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A rapid reversed phase high performance liquid chromatographic method for the determination of docetaxel (Taxotere®) in human plasma using a column switching technique

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Abstract

A rapid, simple and sensitive isocratic high performance liquid chromatography (HPLC) method was developed to measure the concentration of docetaxel in plasma samples with UV detection at 227 nm. The method uses a column switching technique with an Ultrasphere C_{18} column (75 × 4.6 mm ID, 3μ, Altex, USA) as clean-up column and a CSC-nucleosil C_8 column (150 × 4.6 mm ID, 5μ, CSC, Montreal, Canada) as the analytical column. The mobile phase consisted of Phosphate buffer (30 mM, pH = 3)-acetonitrile (47:53, v/v) with the flow rates of 1.1 and 1.3 ml min^{-1} for clean-up and analytical columns, respectively. Paclitaxel was used as an internal standard. The plasma samples were extracted using a solid phase extraction method with Ammonium acetate (30 mM, pH = 5)-acetonitrile (50:50, v/v) as final eluent. The extraction method showed a recovery of 92% for docetaxel. In this system, the retention times of docetaxel and Paclitaxel were 7.2 and 8.5 min, respectively. The detection limit of docetaxel in plasma is 2.5 ng ml^{-1}. This analytical method has a very good reproducibility (7.2% between-day variability at a concentration of 10 ng ml^{-1}). It is applicable in clinical and pharmacokinetic studies. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Reversed phase chromatography; HPLC; Column switching; Docetaxel; Paclitaxel; Plasma

1. Introduction

Docetaxel (N-debenzoil-N-tert-butoxycarbonil-10-deacetyl taxol) (Taxotere®), is a semisynthetic analogue of paclitaxel, prepared from a non cytotoxic precursor extract from the needles of the European yew tree (*Taxus baccata L.*) [1]. It is an inhibitor of microtubule depolymerization and has a broad antitumour activity [2]. It is currently in phase II/III clinical trials in the USA, Europe and Japan [3,4].

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The clinical pharmacokinetics of docetaxel have been reported [5]. Its disposition profile is multiphasic, with rapid initial tissue uptake and large distribution volume. Due to its multi compartmental behaviour and large volume of distribution, a sensitive analytical method is of great importance. Different high performance liquid chromatographic (HPLC) methods for analysing docetaxel or paclitaxel have been described previously, however, the run times of these methods are excessively long, mostly due to the presence of late eluting peaks in biological fluids [6,7]. Attempts have been made to avoid this problem by a long sample treatment procedure [8], or automated sample treatment [9]. This paper describes a practical, specific and sensitive HPLC assay method for docetaxel in plasma using solid phase extraction (SPE) followed by an isocratic column switching HPLC technique.

2. Materials and methods

2.1. Materials

Docetaxel was donated by Rhône-Poulenc Rorer (Paris, France). Paclitaxel solution (Taxol®) was used as an internal standard. Acetonitrile and methanol were of HPLC grade. Analytical grade ammonium acetate, mono potassium hydrogen phosphate and distilled deionized water were used throughout the study. The C_2 microcolumn used for solid phase extraction was purchased from Varian (Mississauga, Canada).

2.2. Methods

2.2.1. Sample preparation

One hundred µl of internal standard solution (Paclitaxel in methanol, 1.5 µg ml^{-1}) and 1 ml acetonitrile–water (30:70, v/v) were added to 900 µl plasma and vortexed for 5 s. With the help of a vacuum, the mixture was then passed through preconditioned C_2 microcolumn (the microcolumn was preconditioned with 1 ml of methanol and 2 ml of water). The microcolumn was then washed with 1 ml of water and 1 ml of methanol–water (50:50). Finally, docetaxel and paclitaxel were eluted by 1 ml of ammonium acetate (30 mM, pH = 5)-acetonitrile (50:50, v/v), while the first 5 drops of elution solvent were not collected. Of the final elution solvent, 150 µl was injected in to the HPLC column.

2.2.2. Chromatography system

The HPLC system consisted of two Shimadzu LC-6A pumps (Shimadzu, Tokyo, Japan), a SPD-6AV Shimadzu UV-VIS Detector adjusted at 227 nm, a C-R5A Shimadzu integrator, a SIL-6A Shimadzu autoinjector, a Shimadzu SCL-6A system controller and a Shimadzu FCV-2AH flow-channel selection valve. The analysis system included two columns: an Ultrasphere C_{18} column (75 × 4.6 mm ID, 3µ, Altex, Berkeley, USA) as a clean-up column, and a reversed phase CSC- nucleosil C_8 (150 × 4.6 mm ID, 5µ, Chromatography Sciences, Montreal, Canada) as an analytical column. The mobile phase was a mixture of Phosphate buffer (KH_2PO_4, 30 mM, pH = 3)-acetonitrile (47:53, v/v) with a flow rate of 1.1 and 1.3 ml min^{-1} through the clean-up and analytical columns, respectively. The flow-channel selection valve allowed the clean-up column to be on-line to the analytical column only within 2.2–4 min of a run to avoid late eluting peaks reaching the analytical column. A Bonda-Pak C_{18} guard column (Waters, Mississauga, Canada) was used before clean-up column to protect it from strongly bonded matters.

3. Results and discussion

Fig. 1 shows typical chromatograms of blank plasma and plasma spiked with docetaxel and paclitaxel. As shown in this figure, there is a very good resolution between these two substances. There are small peaks related to endogenous compounds after paclitaxel peak. They were greater when the mobile phase was used as a final eluent in solid phase extraction. However, they were smaller when another suitable solvent was used as the final eluent. We used ammonium acetate (30 mM, pH = 5)-acetonitrile (50:50, v/v) rather than mobile phase to extract docetaxel and paclitaxel from the extraction microcolumn. The extraction

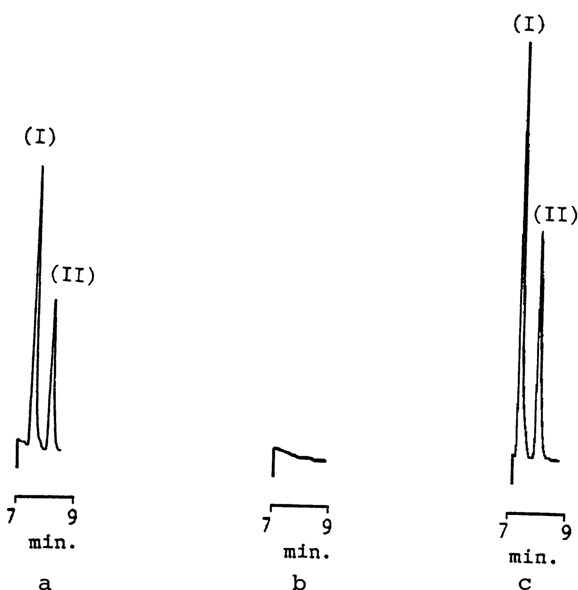

Fig. 1. Chromatograms of docetaxel extracted from plasma samples: (a) spiked plasma; (b) plasma sample of a patient before drug injection; (c) plasma sample of the same patient one hour after injection. The retention time for docetaxel (I) and paclitaxel (II) are 7.2 and 8.5 min., respectively. The integrator started 7 min. after injection.

procedure involved an initial volume of 1 ml plasma and ended with approximately 750 μl of final extraction solvent. However, the recovery of docetaxel in the extraction decreased from 95 to 92 ± 2% and the final volume decreased from 1 ml to 750 μl, since the first five drops of final extraction solvent contained a negligible amount of docetaxel and paclitaxel, the concentration of docetaxel is still higher in the final extract, and a lower concentration of docetaxel in plasma sample than previous reported methods is measurable.

The recovery of internal standard with the final plasma concentration of 150 ng ml^{-1} was 97 ± 2%, which is comparable to the recovery of docetaxel. The retention time of docetaxel and paclitaxel was 7.2 and 8.5 min, respectively, which is shorter than the 23 min reported by Di Song et al. [6] for the detection of paclitaxel in plasma using a column switching technique, and 11 min reported by Vergniol et al. [9].

The late eluting hydrophobic endogenous compounds are a major problem in assays of docetaxel and paclitaxel by HPLC. Different columns to develop a simple assay method for docetaxel in biological fluids were also tried. In spite of a good resolution between docetaxel and paclitaxel and various endogenous interfering compounds, there was still the problem of late eluting peaks after the fourth plasma sample injection. The column switching technique enabled us to have a short run time while avoiding these late eluting peaks in the analytical column.

The use of the 7.5 cm long clean-up column which needed a shorter time to be cleaned during the run time allowed us to have a very short run time without late eluting peaks problem. The clean-up column was on-line with the analytical column during 2–2.4 min of each run, and the sample was introduced to the analytical column for separation. The C_8 nucleosil column which we used as the analytical column, showed a very good resolution between docetaxel and paclitaxel and enabled us to use a high percentage of acetonitrile in the mobile phase (less polar). This led to a reduced band broadening effect resulting from the column switching technique and also a remarkable decrease in retention time and detec-

Table 1
Reproducibilty of the assay method in one and different days (aqueous and plasma samples)

		Aqueous solution		Plasma samples	
Conc. (ng/ml)	*n*	W-d[a] CV%	B-d[b] CV%	W-d[a]CV%	B-d[b] CV%
10	6	3.5	7.2	4.1	7.5
100	6	1.3	4.8	2.5	5.3
300	6	1.2	4.5	2.4	5.0
600	6	1.7	3.9	3.9	6.1
1000	6	1.0	3.1	4.2	8.2

[a] Within-day; [b] Between-day.

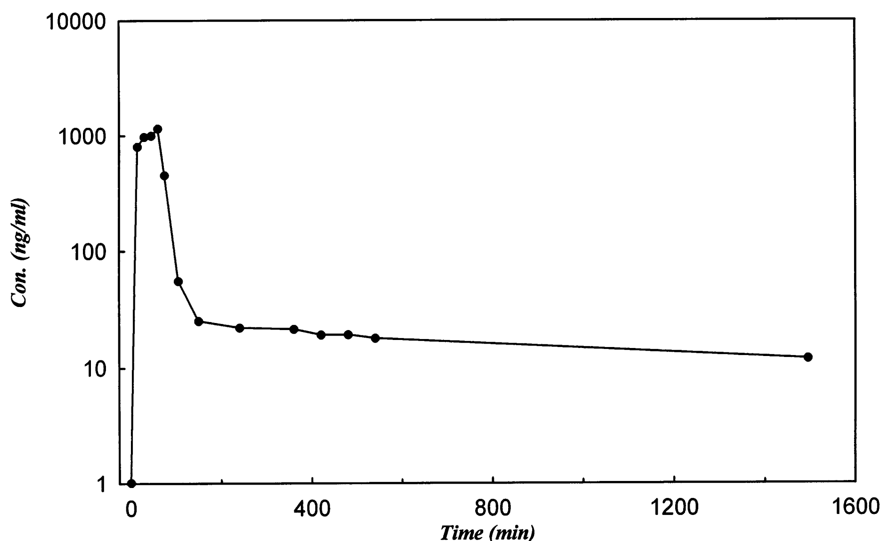

Fig. 2. Plasma-Concentration time profile of patient who received 118 mg docetaxel over one hour as intravenous infusion.

tion limit. The total run time of this method was 9 min, after which, the system was ready for the next injection without needing more wash time. In this system, the retention times of docetaxel and Paclitaxel were 7.2 and 8.5 min, respectively. Using this method, the detection limit was decreased to 2.5 ng ml^{-1} and the limit of quantification to 5 ng ml^{-1}, with a signal to noise ratio of 3 in plasma samples.

Vergniol et al. [9] described a simple isocratic method with a run time of approximately 11 min. However, it required an advanced automated sample processor (AASP), and their method is still less sensitive than ours (detection limit of 5 ng ml^{-1}). Their detection limit increases to 15–20 ng ml^{-1} in manual sample treatment. Column switching was also required to take care of late eluting peaks coming long after major peaks.

Table 1 shows reproducibility of the method in aqueous solution and plasma samples. The calibration curve of data obtained from different day experiments (data obtained during one month), shows a correlation coefficient of 0.996. The CV% for lowest and highest concentrations tested are 7.2 and 3.1%, respectively.

No decrease in peak area of docetaxel during one month of storage in -20°C was detected. Calibration of data obtained from aqueous samples in a one-day experiment in the range of 5–2000 ng ml^{-1}, with plasma samples in the range of 2.5–2000 ng ml^{-1}, have equations: $y = 0.004x - 0.02$, with $r = 0.994$ and $y = 0.004 + 0.002$, with $r = 0.994$, respectively.

Fig. 2 shows the plasma concentration-time profile of a patient having received 118 mg docetaxel at 1 h periods by intravenous infusion.

In conclusion, this described method is a fast, simple, sensitive and reproducible assay for docetaxel. It could be used for the detection of docetaxel in clinical and pharmacokinetic studies.

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