasma after administration of I and no metabolites susceptible to interfere with the analytes were detected. This run was performed to confirm the specificity of the method as the metabolism of I in humans has not yet been fully elucidated.

Sample preparation

Because of the wavelength required for the detection of I and taxol (225 nm), the plasma extracts have to be very clean. This was achieved through the use of a water–methanol (50:50) wash prior to the injection step, which eluted some of the interfering plasma components without affecting the retention of the compounds of interest. Higher concentrations of methanol resulted in partial loss of the analytes.

Recovery

The extraction recovery of I and the internal standard (taxol) was determined by comparing peak heights obtained from direct injections of standard solutions with those obtained after the whole extraction. The recovery of I was 82 ± 7% (n = 6) and 95 ± 2% (n = 6) at 25 and 2500 ng/ml, respectively. The recovery of taxol was 99 ± 3% (n = 12) at 150 ng/ml. The addition of water–acetonitrile to plasma samples prior to extraction increased the recoveries of both I and taxol.

Accuracy and reproducibility

The accuracy and reproducibility were satisfactory over the concentration range 10–2500 ng/ml (Table I). The within-day coefficient of variation varied between 2 and 11%. The limit of detection is 5 ng/ml (signal-to-noise ratio = 3). The day-to-day variation of quality control samples run over a period of six months was 11% at 20 ng/ml and 5% at 1000 ng/ml. Plasma levels up to 5000 ng/ml can be accurately determined using a 0.5-ml plasma sample and performing the calibration under the same conditions. The volume of plasma sample (0.5–1 ml) did not influence the recovery of either I or taxol. The inter-day variation of the linear regression slope of eleven calibration graphs was also satisfactory (slope = 0.0030 ± 0.0001, correlation coefficient > 0.998).

Stability

The stability of I in frozen human plasma was established at 20 and 1000 ng/ml using quality control samples stored at −20°C for six months.

Application of the method

The HPLC method described was used to assess the pharmacokinetics of I during a phase I tolerance study in which I was administered to cancer patients as a 1–2 h constant rate intravenous infusion at escalating doses from 5 to 115 mg/m^2 [8]. Fig. 3 shows a typical plasma concentration–time profile for patient receiving an 85 mg/m^2 dose of I. After the end of infusion, the plasma levels declined tri-exponentially with a terminal half-life of 16.5 h.

This method was also suitable for the determination of I in urine samples with the same operating procedure. The urinary excretion of unchanged I in treated patients was less than 10% of the administered dose.

The method described is less time-consuming and more sensitive than those previously de-

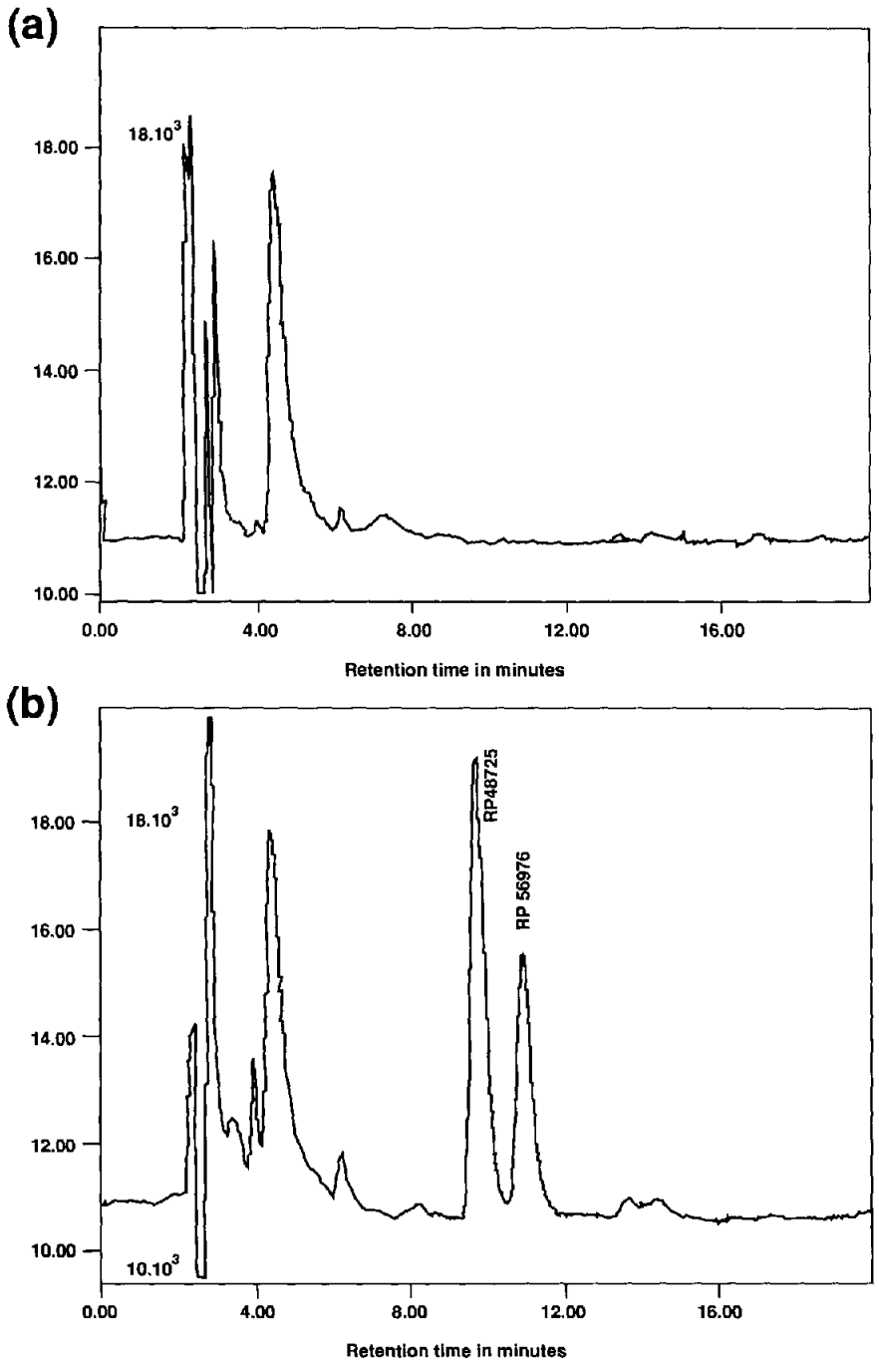

Fig. 2. Chromatograms of extracts of plasma from a cancer patient obtained (a) prior to and (b) 30 min after a 1-h intravenous infusion of I (85 mg/m^2). The concentrations in this specimen were 154 ng/ml for I and 150 ng/ml for the internal standard (taxol).

TABLE I

PRECISION AND ACCURACY OF THE METHOD

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Accuracy (%)
Within-day (n = 6–8)			
10	11.1 ± 0.5	4.1	+11.1
25	24.1 ± 2.7	11.1	−3.6
50	49.9 ± 2.0	4.1	−0.2
100	96.8 ± 1.8	1.9	−3.2
250	254.1 ± 9.7	3.8	+1.6
500	498.5 ± 15.6	3.1	−0.3
1000	1000.0 ± 41.6	4.2	0.0
2500	2509.2 ± 69.8	2.8	+0.4
Inter-day (n = 14)			
20	21.8 ± 2.3	10.6	+9.0
1000	1034.3 ± 51.4	5.0	+3.5

scribed for taxol, used here as the internal standard. The extraction procedures used for taxol involved liquid liquid extraction [4], eventually coupled with solid–liquid extraction [7]. Another approach consisted in performing a deproteinization with acetonitrile [6] coupled with solid–liquid extraction [5]. Some of the methods [4,6] required gradient elution HPLC. All these techniques need a step involving evaporation to dryness of the extraction solvent. The sensitivity of these methods was *ca.* 30 ng/ml.

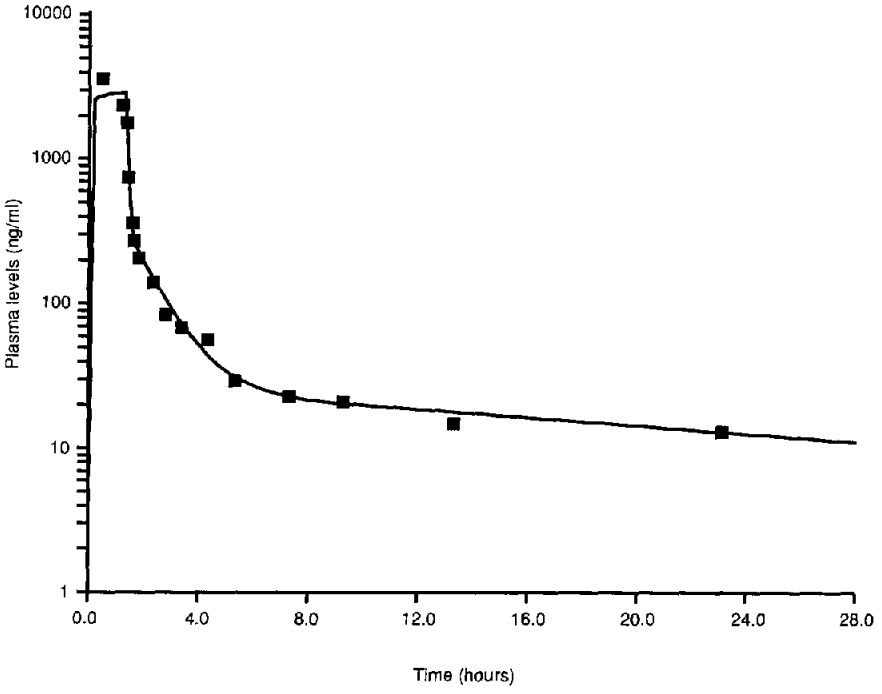

Fig. 3. Typical plasma concentration *versus* time profile of I after a 1-h intravenous infusion of 85 mg/m^2 I to a cancer patient.

Sample preparation of I can also be performed by a manual extraction procedure using C_2 Bond Elut cartridges. The elution is then performed with 250 μl of methanol–water (90:10, v/v) and an aliquot of the eluate (150 μl) is injected into the chromatographic column. This assay, which allows a sensitivity of 15–20 ng/ml to be achieve has been cross-validated with the present method and used in European and American phase I studies.

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