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Quantification of cabazitaxel, its metabolite docetaxel and the determination of the demethylated metabolites RPR112698 and RPR123142 as docetaxel equivalents in human plasma by liquid chromatography–tandem mass spectrometry

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ABSTRACT

We present a sensitive validated LC–MS/MS assay for the simultaneous determination of cabazitaxel and docetaxel in human plasma, with calibration ranges of 1.0–150 ng/mL for cabazitaxel and 0.1–15 ng/mL for docetaxel. Sample pretreatment consisted of liquid–liquid extraction with *tert*-butyl methyl ether. Chromatographic separation was achieved on a Zorbax Extend C_{18} column using a gradient mixture of 10 mM ammonium hydroxide and methanol. Mass detection was carried out by turbo ion spray ionization in positive ion multiple reaction monitoring mode. All inter-day accuracies and precisions were within ±15% of the nominal value and within ±20% at the lower limit of quantitation. Demethylations of cabazitaxel yielding the metabolites RPR112698 and RPR123142 were monitored semi-quantitatively and quantified as ng docetaxel equivalents. Plasma samples of a prostate cancer patient treated with cabazitaxel were analyzed to demonstrate the usefulness of the presented assay for clinical drug monitoring. In conclusion, this method can be applied to support clinical pharmacokinetic studies with the novel anticancer drug cabazitaxel.

1. Introduction

Taxanes are widely used in the treatment of various types of cancer and are highly effective. However, over time patients develop resistance against taxane treatment [1]. Multiple mechanisms of resistance are described, with the induction of the efflux pump P-glycoprotein (P-gp) being one of the most important features [2]. This led the focus to the development of new anticancer agents which are not substrates for P-gp. Cabazitaxel is such a novel anticancer agent with low affinity for P-gp [3,4].

Cabazitaxel, a semi-synthetic derivative of 10-deacetylbaccatin III, is extensively metabolized in the liver mainly by cytochrome P450 3A4/5 resulting in approximately 20 metabolites in vivo including two pharmacologically active demethylated derivatives of cabazitaxel (RPR112698 and RPR123142) and docetaxel [5]. Cabazitaxel is indicated for the second-line treatment of metastasized castration resistant prostate cancer (mCRPC) in combination with prednisone, after treatment with docetaxel.

A liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method to measure cabazitaxel plasma concentrations has been described by De Bruijn et al., using overlapping split calibration lines [6]. Although two calibration lines extend the range of this assay from 1.0 to 4,000 ng/mL cabazitaxel, the assay is forced out of the linear range, resulting in a bias for samples measured in the overlapping range. Furthermore, the need of such a high upper limit of quantitation (ULOQ) can be questioned when an average C_{max} of 535 ± 305 ng/mL has been reported in phase I-III trials [3]. No metabolites, including docetaxel, were part of the

Abbreviations: P-gp, P-glycoprotein; mCRPC, metastasized castration resistant prostate cancer; Cmax, maximum plasma concentration; ACN, acetonitrile; TBME, *tert*-butyl methyl ether; IS, internal standard; AT-IS, 2′,7-bisacetyltaxol; DOC-IS, docetaxel-d9; ESI, electrospray ionization; SRM, single reaction monitoring; S/N, signal to noise; CAL, calibration standard; QC, quality control; LLOQ, lower limit of quantitation; ULOQ, upper limit of quantitation; CV, coefficient of variation; SD, standard deviation.

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assay of De Bruijn et al. [6]. To the best of our knowledge, we here present the first method which combines cabazitaxel and its active metabolite docetaxel in a single assay and has the ability to detect two other active demethylations of cabazitaxel, RPR112698 and RPR123142. The ability to analyze these three active metabolites together with cabazitaxel, makes this assay a very useful tool in clinical pharmacokinetic studies.

2. Experimental

2.1. Chemicals

Cabazitaxel was purchased from Medkoo Biosciences (Chapel Hill, NC, USA). 2′,7-Bisacetyltaxol (AT-IS) was purchased from LKT Laboratories (St. Paul, MN, USA). Docetaxel originated from Sequoia Research Products (Oxford, UK) and its deuterated internal standard docetaxel-d9 (DOC-IS), was obtained from Toronto Research Chemicals (North York, ON, Canada). Methanol and acetonitrile (ACN), both Supra gradient grade, were purchased from Biosolve Ltd. (Amsterdam, the Netherlands). Aqueous ammonia 25% (NH_4OH), *tert*-butyl methyl ether (TBME) and LiChrosolv water for chromatography, all of analytical grade, were from Merck (Darmstadt, Germany). Drug free lithium-heparinized human plasma was obtained from Bioreclamation LLC (Westbury, NY, USA).

2.2. Chromatographic and mass spectrometric conditions

An Agilent HP1100 LC system was used (Agilent Technologies, Palo Alto, CA, USA), consisting of a binary pump, in-line degasser, autosampler and a column oven. A Zorbax Extend C_{18} column (150 mm × 2.1 mm ID, particle size 5 μm; Agilent Technologies) was used, protected with an in-line filter frit (0.5 μm; Upchurch Scientific). The temperature of the column oven was set at 50 °C.

The mobile phase consisted of a mixture of 10 mM ammonium hydroxide (mobile phase A) and 100% methanol (mobile phase B) and was applied at a flow rate of 0.3 mL/min. After an isocratic hold of 4.5 min at 70% methanol, a linear gradient was applied for half a minute to 90% methanol. This eluent composition was kept for 3 min, before returning to the initial conditions within 0.1 min.

The column was allowed to stabilize 4 min prior to the next injection, leading to a total run time of 12 min. Eluent was directed to waste during the first two, and last 4 min of the run, using a switching valve. Samples were kept in a thermostatted autosampler set at 4 °C. An injection volume of 25 μL was applied and prior to each injection the needle was flushed with acetonitrile for 5 s.

A triple quadrupole mass spectrometer (API4000, AB Sciex, Foster City, CA, USA) was used to detect the analytes. Ionization was performed using a turbo ion spray source, operating in positive ion mode. Ion specific parameters were optimized for each compound and are presented in Table 1. Data were acquired and quantified with Analyst software version 1.5 (AB Sciex).

2.3. Preparation of stock and working solutions

Stock solutions were prepared for cabazitaxel, docetaxel and their internal standards (IS), by accurately weighing an amount of 1.0 mg and dissolving it in methanol to obtain 1.0 mg/mL solutions for both the analytes as well as the IS. Since docetaxel plasma concentrations were expected to be low [3,4], combined working solutions were prepared in methanol, using docetaxel in a one-tenth concentration compared to cabazitaxel. IS stock solutions were diluted in methanol to obtain a combined IS working solution containing 250 ng/mL AT-IS and 40 ng/mL DOC-IS. Solutions were stored in polypropylene tubes at −20 °C.

2.4. Preparation of calibration standards and quality controls

Calibration standards (CALs) were prepared by diluting a fixed amount of working solution (1:20, v/v) with control human plasma. The CALs were prepared at nominal concentrations of 1.0, 2.0, 5.0, 10, 25, 50, 125 and 150 ng/mL cabazitaxel. Quality control samples (QCs) were prepared in the same manner at nominal concentrations of 1.0 (LLOQ level), 3.0 (LOW level), 15 (MID level) and 120 ng/mL (HIGH level) cabazitaxel. Docetaxel concentrations were one-tenth of the nominal cabazitaxel concentrations. For validation purposes, additional QCs were made at lower limit of quantitation (LLOQ) (1.0/0.1 ng/mL, cabazitaxel/docetaxel) and above upper limit of quantitation (ULOQ; 1200/120 ng/mL, cabazitaxel/docetaxel). Plasma volumes of 200 μL were transferred to 2.0 mL polypropylene tubes and stored at −20 °C.

2.5. Sample pre-treatment

Plasma samples were allowed to thaw at ambient temperature. A sample volume of 200 μL was transferred to a 2.0 mL vial, additionally spiked with 20 μL IS working solution and vortex mixed

Table 1
Mass spectrometric settings.

Parameter	Setting
Ion source	ESI
Ionization mode	positive
Ionspray voltage (V)	5500
Turbogas temperature (°C)	400
Nebulizer gas (compressed air, gas 1, psi)	60.0
Turbo gas (compressed air, gas 2, psi)	50.0
Curtain gas (N_2, psi)	10.0
Collision gas (N_2, psi)	5.0
Entrance potential (V)	11.0

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell time (msec)	Declustering potential (V)	Collision energy (V)	Collision cell exit potential (V)	Typical retention time (min)
Docetaxel	808.4	527.3	150	56	15	14	3.7
Docetaxel-d9	817.4	527.2	150	56	15	14	3.7
RPR112698	822.4	541.5	150	61	13	16	3.8
RPR123142	822.4	541.5	150	61	13	16	5.4
Cabazitaxel	836.6	555.5	150	61	13	16	5.4
2′,7-bisacetyltaxol	939.4	552.4	150	91	21	14	6.4

Masses for precursor/product ions are nominal.

for 10 s. Double blank samples were spiked with 20 μL methanol instead of IS. Subsequently, analytes were extracted from the samples using liquid-liquid extraction with 1.0 mL TBME. Samples were vortex mixed for 10 s, shaken for 10 min at 1,250 rpm and centrifuged for 10 min at approximately 15,000 rpm at 4 °C, followed by snap-freezing using an ethanol/dry ice bath. The organic upper layer was decanted and evaporated to dryness under a gentle flow of N_2 at 40 °C. Dried extracts were reconstituted in 100 μL methanol:water (1:1, v/v) and vortex mixed during 20 s. Samples were centrifuged for 3 min at 4 °C at approximately 15,000 rpm. The clear supernatant was transferred into a glass autosampler vial with insert.

3. Validation procedures

3.1. Regression model

Before each run, calibration standards were freshly prepared in duplicate at eight concentration levels and analyzed over sixteen independent runs. Calibration curves were fitted using least squares quadratic regression of the peak area ratio (analyte/IS) versus the concentration was weighed by the reciprocal of the squared concentration ($1/x^2$). Back-calculated concentrations should be within ±15% of the nominal concentration value, except for the LLOQ, for which it should be within ±20%. At least 75% of the calibration standards must fulfil this criterion.

3.2. Accuracy and precision

Inter-assay accuracy and precision was assessed by analyzing QCs at LLOQ, LOW, MID, HIGH and above ULOQ levels over sixteen independent analytical runs. Above ULOQ samples were diluted ten times with control human plasma prior to sample processing and measured in two-fold in two analytical runs. The inter-assay accuracy, calculated using Eq. (1), should be within 85–115% of the nominal concentration. At LLOQ, deviations of ±20% in accuracy were permitted, with a minimal signal to noise (S/N) ratio of five compared to the average noise in a blank sample at the retention time of the analyte. The inter-assay bias was assessed by calculating the relative difference of mean back calculated concentrations compared to nominal concentrations (Eq. (2)). The inter-assay precision is expressed as coefficient of variation (CV) using Eq. (3) and should not exceed 15%, except at LLOQ, where the CV should not exceed 20%. The equations used are defined in the supplementary data.

3.3. Specificity and selectivity

Four different batches of control human plasma were used for the sixteen analytical runs. Endogenous interferences co-eluting with analytes where checked by processing double blank samples (without IS) and spiked samples at LLOQ containing cabazitaxel, docetaxel and IS. Interferences co-eluting with analytes or IS should not exceed 20% of the peak area of the analytes at the LLOQ, or 5% of the IS peak area.

Cross analyte and IS interferences were assessed by processing control human plasma samples spiked with only one analyte at its ULOQ level, or an IS at the concentration used in the method. Interferences found in the transitions of the other analytes and IS should not exceed 20% of the analyte peak area at LLOQ level or 5% of the IS areas.

3.4. Carry-over

After each ULOQ sample two blank processed samples were injected to determine the carry-over. Peak area's found in the first blank sample should be less than 20% of the peak area, at LLOQ of the analytes or 5% of the IS areas.

3.5. Total recovery

The total recovery was assessed by spiking samples in two-fold with 5.0 and 50 ng/mL cabazitaxel. Samples were subsequently processed according to the prescribed method. The average response was compared to the peak response of a spiked working solution at the same concentrations.

3.6. Matrix effect

Six double blank samples from different batches of control human plasma were processed to obtain dried extracts containing matrix ions. These extracts were spiked at low and high concentrations, matching the concentration of the analytes in the final extract. The cabazitaxel and docetaxel area and area ratio in the presence of matrix ions were compared to the average peak area and area ratio of working solutions without matrix ions, to determine the absolute and relative matrix factor (internal standard normalized matrix factor). An absolute matrix factor of 1 implies no matrix effects on the ionization of the given analyte. The %CV of the relative matrix factor should be less than 15% for both tested levels.

3.7. Stability

All stability assessments were performed in triplicate with independent dilutions. Analytes extracted from the plasma matrix were considered stable when the average back calculated concentration was within ±15% of the nominal concentration. The recovered concentration in stock solutions should be within ±10%. The stability of cabazitaxel was assessed in the stock solution at -20 °C after nine months of storage. Stability in plasma was tested at low and high concentrations for 4.5 h at ambient temperature (20 °C) and for six months at −20 °C, respectively. Freeze/thaw stability was determined after two cycles. Stability in dried and final extract were tested for ten weeks and four days, respectively. The stability of docetaxel under the tested conditions was reported previously [7].

3.8. Clinical application

Samples were drawn from a patient receiving cabazitaxel intravenously every three weeks, as a one-hour infusion of 25 mg/m^2 for the treatment of mCRPC. Before start of the infusion and at end-of-infusion, whole blood was drawn using tubes containing lithium heparin to prevent coagulation. Samples were centrifuged immediately (5 min at 3000 rpm) and the plasma was stored at −20 °C until analysis. The described procedure was approved by the local Medical Ethics Review Committee. Semi-quantitative analysis of RPR112698 and RPR123142 was performed using docetaxel calibration samples, with the concentrations being expressed as ng docetaxel equivalents/mL.

4. Results and discussion

4.1. Chromatographic and mass spectrometric conditions

4.1.1. Chromatography

A Zorbax Extend C18 column was selected for the combined analysis of cabazitaxel and docetaxel. When considering the ionized state of a basic compound such as cabazitaxel, one would expect to find more ionized molecules in an acidic environment. An acidic eluent, 0.1% formic acid, was therefore tested against 10 mM ammonium hydroxide as aqueous phase, resulting in a two

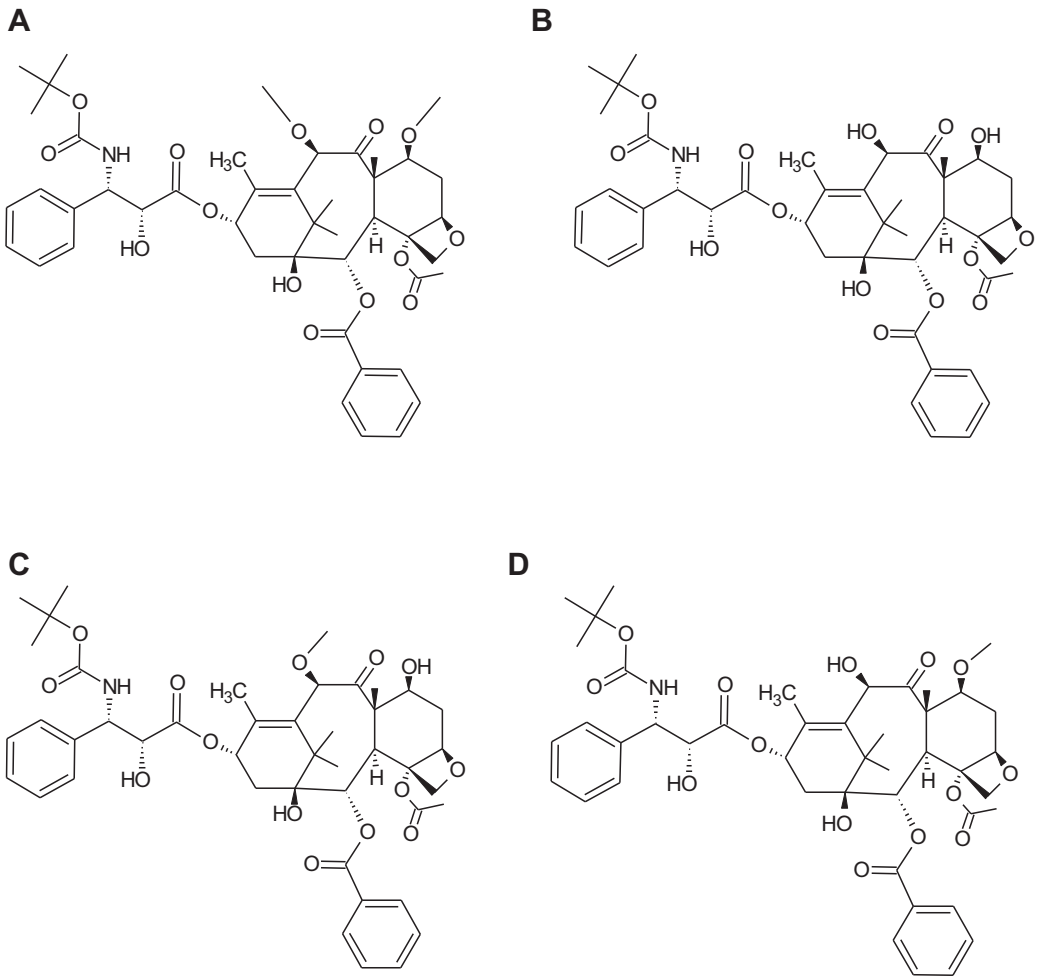

Fig. 1. Chemical structures of cabazitaxel (A), docetaxel (B), RPR112698 (C) and RPR123142 (D).

to threefold decrease in peak intensity for cabazitaxel. Therefore an alkaline aqueous phase was further used. ESI in the positive mode combined with an alkaline eluent, has been shown before to result in an increase in sensitivity for some basic compounds compared to the use of an acidic eluent [7–9]. The gradient chromatographic system exhibited excellent separation of cabazitaxel and the metabolites docetaxel, RPR112698 and RPR123142 (Fig. 1), within an analytical runtime of 12 min.

4.1.2. Mass spectrometry

Mass spectrometric parameters were optimized in positive ionization mode with infusion and flow injection analysis. The full-scan mass spectrum of cabazitaxel (Fig. 2A) showed responses for $[M+H]^+$ at *m/z* 836, $[M+Na]^+$ at *m/z* 858, the loss of two methyl-groups and the benzoic acid from the baccatin III core at *m/z* 400 and di-O-methylated baccatin III core with sodium adduct at *m/z* 595.

Product ions from *m/z* 836.6 were observed at *m/z* 555 (deacetyl-baccatin III core), *m/z* 541 (mono-O-methylated deacetylbaccatin III core), *m/z* 818 (loss of H_2O), *m/z* 730, *m/z* 523 (loss of two methyl-groups from the deacetylbaccatin III core), *m/z* 433 (loss of benzoic acid from the deacetylbaccatin III core). The most abundant product ion of cabazitaxel, i.e. *m/z* 555, was selected for quantification. AT-IS was monitored with the transition *m/z* 939 to 552. The mass spectrometric parameters for docetaxel and DOC-IS were previously established [7] and optimized for the transitions *m/z* 808–527 and *m/z* 817–527, respectively.

4.1.3. Sample pre-treatment

To determine the optimal sample pre-treatment, liquid-liquid extraction (LLE) with TBME and protein precipitation with ACN, ACN-methanol (1:1, v/v) and methanol were tested. LLE with TBME provided the highest S/N ratio and the most clean extracts compared to the other methods. Therefore LLE with TBME was selected as sample clean-up procedure. According to the certificate of analysis, cabazitaxel is photosensitive. In order to determine the grade of photosensitivity, dilutions of working solutions in methanol were stored in triplicate at ambient temperatures in either amber coloured or transparent vials for three and five hours. After 3 h, the cabazitaxel concentration was 0.30% lower in the transparent vial compared to the amber coloured vial ($n = 3$), after 5 h the concentration was decreased by −0.56%. The decrease in concentration was not significant and therefore no precautions were taken to limit the exposure to UV light.

4.2. Validation of the method

4.2.1. Regression model

A prevalidation run was performed with eight CALs ranging from 1 to 500 ng/mL cabazitaxel equally distributed over the range, including QCs at nominal concentrations of 1, 5, 25 and 375 ng/mL cabazitaxel. Excessive non-linear relationship was observed from 150 ng/mL to 500 ng/mL for cabazitaxel, therefore the calibration range was decreased to 1–150 ng/mL cabazitaxel and 0.1–15 ng/mL docetaxel. Eight standards were prepared at concentrations equally distributed over the calibration range, with QCs ranging from the

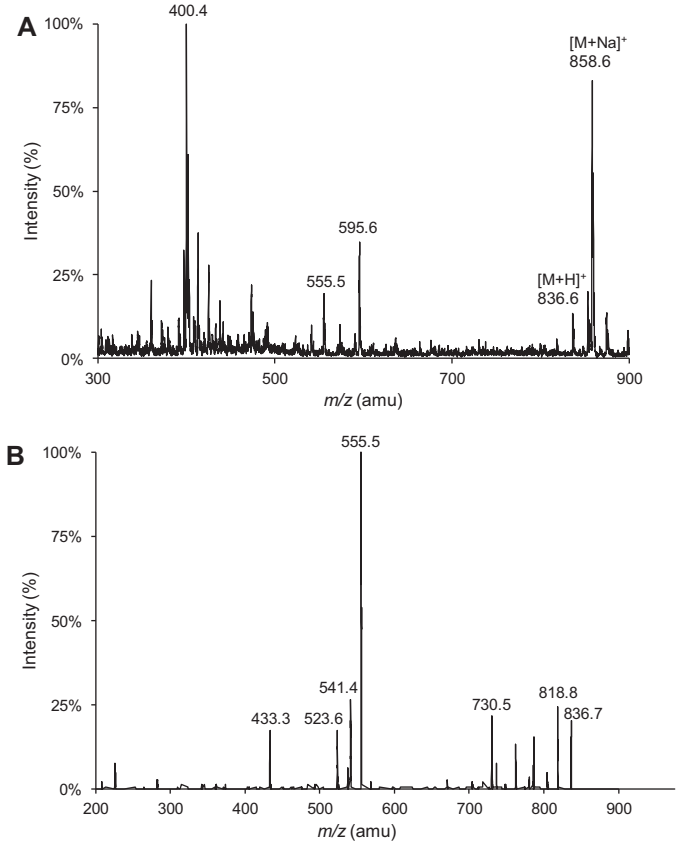

Fig. 2. MS spectra of cabazitaxel (A) and product ion spectrum of *m/z* 836.6 (B).

LLOQ levels to high concentrations levels. Calibration curves were best fitted by quadratic regression with $1/x^2$ as weighting factor. Correlation coefficients (r^2) of 0.990 or higher were obtained for cabazitaxel and 0.996 for docetaxel. The mean deviation of the nominal value was less than 2.90% for cabazitaxel (CV 18.5%) and less than 2.04% for docetaxel (CV 5.84%), for all calibration standards. For the QCs (Table 3), all predefined criteria were met.

4.2.2. Accuracy and precision

A summary of the inter-assay accuracy, bias and precision of cabazitaxel and docetaxel is shown in Table 2. Performance data of the CALs are provided in supplementary Table 1. For the CALs, accuracy ranges from 97.1% to 101% for both analytes at all concentrations, with a precision (%CV) ranging from 5.09% to 18.5% for cabazitaxel and 3.99% to 9.26% for docetaxel. Inter-assay accuracy above ULOQ level was 90.9% for cabazitaxel and 87.7% for docetaxel, with an inter-assay precision of 8.12% for cabazitaxel and 7.66% for docetaxel. The minimal S/N ratio at LLOQ is 47 for cabazitaxel and 7.5 for docetaxel ($n = 32$, data not shown).

4.2.3. Sample dilution

A linear correlation between the number of dilution steps and the observed accuracy has been reported in the literature and was attributed to non-specific binding of cabazitaxel, resulting in a loss of 6.5% cabazitaxel per dilution step [6]. This phenomenon was not seen to this extent, using our method, although we do see a lower accuracy for the ULOQ samples. To obtain more insight into this issue, we spiked lithium heparinised plasma with cabazitaxel working solution at QC HIGH and diluted (1:1, v/v) in triplicate. Dilution step 0 represents QC HIGH (123 ng/mL), subsequent dilutions contain nominally 61.5 ng/mL; 30.8 ng/mL, 15.4 ng/mL and 7.7 ng/mL cabazitaxel. For every dilution step, a sample was set aside and analyzed. The number of dilutions versus cabazitaxel accuracy is depicted in Fig. 4. A modest trend corresponding with the formula $y = -1.66x + 102.5$ ($r^2 = 0.1874$) can be seen, which implies an average decrease in cabazitaxel of −1.7% per dilution step. As a result, we cannot confirm the relation that was found by De Bruijn et al.

4.2.4. Specificity and selectivity

Endogenous interferences were found co-eluting with cabazitaxel in one of the four tested batches of control human plasma, comprising of 0.32% of the LLOQ. In the same batch an interference less than 0.04% of the IS concentrations used in the assay was seen for both AT-IS and DOC-IS. No endogenous interferences were found at the retention time of docetaxel. During cross analyte interference assessment, no interferences where found for the IS of cabazitaxel or docetaxel. For this reason, the specificity and selectivity of the assay was considered to be acceptable for all analytes and IS.

4.2.5. Carry-over

No peaks were detected at the retention time of the analytes or the IS, in the first processed blank sample injected after an ULOQ level. Therefore, the carry-over test was found acceptable for both analytes.

4.2.6. Recovery

The average total recovery of cabazitaxel was 84.9% (CV 15.2%) at 5 ng/mL and 84.0% (CV 10.7%) at 50 ng/mL spiked working solutions. As this method is based on a recently published assay for the analysis of paclitaxel, docetaxel and ritonavir, using the same method of sample pretreatment, the recovery of docetaxel was not tested. Hendrikx et al. found a total recovery of docetaxel between 77.5% and 87.4% (CV < 15%) [7].

Table 2
Assay performance data for cabazitaxel and docetaxel QCs in human plasma (16 runs).

Compound	Nominal conc. (ng/mL)	Mean measured conc. (ng/mL)	Inter-assay accuracy (%)	Inter-assay bias (%)	Inter-assay precision (%)	No. of replicates
Cabazitaxel	1.02	1.03	102	2.17	9.09	26[a]
	3.07	3.08	102	1.93	12.8	32
	15.4	14.9	98.5	−1.47	8.34	32
	123	122	99.6	−0.386	9.07	32
	1230	1120	90.9	−9.10	8.12	4
Docetaxel	0.100	0.103	103	2.75	12.9	26[a]
	0.300	0.307	103	2.86	9.51	32
	1.50	1.56	104	4.00	4.84	32
	12.0	12.2	103	3.02	7.60	32
	120	105	87.7	−12.3	7.66	4

[a] LLOQ samples were not measured during three out of sixteen runs.

Table 3
Stability of cabazitaxel and docetaxel.

Compound	Matrix	Conditions	Initial conc. (ng/ml)	Found conc. (ng/ml)	Bias (%)	CV (%)
Cabazitaxel	Methanol	−20 °C, 9 months	1,025,080	996,397	3.80	5.96
	Plasma	20 °C, 4.5 h	3.07	3.00	−2.90	5.52
			123	107	−13.0	4.86
	Plasma	−20 °C, 6 months	3.07	3.44	12.1	5.24
			123	124	0.813	2.79
	Plasma	2 freeze (−20 °C)/thaw cycles	3.07	2.94	−4.40	0.0554
			123	120	−2.85	0.0296
	Dried extract	2–8 °C, 10 weeks	3.07	1.08	6.21	1.10
			123	119	−2.98	4.30
	Final extract, 50% MeOH (1:1, v/v)	2–8 °C, 4 days	3.07	3.19	3.75	5.55
			123	129	4.88	0.00
Docetaxel	Methanol	−20 °C, 21 months	992,400	931,150	−4.47	2.27
	Plasma	20 °C, 24 h	0.708	0.666	−5.89	4.61
			714	663	−7.10	5.06
	Plasma	−20 °C, 14 months	0.812	0.879	8.19	2.33
		−20 °C, 31 months	34.7	38.8	11.6	3.83
	Plasma	3 freeze (−20 °C)/thaw cycles	0.708	0.692	−2.31	5.25
			714	692	−3.15	0.511
	Dried extract	2–8 °C, 10 weeks	1.50	1.45	−3.11	4.83
			12.0	12.3	2.78	1.24
	Final extract, 50%	2–8 °C, 4 days	0.300	0.330	10.8	5.32
	50% MeOH (1:1, v/v)		12.0	12.6	5.00	1.12

4.2.7. Matrix effect

The overall mean matrix factor for the analytes were around 1.0 for cabazitaxel and 1.2 for docetaxel. This matrix factor indicates that there are negligible matrix effects on the ionization of the analytes in human plasma. The relative matrix factors were calculated and the %CV at each tested concentration for both analytes was less than 15%, indicating an acceptable variability in matrix influence among six batches of control human plasma.

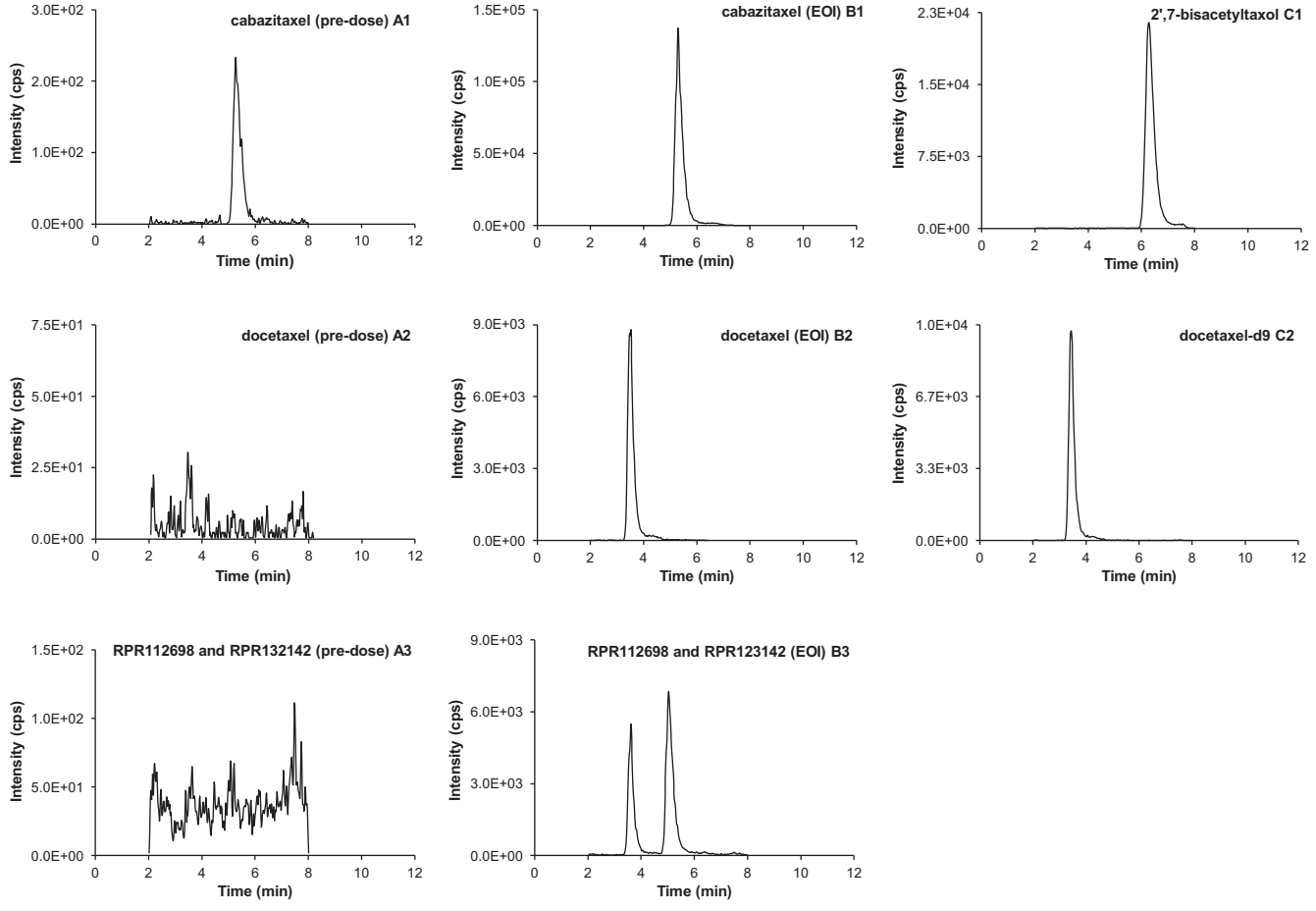

Fig. 3. SRM chromatograms of a pre-dose plasma sample (A), end-of-infusion plasma (EOI) (B) collected from a patient treated with 1 h-infusion of cabazitaxel 25 mg/m^2. Cabazitaxel is monitored from *m/z* 836.6 to 555.5 (A1 and B1), docetaxel from *m/z* 808.4 to 527.3 (A2 and B2) and RPR112698/RPR123142 from *m/z* 822.4 to 541.5 (A3 and B3). The internal standards 2′,7-bisacetyltaxol in the end-of-infusion sample from *m/z* 939.4 to 552.4 and docetaxel-d9 from *m/z* 817.4 to 527.2 (C1 and C2). RPR112698 and RPR123142 were semi-quantitatively analyzed using docetaxel CALs.

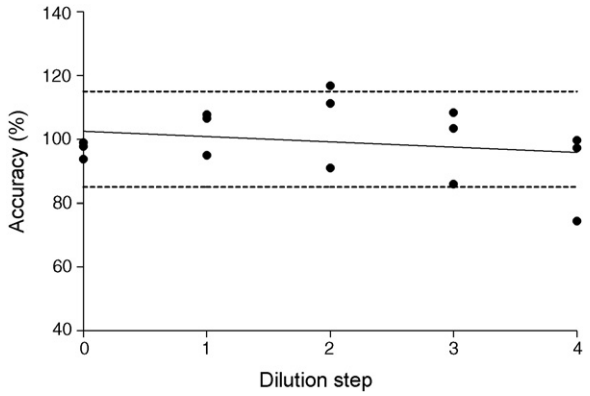

Fig. 4. Correlation between the number of dilution steps versus cabazitaxel accuracy. The average accuracy ($n = 3$) corresponds with the formula $y = -1.66x + 102.5$ ($r^2 = 0.1874$), implying a decrease in cabazitaxel of −1.7% per dilution step, with x being defined as the number of dilution steps.

4.2.8. Stability

In Table 3, results of the investigated stability parameters are presented. Stock solutions of cabazitaxel at a concentration of 1.0 mg/mL in methanol were stable for at least nine months, when stored at −20 °C. After nine months 97.2% of the initial concentration was recovered. Cabazitaxel was stable in plasma after at least 4.5 h at ambient temperatures, after at least six months of storage at −20 °C and during at least two freeze/thaw cycles. Cabazitaxel and docetaxel obtained from plasma samples, were stable as dried extract for at least ten weeks at 2–8 °C and as final extract in 50% methanol for at least four days at 2–8 °C.

4.3. Application of the assay

To demonstrate the applicability of the assay, chromatograms of cabazitaxel, RPR112698, RPR123142 and docetaxel, from plasma samples of a patient treated with cabazitaxel, are depicted in Fig. 3. The pre-dose sample revealed a peak at the retention time of cabazitaxel, but the concentration was below LLOQ. This can be explained by the long elimination half-life of cabazitaxel, that is 95 h [5]. After three weeks, 5.3 times the elimination half-life has passed, resulting in 2.5% of the administered dose being not yet eliminated from the human body, after cabazitaxel administration during a previous course. No metabolites could be observed in the pre-dose samples. At the end of the infusion, 91.0 ng/mL cabazitaxel could be measured. This concentration is lower than the reported average C_{max} found in phase I–III trials, but similar to plasma concentrations reported by De Bruijn et al. and can be explained by the large inter-individual variety in patients. Cabazitaxel metabolites could already be measured at the end of the cabazitaxel infusion. The docetaxel concentration was 4.00 ng/mL and the demethylated derivatives of cabazitaxel yielded concentrations of 2.24 and 3.66 ng docetaxel equivalents/mL for RPR112698 and RPR1234142, respectively. This method is thus sensitive and accurate enough for pharmacokinetic analysis of cabazitaxel and its active metabolites.

5. Conclusion

Herewith, we present a sensitive and selective liquid chromatography-tandem mass spectrometric method which combines cabazitaxel and its active metabolite docetaxel in a single assay and has the ability to detect two other demethylated metabolites of cabazitaxel, RPR112698 and RPR123142. The validated range for cabazitaxel was 1.0–150 ng/mL and for docetaxel 0.1–15 ng/mL, using 200 μL plasma aliquots. RPR112698 and RPR123142 can be determined semi-quantitatively as ng docetaxel equivalents/mL.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2013.02.034.

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