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Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry: A practical solution for non-specific binding

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ABSTRACT

A rapid and sensitive liquid chromatography/tandem mass spectrometry (LC–MS/MS) method has been developed and validated for the quantitative determination of cabazitaxel, a novel tubulin-binding taxane, in 100 μl aliquots of human lithium heparinized plasma with deuterated cabazitaxel as internal standard. The sample extraction and cleaning-up involved a simple liquid–liquid extraction with 20 μl aliquots of 4% ammonium hydroxide, 100 μl aliquots of acetonitrile and 1 ml aliquots of n-butylchloride. Chromatographic separations were achieved on a reversed phase C_{18} column eluted at a flow-rate of 0.20 ml/min on a gradient of acetonitrile. The overall cycle time of the method was 5 min, with cabazitaxel eluting at 3.0 min. The multiple reaction monitoring transitions were set at 836 > 555 (*m*/*z*), and 842 > 561 (*m*/*z*) for cabazitaxel and the internal standard, respectively. The calibration curves were linear over the range of 1.00–100 ng/ml with the lower limit of quantitation validated at 1.00 ng/ml. The within-run and between-run precisions, also at the level of the LLQ, were within 8.75%, while the accuracy ranged from 88.5 to 94.1%. As dilution of samples prior to extraction resulted in a loss of cabazitaxel of approximately 6.5% per dilution step, a second calibration curve ranging from 40.0 to 4000 ng/ml was validated and was also linear. The within-run and between-run precisions in this range were within 4.99%, while the accuracy ranged from 95.8 to 100.3%. The method was successfully applied to samples derived from a clinical study.

1. Introduction

Acquired and intrinsic resistance to docetaxel and paclitaxel (i.e., the two approved first generation taxanes) is still an important concern in daily clinical practice. Therefore, the intravenously available semi-synthetic taxanes, cabazitaxel (Jevtana®; XRP6258; TXD258; RPR116258A) and larotaxel (RPR109881A) were selected for clinical development as a result of their efficacy in a broad range of cell-lines and tumor models of mouse and human origin. Also, both compounds showed greater potency than docetaxel in cell lines expressing the drug transporter p-glycoprotein (reviewed in [1–3]).

While larotaxel is currently still under clinical evaluation, cabazitaxel has been approved in the US by the Food and Drug Administration (FDA) in June 2010 [3] and in Europe by the European Medicines Agency (EMA) in January 2011 [4] in combination with prednisone for the treatment of patients with castration-resistant metastatic prostate cancer whose disease progresses after docetaxel treatment, based on the results of the TROPIC trail investigating cabazitaxel plus prednisone versus mitoxantrone plus prednisone following docetaxel failure [5]. Cabazitaxel is currently being investigated in the setting of metastatic breast cancer progressing after taxane or anthracycline based chemotherapeutic regimens [6,7].

A population pharmacokinetic model was developed using pharmacokinetic data from five different studies [4], from which two currently have been published as peer reviewed manuscripts [7,8]. The pharmacokinetics of cabazitaxel are linear in the studied dose-range of 10–30 mg/m^2 given as 1 h infusions and are consistent with a three-compartment pharmacokinetic model with half lives in the initial, intermediate and terminal phase of approximately 4.4 min, 1.6 h, and 95 h, respectively. The drug has a fast plasma clearance estimated to be 48.5 l/h in the studied population and has a large volume of distribution of 4870 l. The *ex vivo* protein binding was 91.6%, mainly to albumin and lipoproteins, while the

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drug displays low binding to α1-acid glycoprotein. Cabazitaxel is extensively metabolized by cytochrome P450 iso-enzymes CYP3A4 and CYP3A5, with CYP2C8 playing a minor role in its metabolism. Cabazitaxel and its metabolites are mainly excreted via the feces (76% of the dose) and to a lesser extent through the urinary pathway (3.7% of the dose).

Neutropenia is the principle dose-limiting and most commonly observed toxicity in cabazitaxel treatment when administered as 1 h infusion every 3 weeks [8]. In the phase III trial comparing the efficacy of prednisone plus cabazitaxel to mitoxantrone, grade ≥3 neutropenia was observed in 82% of the patients in the cabazitaxel arm, with 8% of the patients experiencing febrile neutropenia [5]. Of the non-hematological toxicities diarrhea is the most commonly observed side effect seen in this regimen [5,8]. Overall, diarrhea occurred in 47% of the patients with 6% experiencing grade ≥3 diarrhea [5]. In a weekly schedule, diarrhea was even more pronounced and considered a dose-limited toxicity [9].

As cabazitaxel is a promising new anticancer agent for taxanes-resistant tumors, it is expected that numerous subsequent clinical studies investigating both single agent and cabazitaxel-based combinations will be initiated. For this purpose, quantitation of cabazitaxel is imperative.

To the best of our knowledge, no reports have been published describing a validated bioanalytical method for the quantitation of cabazitaxel. We have developed and validated a sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay for cabazitaxel in human plasma, according to the Guidance for Industry, Bioanalytical Method Validation, as specified by the FDA. In addition, we discuss the non-specific binding of cabazitaxel observed during sample preparation and provide a simple and practical solution to deal with this phenomenon during pharmacokinetic analysis.

2. Experimental

2.1. Chemicals

Cabazitaxel (purity 92.9%) and the deuterated internal standard, $^{2}H_{6}$-cabazitaxel (purity 97.4%), were kindly supplied by Sanofi-Aventis (Frankfurt am Main, Germany). All chemicals were of analytical grade or higher. Acetonitrile, methanol and water were purchased from Biosolve BV (Valkenswaard, The Netherlands). Dimethyl sulfoxide, ammonium formate and n-butylchloride were from Sigma–Aldrich (Zwijndrecht, The Netherlands). Formic acid and ammonium hydroxide were obtained from J.T. Baker (Deventer, The Netherlands) and 2-propanol from Merck (Darmstadt, Germany). Blank lithium heparinized plasma was purchased from Biological Specialty Corporation (BSC, Colmar, PA, USA).

2.2. Preparation of stock solutions, calibration standards and quality control samples

Cabazitaxel stock solutions were prepared at 1 mg/ml free base in dimethyl sulfoxide. Stock solutions were aliquotted and stored at $T < -70\,^{\circ}C$. Separate stock solutions were prepared for the construction of the calibration curve standards and the pools of quality control samples. The internal standard stock solution was prepared at 1 mg/ml in dimethyl sulfoxide, which subsequently was aliquotted and stored at $T < -70\,^{\circ}C$. Internal standard working solutions were prepared at concentrations of 100 and 1000 ng/ml in acetonitrile, which were stored at $T < 8\,^{\circ}C$ for a maximum of 3 months.

Calibration standards were prepared in duplicate on the day of analysis, by addition of 10 μl aliquots of appropriate dilutions of cabazitaxel stock solution in acetonitrile/DMSO (1:1, v/v) to 190 μl aliquots of human lithium heparinized plasma with concentrations of 1.00, 2.50, 10.0, 25.0, 50.0, 75.0, 90.0 and 100 ng/ml for quantitation of cabazitaxel in the concentration range of 1.00–100 ng/ml and 40.0, 120, 500, 1000, 2500, 3600 and 4000 ng/ml for concentrations of cabazitaxel in the range of 40.0–4000 ng/ml.

Pools of QC samples were prepared in human lithium heparinized plasma at concentrations of 1.00 ng/ml (lower limit of quantitation, LLQ), 3.00 ng/ml (QC low), 40.0 ng/ml (QC middle) and 80.0 ng/ml (QC high) for calibration standard curve in the range of 1.00–100 ng/ml and at 40.0 ng/ml (LLQ; i.e., QC middle above), 120 ng/ml (QC low), 1500 ng/ml (QC middle) and 3000 ng/ml (QC high) for calibration standard curve in the range of 40.0–4000 ng/ml. Pools of QC samples were aliquotted and stored at $T < -70\,^{\circ}C$ and $T < -20\,^{\circ}C$ upon processing.

2.3. Sample preparation

For both calibration ranges, aliquots of 20 μl 4% ammonium hydroxide and 100 μl of internal standard working solution (100 ng/ml for the range of 1.00–100 ng/ml and 1000 ng/ml for the range of 40.0–4000 ng/ml) were added to 100 μl of plasma samples in 2 ml microcentrifuge tubes followed by 1 ml aliquots of n-butylchloride. Hereafter, the samples were vigorously mixed for 10 min and then centrifuged at 18,000 × *g* at ambient temperature for 10 min. Aliquots of 1 ml of the organic phase were transferred into 4.5 ml glass tubes and evaporated under nitrogen at $T = 70\,^{\circ}C$. The residues were resuspended in 100-μl aliquots of acetonitrile/water/ammonium formate (40:60:0.2, v/v/v). After 5 min of centrifugation at 3000 × *g*, the supernatants were transferred into 350 μl 96-well plates, which were placed into the chilled ($T = 10\,^{\circ}C$) autosampler, from which aliquots of 50 μl were injected onto the HPLC column for the low-range method (1.00–100 ng/ml) and 10 μl for the high-range method (40.0–4000 ng/ml).

2.4. Equipment

The LC–MS/MS system was purchased from Waters Chromatography B.V. (Etten-Leur, The Netherlands) and consisted of a Waters 2795 Separation Module coupled to a Quatro micro API Mass Spectrometer. The MassLynx V4.1 SCN627 software package was used for the acquisition and processing of data. Quantification was performed using QuanLynx as implemented in the MassLynx software.

2.5. Chromatographic conditions

Analytes were separated on an Alltima HP C_{18} HL column 3 μm, 50 mm × 2.1 mm, (Grace, Breda, The Netherlands) thermostatted at $T = 40\,^{\circ}C$. A gradient at a flow-rate of 0.20 ml/min was achieved with mobile phase A, composed of 2 mM ammonium formate and mobile phase B, composed of acetonitrile. A linear gradient was used, with 50–15% mobile phase A, from 0 to 1.5 min, followed by holding on 15% mobile phase A (i.e., 85% mobile phase B) for 1.5 min. This was succeeded by a linear gradient back to 50% mobile phase A from 3.0 to 3.1 min, which was held for 1.9 min to re-equilibrate. The overall run time of the assay was 5 min. A pre-column volume of 300 μl was applied and a parallel injection was enabled. The needle wash solvent was composed of acetonitrile/methanol/water/2-propanol/formic acid (25:25:25:25:0.1, v/v/v/v/v).

2.6. Mass spectrometry

Tandem mass spectrometry was performed in the positive ion electrospray ionization mode. Mass transitions of *m*/*z* were optimized for cabazitaxel and $^{2}H_{6}$-cabazitaxel (internal standard) by infusion of the respective analytes in acetonitrile/water/formic acid (40:60:0.1, v/v/v) via a T-union. Optimal MS settings were manually adjusted. The desolvation gas was set at 800 l/h and the cone