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Rapid analysis of docetaxel in human plasma by tandem mass spectrometry with on-line sample extraction

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Abstract

A simple, rapid, and sensitive analytical method for the measurement of docetaxel in human plasma was developed and validated. The method is based on positive electrospray ionization tandem mass spectrometry (ESI^+–MS–MS) with on-line sample extraction. It uses paclitaxel as internal standard for calibration. The on-line sample extraction minimizes sample handling and is readily adopted for automation. Quantitation of plasma docetaxel was done by the multiple reaction monitoring (MRM) mode. The method had a linear calibration range of 1.00–3000 ng/mL with a correlation coefficient >0.9999. The limit of quantitation (LOQ) for docetaxel in plasma was 1.00 ng/mL. The on-line extraction recovery of docetaxel was between 86.1–94.7%, with %CV $\leq$ 6.1%. This method has high accuracy (90.1–96.3%), and excellent intra-assay (0.6–3.8%) and inter-assay (2.0–5.7%) precision. Its applicability to clinical samples was demonstrated by measuring patient plasma samples after treatment of weekly docetaxel at 25 mg/m^2 as 60-min infusion.

Keywords: Docetaxel; Taxotere®; Tandem mass spectrometry; On-line extraction

1. Introduction

Taxanes including paclitaxel and docetaxel are potent microtubule poisons that promote microtubule assembly from tubulin dimmers without depolymerization. This disrupts the normal dynamic reorganization of the microtubule network, which is essential for the vital interphase and mitotic function [1–3].

Clinically, docetaxel has shown considerable antitumor activity toward a variety of cancers when used as a single agent and in combination with other cytotoxic agents [4–12], even though the optimal regimen of docetaxel in the neoadjuvant and adjuvant treatment of cancer has yet to be defined [13].

The standard regimen for docetaxel administration is a one-hour infusion every three weeks. Docetaxel has also been studied as a single agent or in combination in a variety of schedules including monthly, bi-weekly and weekly [14–17]. To improve antitumor activity and reduce toxicity, recent clinical trials using docetaxel as a single agent or in combination with others have focused on a weekly therapy with low doses, and this has led to an increased interest in measuring the drug at low concentrations [18].

Quantitative determination of docetaxel in plasma at low concentrations is crucial to establish its pharmacokinetic and pharmacodynamic profiles. Several analytical methods for docetaxel quantitation have been described previously [18–26]. The majority of them are HPLC methods with UV absorbance detection [20–26], which do not have very high sensitivity. Furthermore, these assays require elaborate sample preparations involving either off-line solid-phase extraction [18–24] or liquid–liquid extraction [25,26], and they are difficult to automate; therefore, resulting in relatively low throughputs.

We have developed and validated a rapid and sensitive method for the quantitative determination of docetaxel in

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human plasma. Its applicability to biological matrices was successfully tested using plasma samples from patients participating in a phase II clinical trial. This method relies on a fully automated on-line sample extraction procedure followed by positive electrospray ionization—tandem mass spectrometry detection. The method provides the rapidity, sensitivity, and specificity for quantifying docetaxel in patient samples from the investigational regimen of low-dose chemotherapy.

2. Experimental

2.1. Chemicals and solutions

Docetaxel (Taxotere®) was provided by Aventis Pharmaceuticals (Parsippany, NJ, USA). Paclitaxel was purchased from Sigma (St. Louis, MO, USA). Methanol (Optima grade), acetonitrile (HPLC grade), and formic acid were purchased from Aldrich (Milwaukee, WI, USA). Deionized water was obtained from a NANOpure system (Barnstead, Dubuque, IA, USA).

Stock solutions of docetaxel and paclitaxel (1.00 mg/mL in methanol) were stored at −20 °C. Working solutions of docetaxel (0.0100, 0.0250, 0.0500, 0.100, 0.250, 0.500, 1.00, 2.50, 5.00, 10.0, 30.0 μg/mL) were prepared in methanol. The highest concentration of the working solution was prepared by a 1–33.3 dilution in methanol from the stock docetaxel solution and used for preparing the others by serial dilutions. A working internal standard solution of 2.50 μg/mL paclitaxel in methanol was prepared by a proper dilution of the stock solution.

2.2. Blood sampling

Blood samples were collected from patients participating in an ongoing phase II clinical trial of weekly docetaxel administered intravenously as a 60-min infusion at 25 mg/m^2. Blood samples were drawn from contralateral arm at various predetermined time points into Becton–Dickinson Vacutainer tubes containing EDTA potassium salt, following the drug administration. Immediately after collection, the samples were gently inverted a few times for complete mixing with the anticoagulant and placed on ice. Within 30 min of collection, samples were centrifuged at approximately 2000 × *g* at 4 °C for 5 min. The resultant plasma samples were transferred to polypropylene screw-capped tubes, and were immediately stored at ≤−70 °C until analysis.

2.3. Preparation of plasma calibrators, controls and patient samples

Human plasma from voluntary blood donors containing no detectable docetaxel and paclitaxel was obtained from the Blood Bank at the Cleveland Clinic Foundation and used as blank plasma for this work.

To prepare plasma calibrators, 50 μL of each docetaxel working solution and 10 μL of paclitaxel working solution (2.50 μg/mL) were transferred into 1.5-mL Eppendorf tubes. After evaporation of solvent in a DNA 120 SpeedVac® (ThermoSavant, Holbrook, NY, USA) at room temperature for 25 min, 500 μL of blank plasma were added to each tube. The tubes were briefly vortexed to allow mixing and dissolution of docetaxel and paclitaxel in plasma. The resultant plasma solutions (containing docetaxel at 1.00, 2.50, 5.00, 10.0, 25.0, 50.0, 100, 250, 500, 1000 and 3000 ng/mL, and internal standard paclitaxel at 50.0 ng/mL) were used as plasma calibrators/controls.

Patient samples were prepared by adding 500 μL of patient plasma to Eppendorf tube containing 10 μL of paclitaxel working solution (2.50 μg/mL) that was previously dried in the SpeedVac®. The plasma samples were mixed well with the internal standard by vortex prior to analyses.

2.4. Recovery studies

The recoveries of docetaxel from plasma samples by on-line extraction were determined by comparing the mean peak area ratios of docetaxel to the internal standard obtained from mass chromatograms of plasma samples with those obtained from mass chromatograms of aqueous samples at four known concentrations.

2.5. Instrumentation

The instrument system used for this work is consisted of an HP1100 pump and an HP1100 autosampler (Hewlett-Packard, Palo Alto, CA, USA), a stainless steel in-line filter (0.5 μm pore, 0.23 μL dead volume, Upchurch Scientific, Oak Harbor, WA, USA), an Oasis® HLB extraction column (2.1 mm × 20 mm, Waters, Milford, MA, USA), a SelectPro® 2-position 6-port automated switching valve (Alltech, Deerfield, IL, USA), a stainless steel splitting tee (1/16 in. × 0.25 mm, Valco, Houston, TX, USA), and a Quattro II triple quadrupole mass spectrometer controlled by a PC station with MassLynx (version 3.3) software (Micromass, Manchester, UK). PEEK tubing (1/16 in. × 0.01 in.) was used for connections except for between the splitting tee and ESI source, where 0.005 in i.d. PEEK tubing was used.

2.6. On-line sample extraction

On-line extraction of docetaxel and paclitaxel from plasma samples was performed using Oasis® HLB extraction column, and the fluidic profile of the entire analysis is given in Table 1. Firstly, the column was equilibrated with the extraction solvent (H_2O) for 1 min at a flow rate of 2.0 mL/min with the switching valve at the position A (Fig. 1). Then, 20 μL of plasma sample was injected by the autosampler and carried onto the extraction column by the extraction solvent at the same flow rate. While the analytes

Table 1
The fluidic profile of each sample analysis

Time (min)	H_2O (%)	CH_3OH (%)	Flow rate (mL/min)	Switching valve position
0.0	100	0	2.0	A
1.0	100	0	2.0	A
1.1	5	95	2.0	A
1.3	5	95	2.0	A
1.4	5	95	0.25	B
5.1	5	95	0.25	B
5.2	5	95	2.0	A
5.6	5	95	2.0	A
5.7	100	0	2.0	A
7.0	100	0	2.0	A

were retained on the extraction column, the plasma matrix was excluded to the waste. At 1.1 min after sample injection, the extraction solvent was switched to the elution solution (methanol:water 95/5, v/v) at a flow rate of 2.0 mL/min. At 1.4 min after the sample injection, the switching valve was turned to the position B, and the flow rate was reduced to 0.25 mL/min, simultaneously. With a post column flow split ratio of 2:1, one-third of eluant was diverted to tandem mass spectrometer for instrument analysis. At 5.2 min after sample injection, the switching valve was turned to the position A, and the elution solution was pumped at a flow rate of 2.0 mL/min to wash the extraction column and eliminate any carryover from the previous sample until 5.6 min after sample injection. At 5.7 min after sample injection, the elution solution was switched to extraction solvent with a flow rate of 2.0 mL/min for re-equilibrating the extraction column. At 7 min after the first sample injection, the system was ready for the next injection.

2.7. Mass spectrometric detection

The Quattro II triple quadrupole mass spectrometer was operated under the positive electrospray ionization mode (ESI^+). The optimal ionization conditions were tuned by infusing a mixture of docetaxel and paclitaxel (500 ng/mL each) in methanol/water (95/5, v/v) at a flow rate of 3.0 μL/min with a syringe pump (Harvard Apparatus, South Natick, MA, USA); they were: nitrogen nebulising and drying gas at 10 and 350 L/h, capillary at 3.5 kV, ion source temperature at 130 °C, low- and high-mass resolution at 15, and multiplier at 650 V.

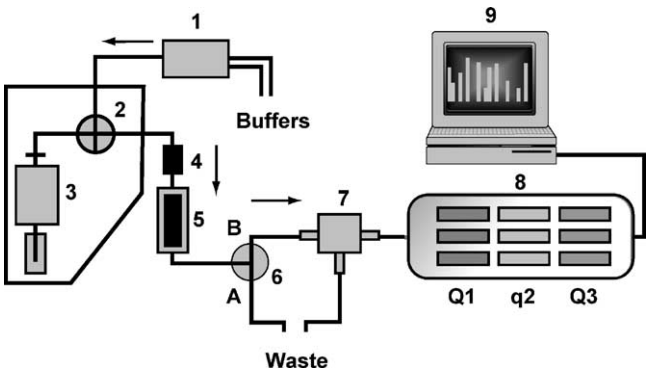


Fig. 1. The instrumentation system: (1) pump; (2) autosampler; (3) injector; (4) in-line filter; (5) extraction column; (6) automated switching valve; (7) splitting tee; (8) triple quadrupole; (9) data acquisition. The arrows show the fluid flow direction when the system is running.

Full scan spectra were acquired over the *m/z* range of 200–900. Multiple reaction monitoring (MRM) mode was used for analyte quantitation with the following parameters: *m/z* 830 > 304 for docetaxel Na^+ adduct, *m/z* 876 > 308 for paclitaxel Na^+ adduct, collision energy at 18 V, argon collision gas at 2.0 μbar, cone voltage at 40 V for docetaxel, and at 60 V for paclitaxel, low- and high-mass resolution at 15 for both quadrupoles 1 and 3, dwell time at 0.6 s, and inter-scan delay at 0.03 s.

2.8. Data analysis

Micromass Masslynx software (version 3.3) was used for data acquisition and processing. The peak area ratios of docetaxel to internal standard paclitaxel were plotted versus the known docetaxel concentrations for calibration curve. Each datum point on the calibration curve represented a mean value of triplicate measurements. Microsoft Excel software was used to perform the linear regression. Docetaxel concentrations of unknown samples were determined by the regression equation after obtaining the peak area ratios of the analyte to the internal standard.

3. Results and discussions

3.1. Preparation of plasma calibrators and samples

Since docetaxel and paclitaxel have relatively low solubilities in water, the stock and working solutions of standards were prepared in 100% methanol. Our experiments showed that the addition of as little as 5 μL of methanol in 500 μL of plasma could cause protein precipitation in plasma and resulted in poor precision of the measurements. Therefore, the methanol was evaporated prior to the addition of blank plasma and patient samples.

3.2. On-line sample extraction

On-line solid-phase extraction (SPE) was the method of choice for extraction of docetaxel and paclitaxel from plasma samples. In comparison with liquid–liquid extraction or offline solid-phase extraction techniques, the on-line SPE requires minimum sample handling, is time efficient and readily adopted for automation.

In this work, Waters Oasis® HLB extraction column was used. The packing material is poly(divinylbenzene-co-*N*-vinylpyrrolidone), a macroporous copolymer, which can retain both hydrophilic and hydrophobic compounds under

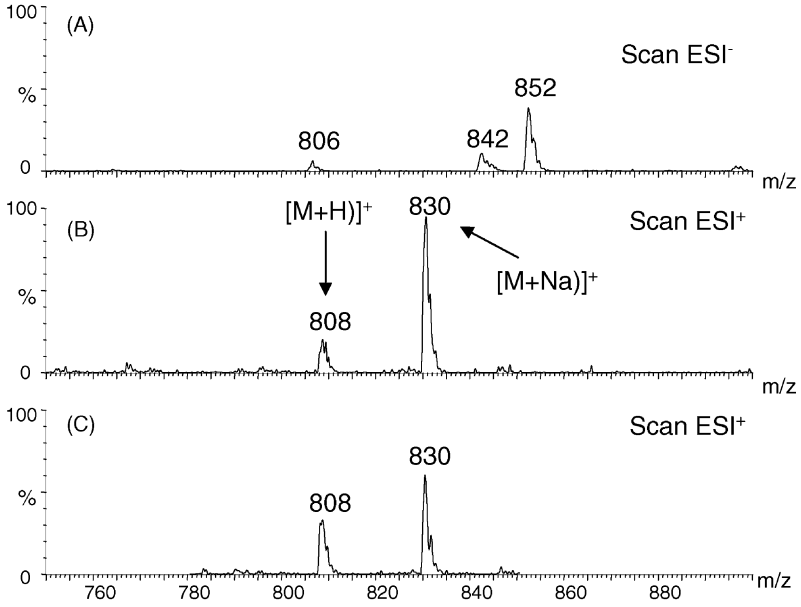


Fig. 2. Full scan spectra of docetaxel (raw data) by negative (A), and positive (B, C) electrospray ionization modes. Docetaxel, 2.0 μg/mL in methanol/H_2O (70%/30%) for (A) and (B), and additional 0.1% formic acid for (C). Other conditions were as described in Section 2.7.

various conditions. Previous studies [27–29] showed that the column had excellent performance toward a variety of pharmaceutical compounds in plasma matrix. While the analytes of interest were retained on the column, the plasma proteins, as well as other macromolecules, could be easily excluded. The recovery studies of this work showed that this column is also well suited for the extraction of docetaxel and paclitaxel from plasma.

Compared to a conventional liquid chromatography system the on-line sample extraction system required an automated switching valve that could divert the unwanted sample matrix to waste. Once it was connected to the system, the automated switching valve could be controlled by the PC system with the MassLynx software. Sample carryover from the previous injection could be eliminated by a post-extraction washing step in the fluidic profile (Table 1). The in-line filter could last for over 50 sample runs, and the extraction column could be re-used for at least 300 extractions if it was stored in 50% acetonitrile aqueous solution after used.

3.3. ESI–MS–MS detection

Docetaxel and the internal standard paclitaxel could be ionized under either positive (ESI^+) or negative (ESI^-) electrospray ionization conditions. The full scan mass spectra of docetaxel showed that the higher detection sensitivity was achieved by the ESI^+ mode (Fig. 2B) than the ESI^- mode (Fig. 2A). The spectra also revealed that docetaxel forms Na^+ adduct more easily than H^+ adduct in ESI^+ mode (Fig. 2B), even if it was in an acidic condition that was favorable to the formation of H^+ adduct (Fig. 2C). Therefore, the ESI^+ mode was used for analyte quantitation.

As shown in Fig. 3A, under ESI^+ conditions the prominent quasi-molecular ions for docetaxel and paclitaxel were [docetaxel + Na]$^+$ at *m/z* 830 and [paclitaxel + Na]$^+$ at *m/z* 876, respectively. Hence, these quasi-molecular ions were

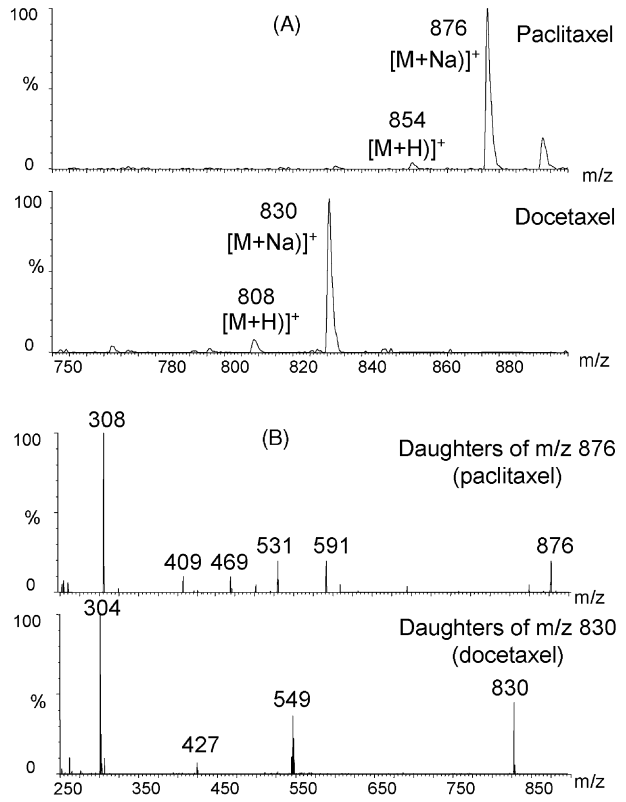
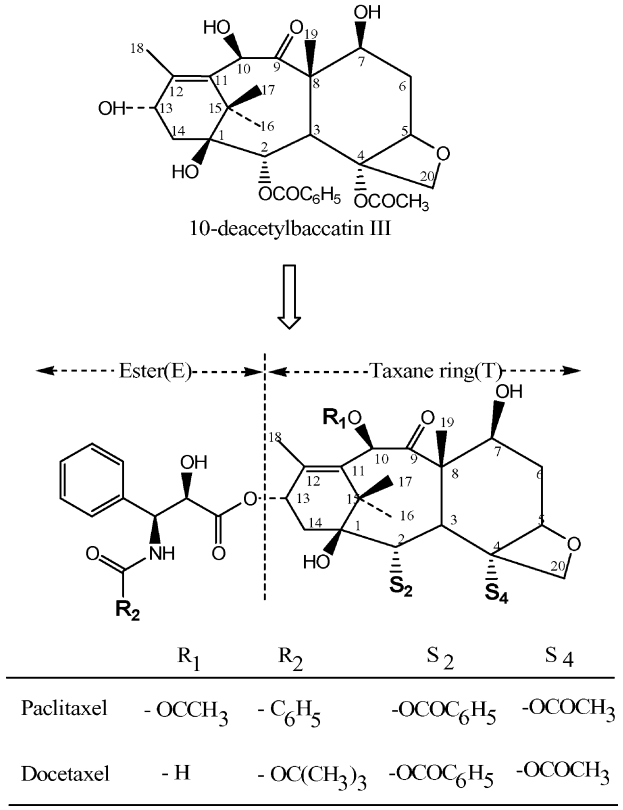


Fig. 3. Mass spectra of parent ions (A) and their daughter ions (B) by positive electrospray ionization mode. Analyte concentration: 500 ng/mL each. Other conditions were as described in Section 2.7.



	R_1	R_2	S_2	S_4
Paclitaxel	$-OCCH_3$	$-C_6H_5$	$-OCOC_6H_5$	$-OCOCH_3$
Docetaxel	-H	$-OC(CH_3)_3$	$-OCOC_6H_5$	$-OCOCH_3$

Fig. 4. The chemical structures of 10-deacetylbaccatin III, and its derivatives docetaxel and paclitaxel.

Table 2
Characteristic fragment ions of paclitaxel and docetaxel

Compound	$[MNa]^+$ of parent (*m/z*)	$[MNa]^+$ of daughter (*m/z*)	Fragment ion
Paclitaxel	876	591	$[TNa]^+$
		531	$[(T\text{-}R_1OH)Na]^+$
		469	$[(T\text{-}S_2H)Na]^+$
		409	$[(T\text{-}R_1OH\text{-}S_2H)Na]^+$
		308	$[ENa]^+$
Docetaxel	830	549	$[TNa]^+$
		427	$[(T\text{-}S_2H)Na]^+$
		304	$[ENa]^+$

chosen as parent ions for acquisition of daughter fragments (Fig. 3B). Since docetaxel and paclitaxel are ester derivatives of 10-deacetylbaccatin III (Fig. 4), the possible identities of the daughter ions in Fig. 3B could be assigned in Table 2. Among them, $[ENa]^+$ ions at *m/z* 304 and 308 were the major daughter ions for docetaxel and paclitaxel, which were chosen as the quantitation ions in subsequent MRM mode detection.

The specificity and sensitivity of a tandem mass spectrometer are warranted by its dual-mass selection processes. Fig. 5 showed the representative mass chromatograms of docetaxel and paclitaxel in plasma by MRM mode detection, which clearly illustrated the inherent selectivity and specificity of the ESI^+–MS–MS method.

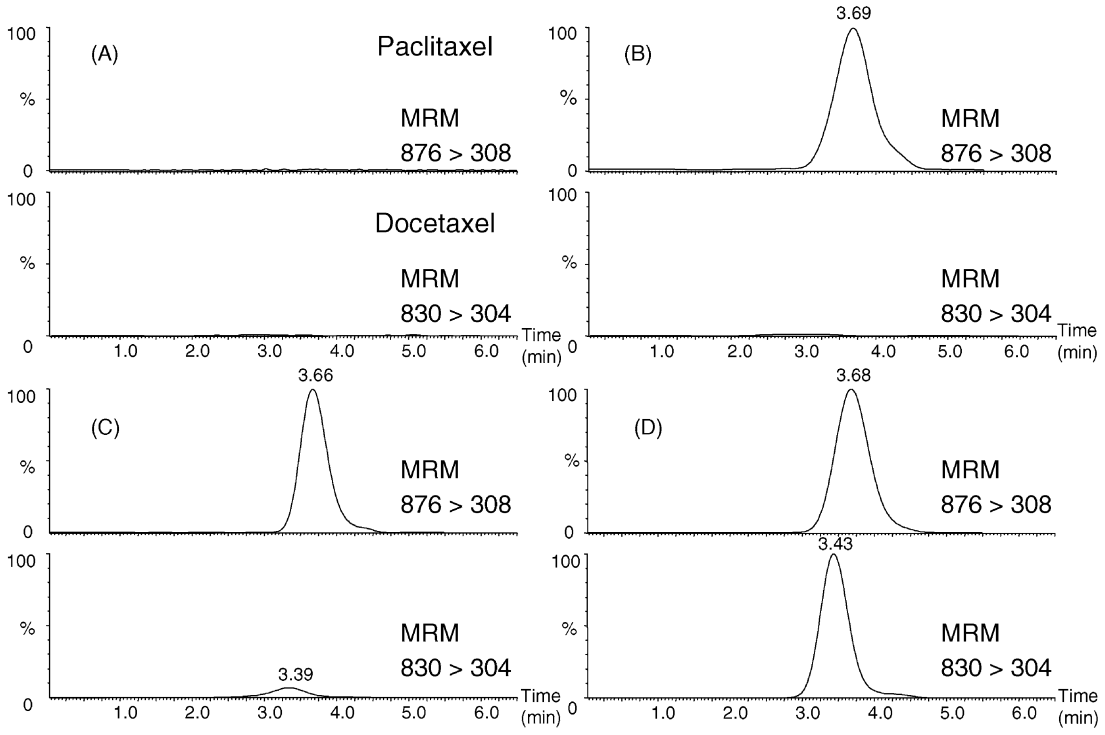


Fig. 5. Mass chromatograms of MRM detection mode: (A) blank human plasma; (B) human plasma containing 50.0 ng/mL internal standard; (C) human plasma containing 50.0 ng/mL internal standard and 1.00 ng/mL docetaxel; (D) human plasma containing 50.0 ng/mL internal standard and 50.0 ng/mL docetaxel.

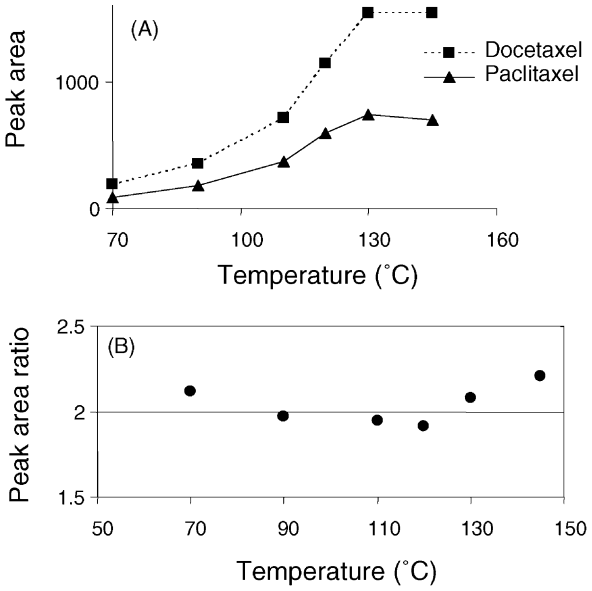


Fig. 6. Effect of ion source temperature on the signal responses of the analytes by MRM mode. Docetaxel, 100 ng/mL; paclitaxel, 50.0 ng/mL.

3.4. Ion source temperature

Ion source temperature had a remarkable influence over the sensitivity of the detection. For docetaxel and paclitaxel, the peak areas increased significantly at higher temperatures (Fig. 6A), while the peak area ratios of docetaxel to paclitaxel remained relatively constant (Fig. 6B). For this work, the temperature of the ion source was set at 130 °C.

3.5. Analytical performance

The recovery of docetaxel was assessed by two steps at four different concentrations of docetaxel (5.00, 50.0, 250 and 1000 ng/mL). Firstly, the aqueous standard solutions of docetaxel were analyzed with both direct injection (without sample extraction) and on-line sample extraction by the ESI^+–MS–MS. No measurable differences in the signal responses have been observed between the non-extracted and extracted aqueous standard solutions; therefore, the aqueous standard solutions of docetaxel with on-line sample extraction were used as references in the recovery studies for docetaxel in plasma. The results are summarized in Table 3. Based on triplicate measurements at each concentration, the recovery of docetaxel in plasma were from 86.1 to 94.7% with %CV of 6.1 or less.

Table 3
Recovery of docetaxel in human plasma

Plasma docetaxel concentration (ng/mL)	5.00	50.0	250	1000
Recovery (%)	86.1	91.4	90.7	94.7
CV (%)	6.1	3.3	2.0	2.0

All samples contained 50.0 ng/mL of internal standard paclitaxel.

Table 4
Precision and accuracy of the method

Plasma docetaxel concentration (ng/mL)	5.00	50.0	250	1000
Accuracy (%)	93.0	90.1		96.3
Intra-assay precision (%CV, $n = 5$)	3.8		1.6	0.6
Inter-assay precision (%CV, $n = 5$	5.7		3.4	2.0

All samples contained 50.0 ng/mL of internal standard paclitaxel.

The reproducibility of the method expressed in terms of intra- and inter-assay precision was determined using plasma calibrators at three different concentrations (5.00, 250, 1000 ng/mL) (Table 4). Based on five replicate measurements at each concentration, the %CVs for the intra- and inter-assay measurements were ≤3.8 and 5.7%, respectively.

A linear relationship between the peak area ratios docetaxel to internal standard and plasma docetaxel concentrations was found over the concentration range of 1.00–3000 ng/mL.

The linear regression equation was $Y = 0.0189X + 0.0307$ with Y as the peak area ratio and X as the concentration of docetaxel, and the correlation coefficient (r^2) was greater than 0.9999.

The accuracy of the method defined as the measured docetaxel concentration of the plasma control divided by its accepted concentration times 100% was determined at three concentrations (5.00, 50.0 and 1000 ng/mL), corresponding to the low, median, and high region of the linear calibration curve. The results ranged from 90.1 to 96.3% (Table 4).

The limit of quantitation (LOQ) for this method defined as the lowest point in the calibration curve was 1.00 ng/mL (%CV = 7.0, $n = 3$), which was at least 5–12.5 times lower than those of HPLC methods [20–26].

Stability studies of docetaxel in plasma were carried out by sampling aliquots of plasma controls stored at −20 °C over two months. Our data showed that there was no perceptible loss of docetaxel in plasma at the frozen state (%CV < 1.6%). The stability of the drug under repetitive freeze–thaw cycles was determined with five consecutive cycles, and the losses were less than 2.0% at four different concentrations of plasma controls (5.00, 50.0, 250, and 1000 ng/mL). When a plasma control was left at room temperature, a loss of 2.5% of docetaxel could be found after 5 h. This loss increased to 20–25% after 12 h. Consequently, the total sample run time was established in ≤5 h. Within this time frame, over 40 samples could be analyzed by our method. For analyses which last longer than 5 h, a refrigerated autosampler is recommended to ensure the stability of the drug while waiting to be analyzed.

3.6. Applicability to biological samples

The validated method was used to determine plasma concentrations of docetaxel in a phase II trial of weekly docetaxel at 25 mg/m^2 given as 60-min infusion. Prior to the drug administration, the patients were informed of the

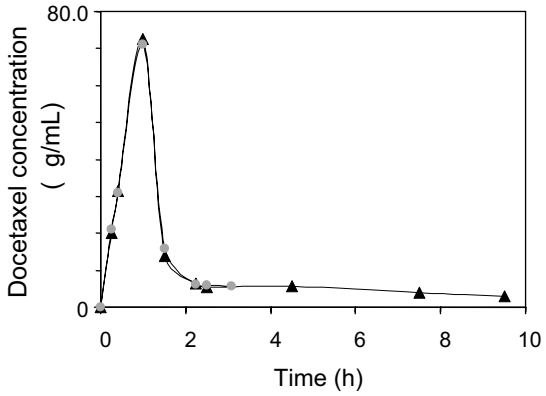


Fig. 7. The plasma concentration profiles of docetaxel during and after 60-min infusion of docetaxel at 25 mg/m^2: (▲) patient 1; (●) patient 2.

investigational nature and consent forms were obtained in compliance with the Cleveland Clinic Foundation and FDA guidelines. In accordance with the protocol for patient sampling, blood samples were collected: 0 (prior to drug infusion), 15, 25, 60, 105, 150, 240, 420, and 540 min following initial drug infusion. Each sample was processed and analyzed as described in the Experimental section. The time-concentration profiles of docetaxel in human plasma are plotted in Fig. 7.

It is worth pointing out that the two patients for whom samples were analyzed were on a combined chemotherapy of temozolomide and docetaxel and they were pretreated with diphenhydramine and dexamethasone prior to the infusion. None of the drugs taken by the patients was found to interfere with the quantitation of docetaxel in plasma, which further attested the specificity of the method.

4. Conclusions

A simple, rapid and sensitive analytical method for the quantitative measurement of docetaxel in human plasma has been developed and validated. This method combines on-line sample extraction with ESI$^+$–MS–MS detection to achieve the selective and specific quantitation of docetaxel. Plasma samples after adding internal standard can be directly injected and be analyzed by the analytical system within 7 min. This method has wide linear calibration range (1.00–3000 ng/mL), high precision (%CV $\leq$ 5.7) and accuracy ($\geq$90.1%). It has been proven useful for analyzing patient plasma samples in a phase II trial of weekly docetaxel at 25 mg/m^2 given as 60-min infusion.

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