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# Determination of paclitaxel in biological fluids by micellar electrokinetic chromatography

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**Abstract**

A method has been developed for the determination of paclitaxel (Taxol) in plasma and urine using capillary electrophoresis with sodium dodecyl sulfate as additive in the run buffer. The samples are extracted and preconcentrated with *tert.*-butyl methyl ether. Taxotere has been used as the internal standard. The limit of detection for paclitaxel is 20 ng/ml. In comparison to high-performance liquid chromatography, the capillary electrophoresis method is simple and needs less organic solvents.

*Keywords:* Paclitaxel; Taxol; Taxotere

## 1. Introduction

Paclitaxel (Fig. 1a; trade name Taxol) is a novel anticancer drug isolated from the bark of *Taxus brevifolia*. It shows activity against a wide range of tumor types and phase I studies have been carried out in children with solid tumors [1]. Paclitaxel displays nonlinear pharmacokinetics [2]. This means that there is no linear relationship between dose and the peak plasma concentrations. Thus, the plasma concentrations should be monitored in children to prevent side effects from overdosing. Our aim is to develop a limited sampling model to calculate the individual pharmacokinetic data from only a few plasma samples. For the determination of paclitaxel in plasma and urine, several methods using high-performance liquid chromatography (HPLC) are described [3–7]. All these methods require much organic solvents. Chan et al. [8] used capillary electrophoresis (CE) with sodium dodecyl sulfate (SDS) as additive in the run buffer for the separation of paclitaxel and related compounds in plant extracts from *Taxus* species, but not in human plasma or urine.

CE is a very promising technique for the determination of drugs in biological fluids. In comparison to HPLC, the method shows a higher separation power and is more insensitive to endogenous compounds from the biological matrix [9]. In CE, only a few nanoliters of the sample are necessary. However, due to the small sample volumes, the sensitivity is often not sufficient for the determination of drugs in plasma. For paclitaxel, plasma concentrations are in the range of about 50 to 10 000 ng/ml for the first 24 h after a 3 h infusion of 250 mg/m$^2$ [2,10]. To achieve a sufficient sensitivity, we have modified the separation conditions of Chan et al. [8] by changing the buffer conditions to get sharper peaks. Furthermore, a capillary with an extended detector window [11] (bubble cell) has

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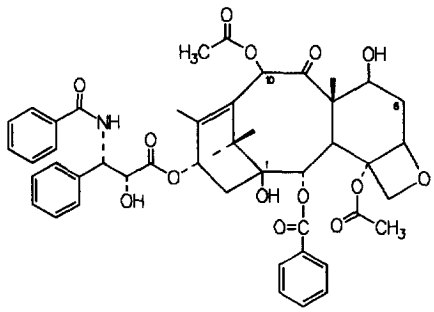
a: Paclitaxel



b:Taxotere

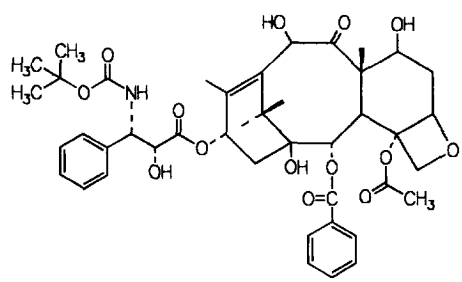


Fig. 1. Chemical structures of the compounds involved.

been used. The samples are preconcentrated ten-fold by liquid–liquid extraction using *tert*-.butyl methyl ether. To date, the method is used to investigate the pharmacokinetics in children treated with paclitaxel.

## 2. Experimental

### 2.1. Chemicals

Paclitaxel was provided by Bristol Myers-Squibb, Munich, Germany. Taxotere was kindly supplied by Rhône-Poulenc Rorer, Vitry sur Seine, France. All solvents used were of HPLC grade. Water was prepared with a Milli-Q-Plus unit (Millipore, Eschborn, Germany). All solutions for CE were filtered through a 0.45 $\mu$m cellulose acetate filter.

Stock solutions of paclitaxel and the internal standard were prepared in methanol and stored at −18°C. Standard solutions were prepared by dilution of the stock solution with the electrophoresis buffer every week. Solutions for calibration and quality control samples were prepared by spiking blank human plasma with the standard solutions to achieve concentrations from 50 to 5000 ng/ml. For urine samples the concentrations of the solutions for calibration were three times higher. Here standard solutions were prepared with blank urine.

### 2.2. Capillary electrophoresis

A Beckman P/ACE 5510 system (Beckman Instruments, Munich, Germany) equipped with an Beckman UV detector and a 230 nm filter was used. A 80.5 cm×50 $\mu$m I.D. long, uncoated fused-silica capillary with an extended light path (Hewlett-Packard, Waldbrunn, Germany) was cut off at 67 cm and inserted in a Beckman capillary cartridge (effective length 60 cm). Samples were introduced into the capillary by pressure injection with 0.5 p.s.i. (1 p.s.i.=6894.76 Pa) for 12 s. The electrophoresis buffer was prepared as follows: 100 mmol/l SDS was dissolved in a 25 m*M* Tris–phosphate buffer (pH 8.5). To this solution, 35% (v/v) of acetonitrile (ACN) was added. After each run the capillary was flushed with 0.1 *M* NaOH for 2 min and the electrophoresis buffer for 3 min. The applied voltage was 418 V/cm.

### 2.3. Sample preparation

To 0.5 ml of plasma, 50 $\mu$l of a solution of taxotere (18.8 $\mu$g/ml in methanol), 450 $\mu$l of water and 4 ml of *tert*.-butyl methyl ether were added. The mixture was shaken for 2 min and stored at −18°C until the bottom layer was frozen. The organic layer was decanted and evaporated under a stream of nitrogen at 35°C. The residue was reconstituted in 50 $\mu$l of the electrophoresis buffer. Urine samples were prepared in the same way. Here 200 $\mu$l urine was diluted by adding 50 $\mu$l of the internal standard solution and 750 $\mu$l of water before extraction.

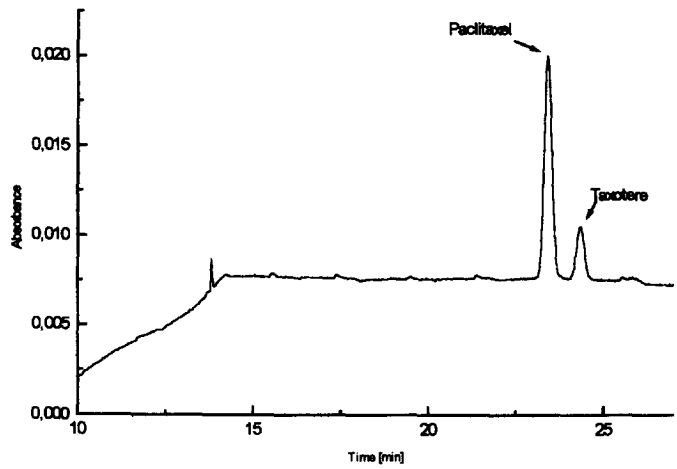
## 3. Results and discussion

### 3.1. Capillary electrophoresis

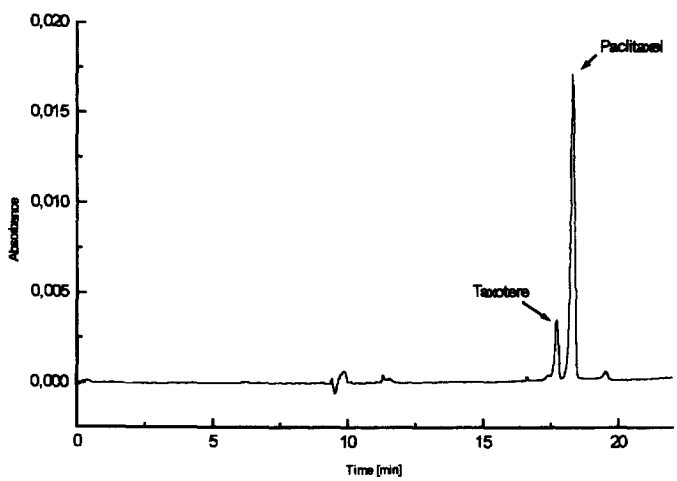
Initially the method of Chan et al. [8] was used, but under these conditions paclitaxel was not sepa-

rated from endogenous plasma constituents. By increasing the concentration of SDS a good separation was achieved. To reduce the analysis time we used a 67 cm capillary instead of a 87 cm capillary. The sensitivity was improved three-fold using a capillary with an extended light path (bubble cell). However, in comparison to a normal 50 $\mu$m capillary the separation was slightly deteriorated.

Most of the procedures described for the determination of paclitaxel use the external standard method for quantification [3–5,7]. Others use an internal standard with a chemical structure not related to paclitaxel [6]. Due to the small sample volumes in CE, the reproducibility and accuracy of the quantification can often be improved by using an internal standard. Taxotere (Fig. 1b) was chosen as




a: pH 8,5, 100mM SDS, 22 % ACN




b: pH 8,5, 100mM SDS, 35 % ACN

Fig. 2. Separation of paclitaxel under different conditions. Concentration: paclitaxel 50 $\mu$g/ml and taxotere 18.8 $\mu$g/ml dissolved in run buffer.

the internal standard because of its similar structure and its similar behavior in the extraction and electrophoresis.

To achieve a good separation between paclitaxel and taxotere, the amount of ACN in the run buffer was modified. With 22% ACN, the two compounds are completely resolved, but the peak shape was not sufficient (Fig. 2a). By increasing the amount of ACN to more than 30%, the migration order of paclitaxel and taxotere is reversed (Fig. 2b), possibly indicating a change in the structure of the SDS micelles.

### 3.2. Reproducibility of the assay

#### 3.2.1. Linearity

Plasma spiked with six different concentrations of paclitaxel and 18.8 μg/ml of the internal standard (I.S.) were extracted and analysed in duplicate. A linear correlation was found between the peak-height ratios of paclitaxel and the I.S. and the concentration in the range from 0.05 to 5 μg/ml (slope: 0.89301±0.01888, intercept: 0.07294±0.04439, correlation coefficient: 0.9959). Without I.S. the linearity was not sufficient ($r \leq 0.99$) due to deviations from the extraction procedure and the injection onto the capillary.

#### 3.2.2. Recovery

The extraction efficiency was determined by analysing plasma and urine samples spiked with three different concentrations of the analyte and comparing the peak heights to peak heights from standard solutions (external standard method). The results are shown in Table 1. The mean recovery for paclitaxel was found to be about 74% for both plasma and urine. All calculations from patient samples were carried out with calibration graphs prepared with spiked plasma or urine solutions, respectively, using the internal standard method. Thus, deviations in the recovery from day to day do not influence the results.

Table 1
Recovery of paclitaxel from plasma and urine

| Matrix | Conc. added (μg/ml) | Conc. found (μg/ml) | Recovery (%) | R.S.D. (%) | n |
|---|---|---|---|---|---|
| Plasma | 5 | 3.76 | 75.2 | 12.1 | 6 |
| | 2 | 1.63 | 78.6 | 9.1 | 5 |
| | 0.1 | 0.0713 | 71.3 | 16.9 | 5 |
| Urine | 10 | 7.89 | 78.9 | 6.9 | 4 |
| | 5 | 3.41 | 70.9 | 6.9 | 4 |
| | 0.5 | 0.35 | 70.0 | 10.2 | 4 |

Table 2
Precision and accuracy of the assay (plasma samples)

| Concentration added | Mean Conc. found (μg/ml) | Accuracy (%) | R.S.D. (%) | n |
|---|---|---|---|---|
| 2.8100 | 2.7369 | 97.40 | 3.29 | 5 |
| 0.7030 | 0.6927 | 98.54 | 3.03 | 5 |
| 0.0937 | 0.0862 | 91.98 | 17.46 | 5 |

#### 3.2.3. Precision and accuracy

Plasma samples spiked with three different concentrations of paclitaxel were analysed with five replicates and the concentrations were calculated from the peak-height ratios of paclitaxel and the I.S. The results are shown in Table 2. The precision and accuracy should always be within ±15% for bioanalytical methods except at the lower limit of quantification, where they should not deviate by more than 20% [12]. Variations in the migration times are a common problem in CE. As pointed out by Thormann et al. [9], detection times are unreliable parameters for the identification of peaks because different matrices lead to different detection times. The relative standard deviations of the migration times are shown in Table 3. Due to matrix effects, deviations of up to 5% are common. However, using the relative migration time calculated from the I.S., the identification of the paclitaxel peak is possible. In micellar electrokinetic chromatography, proteins should not influence the reproducibility of the assay [13]. In contrast, we observed a slight increase in the migration times after injection of several plasma samples.

Table 3
R.S.D.s for the migration times on one day ($n=6$)

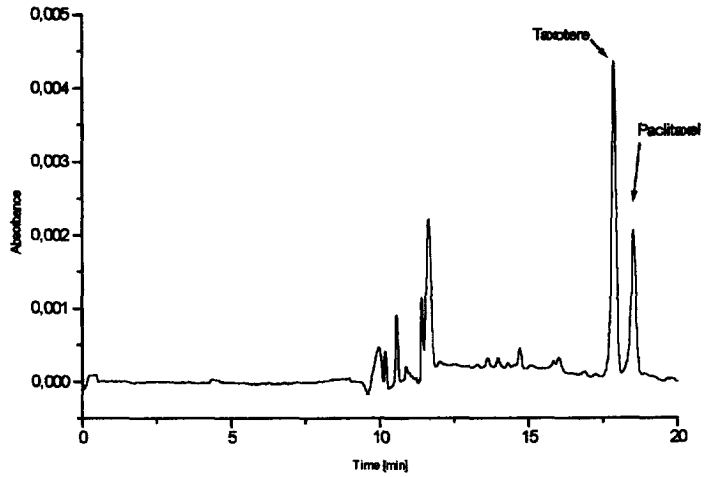
| Matrix | R.S.D. (%) | | |
|---|---|---|---|
| | Paclitaxel | I.S. | Paclitaxel/I.S. |
| Plasma | 4.48 | 4.35 | 0.34 |
| Urine | 3.56 | 3.53 | 0.06 |
| Buffer | 1.00 | 0.93 | 0.08 |

### 3.2.4. Sensitivity

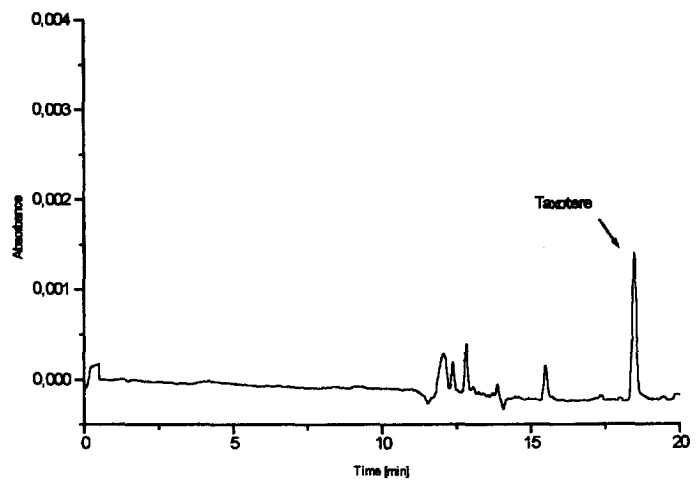
The limit of detection, defined as the concentration where the signal-to-noise ratio is 5 was found to be 20 ng/ml for plasma and 50 ng/ml for urine. The limit of quantification, defined as the lowest concentration which can be measured with acceptable precision and accuracy, was determined to be 50 ng/ml for plasma and 125 ng/ml for urine samples.

### 3.3. Application to patient plasma and urine samples

Fig. 3a shows an electropherogram from plasma of a patient after treatment with 170 mg/m$^2$ paclitaxel as a 3 h infusion. In blank plasma, no interfering peaks were observed (Fig. 3b). Most of the plasma constituents migrate slower than the analyte and the



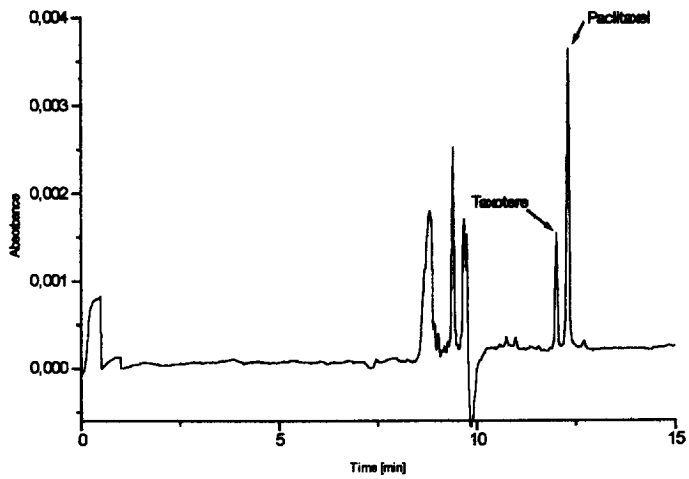

a: Plasma 5 hours after the end of infusion. Paclitaxel conc. in plasma 0.40 µg/ml



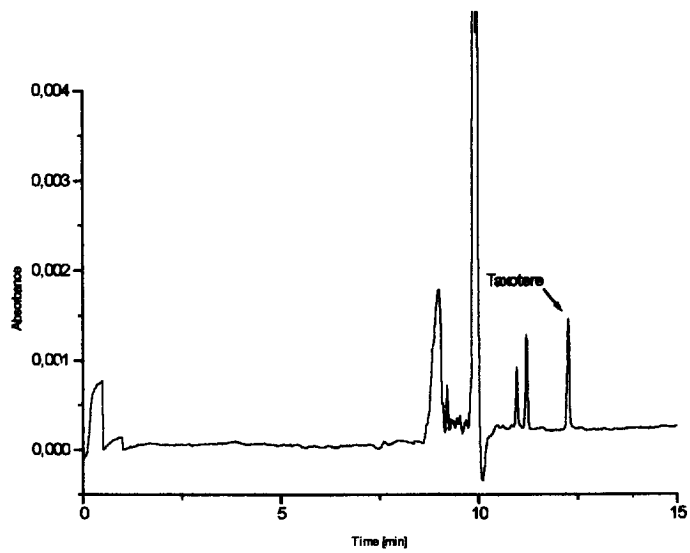

b: Blank plasma

Fig. 3. Electropherograms of patient plasma samples. For conditions see Section 2. Concentration: taxotere 18.8 $\mu$g/ml.

I.S., so that the analysis can be stopped immediately after the paclitaxel peak. Some problems arose with deviations in the migration times possibly due to proteins adsorbing to the capillary wall. Therefore, the capillary was rinsed with sodium hydroxide for two min and with the run buffer for three min between each run. Fig. 4 shows electropherograms of urine samples. In comparison to plasma, the urine samples contain no compounds, which might interfere with the analyte. Thus, a shorter analysis time




a: Urine from 3 to 6 hours after end of infusion. Paclitaxel conc. 12 µg/ml




b: Blank urine

Fig. 4. Electropherograms of urine. Conditions: capillary length 40/47 cm, buffer pH 8.5, 100 m*M* SDS, 35% ACN, 20 kV; concentration taxotere 18.8 $\mu$g/ml.

was achieved using a shorter capillary and different electrophoresis conditions. Currently, validation work with urine is in progress.

To date, we are investigating samples from children treated with different schedules of paclitaxel. The results will be reported elsewhere.

## 4. Conclusion

A simple and sensitive method for the determination of paclitaxel in plasma has been developed. The precision and accuracy is good and the limit of detection in plasma is 20 ng/ml. Thus, plasma concentrations of paclitaxel can be monitored up to 36 h after the end of an infusion. Furthermore, paclitaxel can be determined in urine with this method. Under these conditions, paclitaxel metabolites should also be detectable.

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