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Short communication

On-line solid-phase extraction and determination of paclitaxel in human plasma

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Abstract

The application of coupled-column liquid chromatographic analysis to pharmacokinetic studies eliminates the need for sample clean-up from plasma. Considering lipophilic antineoplastic agents, we tested this approach to analyze paclitaxel under unfavourable circumstances (i.e., weekly low-dose regimen, plasma protein binding >90%, UV detection at 229 nm). The excellent quality control data (recovery: 95.6–100.7%, inter-assay relative standard deviation on 5 days: 1.3–3.2%, accuracy: 0.9–2.7%) and the detection limit of 19 n*M* indicates the usefulness of this method for the analysis of paclitaxel in plasma using on-line solid-phase extraction.

Keywords: Paclitaxel

1. Introduction

The applications of clinical pharmacokinetics to oncologic studies are limited by the substantial efforts needed for the acquisition of reliable analytical data. It is well known that sample clean-up is a time consuming step, which is prone to errors when complex purification procedures are required. In addition to the inevitable loss of analyte during sample clean-up, methods without internal standard calibration may be challenging to standardize.

One possible alternative is the direct injection of plasma samples onto coupled-column liquid chromatographic systems combining on-line solid-phase extraction and analytical separation. Using a column-switch technique, the analyte is quantitatively adsorbed on a restricted access silica pre-column packing such as alkyl-diol silica (ADS), a spherical silica gel possessing two different chemical binding surfaces. The pore size of this material excludes plasma proteins, whereas the selective retention of lipophilic analytes occurs on the hydrophobic internal surface. In the next step, the analytes are transferred to the analytical column and monitored after separation. This method has been successfully applied to analyze a variety of drugs in biological fluids [1] including the enantioselective analysis of atenolol [2].

One of the first drugs analyzed by on-line solid-phase extraction were epirubicin and its metabolites by Rudolphi et al., who pioneered the use of ADS materials in clinical applications [3]. In general, the cytostatic compound epirubicin and other anthracyclines are analyzed in plasma after solid-phase

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extraction, solvent evaporation and injection onto a high-performance liquid chromatography (HPLC) system after reconstitution of the sample [4,5].

The lipophilic nature of the anthracyclines is a common characteristic of several anticancer drugs and may thus be exploited for the on-line analysis from biological fluids. Since anthracyclines are easily monitored by fluorescence detection, we questioned the possibility of this approach to analyze paclitaxel. This drug is monitored in the low-UV range and has been analyzed in plasma using solid-phase extraction techniques [6,7]. To prove the feasibility of the coupled-column liquid chromatographic analysis for paclitaxel in plasma, we present a reliable on-line technique to evaluate a drug characterized by (i) a moderate molar absorptivity, (ii) an absorption maximum in the low-UV range, i.e., 229 nm, which is (iii) administered in a low dose regimen (70 mg paclitaxel/m^2) when compared with standard regimens (175 mg paclitaxel/m^2 and higher), and (iv) a plasma protein binding >90%. Even under these conditions, coupled-column liquid chromatographic analysis of paclitaxel in plasma has proven to be an excellent tool for pharmacokinetic investigations.

2. Experimental

2.1. Chemicals and reagents

Paclitaxel was obtained as a reference substance for chromatography (ICN Pharmaceuticals, CA, USA) and for therapeutic administrations (kindly supplied by EBEWE Pharmaceuticals, Austria). Water, acetonitrile, and methanol were of HPLC grade (LiChrosolv, Merck, Germany); ammonium acetate and glacial acetic acid were of analytical grade (Merck).

Solvent A was a mixture of methanol–water (5:95, v/v), solvent B was 20 m*M* ammonium acetate buffer (adjusted to pH 5.0 with glacial acetic acid)–acetonitrile (50:50, v/v).

2.2. Plasma samples

Plasma samples were obtained from patients with advanced breast cancer participating in a pharmacokinetic dose escalation study with weekly administration of epirubicin (starting at 20 mg/m^2) and paclitaxel (starting at 70 mg/m^2). Volumes (3 ml) of blood were collected in tubes containing EDTA as anticoagulant. Plasma was separated from blood immediately by centrifugation (5 min at 1300 *g*) and stored in polypropylene tubes under light protection at −20 °C until analysis.

2.3. Preparation of calibration standards

7 m*M* paclitaxel was diluted in methanol–water (solvent A) to yield concentrations of 50, 100, 1000, and 10 000 n*M*. The same concentrations of paclitaxel in plasma were obtained by using fresh frozen plasma as a diluent.

2.4. Equipment

A Model 1090M liquid chromatograph from Agilent equipped with a diode-array detector was used in combination with an automatic column-switching valve (Model 2870; Vici, Switzerland) as previously described [3]. The sample was loaded onto the C_4-ADS clean-up column (LiChrospher RP4-ADS, 25 μm particles, 25×4 mm I.D.; Merck) using an isocratic pump (Model 305; Gilson, France). The analytical column and the protecting pre-column were filled with C_8 reversed-phase material (LiChrospher 100 RP-8e, 5 μm particles, 250×4 mm I.D. and 4×4 mm I.D., respectively; Merck). An additional in-line filter was used to protect the analytical system from solid particles (Merck).

2.5. HPLC method and chromatographic conditions

Frozen samples were thawed under light protection, vortex-mixed, and centrifuged 2 min at 20 000 *g*. A 100-μl volume from the clear supernatant was directly injected onto the HPLC system and loaded onto the C_4-ADS clean-up column with a constant flow of 1 ml solvent A (methanol–water)/min. After column-switching 10 min later, paclitaxel was transferred in backflush mode onto the analytical column delivering solvent B (ammonium acetate buffer–acetonitrile) at a constant rate of 1.2 ml/min. The analyte was then eluted isocratically with a typical

retention time of 10.7 min (20.7 min including the sample loading time onto the C_4-ADS clean-up column) from the analytical C_8 column and monitored at a wavelength of 229 nm (bandwidth: 10 nm). The overall analysis time of 25 min was then completed by a post-run interval of 5 min (conditioning of the clean-up column) prior to the following injection.

To increase sample throughput, reconditioning of the C_4-ADS clean-up column may start 5 min after the transfer of the analyte thus eliminating the necessity of a post-run interval.

3. Results and discussion

3.1. Sample loading

Plasma samples were loaded onto the C_4-ADS clean-up column using 5% methanol in water (solvent A). Although concentrations up to 15% methanol in water did not elute paclitaxel from the C_4-ADS material within 60 min, there was no difference in the elution profile of the plasma matrix when comparing 5, 10, and 15% methanol in water. To avoid the precipitation of plasma proteins, 15% methanol in water is the upper limit of organic solvent for the loading of human plasma as suggested by the manufacturer.

3.2. Linearity of the standard curve

The standard curve was linear up to 10 000 n*M* paclitaxel in methanol–water (diluent A) and in plasma, when assaying four calibration standards. Each calibrator was injected five times. The coefficient of correlation was 1.0 in both matrices when calculated at an accuracy of four digits.

3.3. Recovery

Comparing the calibration standards in methanol–water (diluent A) with that in plasma, the mean overall recovery was 98.8%. The recoveries of the single calibrators are shown in Table 1.

Table 1
Recovery of paclitaxel from plasma ($n=5$)

Concentration of paclitaxel (nmol/l plasma)	Recovery (%)
50	99.8
100	100.7
1000	99.5
10 000	95.6

3.4. Repeatability

The repeatability of the method was estimated by evaluation of the calibration standards in plasma within a sequence ($n=5$) and on 5 consecutive days. The relative standard deviation (RSD) was below 3% with one exception only (Table 2).

3.5. Accuracy

The accuracy of the method was proven by analysis of four concentrations of paclitaxel in plasma on 5 consecutive days. The mean overall accuracy of the method was +1.4% deviation from the target value (Table 3).

3.6. Sensitivity

Considering a signal-to-noise ratio of 3:1, the limit of detection was calculated to be 19 nmol paclitaxel/l plasma (=11 ng/ml). Since 50 nmol paclitaxel/l plasma was already accurately and precisely evaluated by the method, the limit of quantification was estimated to be lower than 50 n*M*.

3.7. Specificity

When assaying plasma samples from patients participating in the pharmacokinetic study, no interferences from plasma with paclitaxel were observed (Fig. 1). Moreover, concomitant medications, e.g., dexamethasone, serotonin antagonists such as ondansetron and tropisetron, diazepam, tramadol, ranitidine, and omeprazole did not interfere with the analysis of paclitaxel. Due to the nature of the chromophore of paclitaxel with a single absorption maximum at 229 nm, we were not able to check the peak purity by assessing the ratio of two different wavelengths. When analyzing paclitaxel in plasma

Table 2
Repeatability of the analytical method ($n=5$)

Concentration of paclitaxel (nmol/l plasma)	Intra-assay variance (RSD, %)	Inter-assay variance (RSD, %)
50	1.5	1.3
100	0.7	2.0
1000	1.6	3.2
10 000	0.3	2.4

Table 3
Accuracy of the analytical method ($n=5$)

Concentration of paclitaxel (nmol/l plasma)	Mean result of the analysis (nmol/l plasma)	Accuracy (%)
50	51.4	+2.7
100	101.2	+1.3
1000	1006.7	+0.7
10 000	10 094.7	+0.9

samples, however, the spectra recorded at the inflection points and the apex of the peak were completely congruent with that of the reference compound. Together with the quality control data, this strongly suggests this method to be specific for paclitaxel.

3.8. Application and limits of the analytical method

This method has been developed to reliably detect paclitaxel when administered under a weekly low-dose regimen (70 mg paclitaxel/m^2 given as an i.v. infusion with a duration of 3 h). Using this protocol, we were able to detect paclitaxel up to 44 h after the start of the infusion, which definitely suffices to calculate the pharmacokinetic parameters of the drug [8]. Considering the low dose of paclitaxel and the small amount of the plasma sample (100 μl), the metabolites (hydroxypaclitaxel derivatives of the parent drug) could not be detected. This would require higher sample volumes and higher paclitaxel doses (175 mg/m^2) as shown by Huizing et al. [7].

The direct injection of 500 μl plasma has been suggested using this on-line technique [9]. Injecting large sample volumes, one may then expect to detect metabolites of paclitaxel, which circulates only in minor amounts in blood. The increased injection volume, however, considerably shortens the lifetime of the ADS pre-column [10].

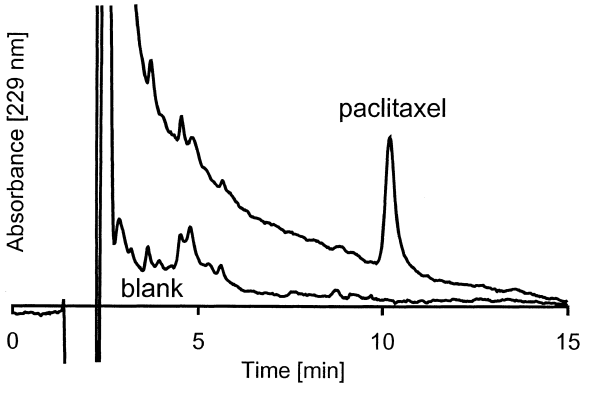

Fig. 1. Chromatograms of patient samples. Plasma blank vs. plasma sample taken during i.v. infusion of 70 mg paclitaxel/m^2 (infusion time: 3 h).

4. Conclusions

Coupled-column liquid chromatography allows for the on-line solid-phase extraction and analysis of paclitaxel in human plasma. Even under difficult circumstances such as low-dose regimens and unfavourable detection conditions, the merits of the described analytical method are a rapid evaluation combined with an excellent performance.

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