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Simple and rapid docetaxel assay in plasma by protein precipitation and high-performance liquid chromatography–tandem mass spectrometry

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Abstract

A simple, rapid and low cost sample preparation method was developed for quantification of docetaxel in mouse plasma by high-performance liquid chromatography/tandem mass spectrometry with paclitaxel as the internal standard. A small volume of plasma (40 μl) and one-step protein precipitation using methanol and acetonitrile (1:1 (v/v)) were used for sample preparation. The calibration curve for docetaxel in mouse plasma was linear over the range 25–2500 nM. The detection limit was 8 nM. The lower limit of quantitation is 25 nM. The intra- and inter-day precisions (CV) of analysis were 9.5 and 9.7% for the low quality control (LQC), 5.5 and 4.9% for the medium quality control (MQC) and 3.9 and 6.3% for the high quality control (HQC), respectively. The accuracy was 102.5% for LQC, 97.9% for MQC and 108.8% for HQC. This assay has now been applied to evaluation of mouse pharmacogenetics and other clinical pharmacology applications.

Keywords: Docetaxel

1. Introduction

Docetaxel is a semi-synthetic analogue of paclitaxel, prepared from a non-cytotoxic precursor extract from the needles of the European yew tree (*Taxus baccata L.*) [1]. It is an inhibitor of microtubule depolymerization [2] and has a broad antitumour activity against various solid tumors including breast, non-small cell lung, head and neck and ovarian carcinomas [3,4]. Large scale pharmacokinetic analysis has identified a relationship between docetaxel plasma pharmacokinetics and both toxicity and antitumor activity [5]. However, limited volumes of blood samples are often used not only for pharmacokinetic studies, but also for pharmacogenomic investigations. This is especially true in the preclinical setting where only semi-micro (<0.1 ml) sample volumes are available for research in small animal models. Therefore, sensitive and selective assays are required to evaluate the pharmacokinetics of docetaxel in this setting. Current methods for the determination of docetaxel are high-performance liquid chromatography (HPLC) with UV [6–15], HPLC/MS [16] and HPLC/MS/MS [17–19]. These assays employ manual solid-phase extraction (SPE), liquid–liquid extraction, or a combination of liquid and solid extraction. The solid-phase extraction often involves multi-step purification and nitrogen evaporation, and 0.5–1 ml plasma is usually needed. The liquid–liquid extraction often needs to use high-purity organic solvents, is hard to handle and nitrogen evaporation is needed. It is often also expensive and time-consuming. So far there are only a few papers published [15,17] in which a small volume of plasma (at least 50 μl) can be used and both use liquid–liquid extraction for sample preparation.

This paper describes HPLC/MS/MS method for the determination of docetaxel in mouse plasma with a micro-sample volume using one-step protein precipitation. The sample preparation is very simple, fast and low cost. This HPLC/MS/MS method is very specific compared with commonly used HPLC/UV methods and no interfering plasma peaks were found for the docetaxel assay.

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2. Experimental

2.1. Chemical and reagents

Docetaxel (purity 99%) and paclitaxel (purity 99%) were obtained from Hande Tech Development Co. (Houston, TX, USA). Methanol and acetonitrile (HPLC grade) were from Fisher Scientific. (Houston, TX, USA). Whatman nylon membrane filters, 47 mm (Maidstone, UK), 0.2 μm filter was used for filtering the organic solvents. Millipore 0.22 μm GP Express plus membrane (Bedford, MA, USA) was used for filtering water-based solution for HPLC mobile phase. Minimum 95% formic acid was from Sigma (St. Louis, MO, USA). Milli-Q deionized water was used throughout the study.

2.2. HPLC/MS/MS system and operating conditions

HPLC pump LC-10Advp and controller SCL-10Avp (Shimadzu Scientific Instruments, Columbia, MD, USA) were used to deliver the mobile phase to a Waters XTerra MS C18 3.5 μm, 2.1 mm × 50 mm column. The guard column was Phenomenex C18 2 mm × 4 mm. The mobile phase was a linear gradient of 20–100% solvent B in 5 min, and then equilibrated with 20% solvent B for 5 min before the next injection. The solvent A was 0.1% formic acid in water and solvent B was 0.1% formic acid in acetonitrile. Flow rate is 0.25 ml/min. Samples were injected with a Shimadzu SIL-10Advp autosampler. In line MS/MS was performed using a Micromass Quattro Micro mass spectrometer with a Z-spray electrospray interface in the positive mode, specifically with a capillary voltage of 3.7 kV and cone voltage of 25 V for docetaxel and paclitaxel. Nitrogen was used for both the cone gas (150 l/h) and the desolvation gas (650 l/h), with the source and desolvation temperatures being held at 120 and 350 °C, respectively. Argon was used as the collision gas and the collison energy is 10 eV for docetaxel and 18 eV for paclitaxel. Docetaxel was quantitated in multiple reaction monitoring mode in the presence of the internal standard paclitaxel. The transition for docetaxel is 808.2 > 527.05 and 854.2 > 285.9 for paclitaxel. A calibration curve was constructed with five-point calibrators in duplicate using docetaxel and paclitaxel (internal standard) concentration ratios.

2.3. Stock solution

Stock solutions of 1 mM paclitaxel and 2 mM docetaxel were prepared in methanol. The 2 mM docetaxel stock solution for the standard curve and quality control samples were separately prepared. Working standard solutions of paclitaxel and docetaxel were prepared by appropriate dilutions of the stock solutions in methanol. The working standard solution of docetaxel for calibration standards (250 nM, 1.25, 5, 12.5 and 25 μM) and quality control (500, 4000 and 20 000 nM) were prepared in 1:1 methanol and water. 1.5 μM paclitaxel was prepared by diluting working standard in 1:1 methanol and water. The solutions were stored at −20 °C until needed. The docetaxel stock solution was stable for more than 1 year when stored at −20 °C [7].

2.4. Preparation of standard and quality control samples

Heparin prepared mouse plasma for standard curve and quality control was purchased from Biomeda (Foster City, CA, USA). For preparation of the standards used for construction of calibration curve, 10 μl of working standard solution of docetaxel (250 nM, 1.25, 5, 12.5 and 25 μM) was added to 90 μl of blank mouse plasma followed by 1 min vortex. The calibration samples for docetaxel are 25, 125, 500, 1250 and 2500 nM (final concentration). Quality control samples were prepared by adding 10 μl of working standard solution of docetaxel (500, 4000 and 20 000 nM) to 90 μl of blank mouse plasma and vortex 1 min. The final concentration of the quality control samples was 50 nM for low quality control (LQC), 400 nM for medium quality control (MQC) and 2000 nM for high quality control (HQC). All these spiked plasma were used immediately.

2.5. Mouse plasma samples

Female mice were obtained from The Jackson Laboratory. The following strains were used in this study: 129/SvImJ, FVB/nJ, and NZB/B1nJ. All animals were aged 6–8 weeks.

Two females from each strain were injected intra-peritoneally (I.P.) with 100 μl of a solution containing 20 mg/kg docetaxel in DMSO. One hour post-injection, blood was obtained by cardiac puncture and collected in microtainer plasma separator tubes with lithium heparin (Becton Dickinson). Samples were spun at 3500 RPM in a microcentrifuge for 10 min. Plasma was then collected and immediately stored at −80 °C. Previous studies have shown that plasma samples were stable for more than 1 year at −80 °C [7]. Up to five freezing–thawing cycles resulted in a <3% loss in spiked plasma [7].

2.6. Sample preparation

Forty microlitres of sample mouse plasma or docetaxel spiked standard or quality control plasma was added to 1.5 ml micro-centrifuge tubes. Ten microlitres of 1.5 μM paclitaxel internal standard was added and the tubes were vortexed for 1 min. One hundred microlitres methanol/acetonitrile (1:1 (v/v)) was added and tubes was vortexed 30 s to precipitate protein. The samples were centrifuged at 10,000 × *g* for 10 min and the supernatants were transferred to HPLC vials with inserts, and 10 μl was injected into HPLC/MS/MS system.

When the docetaxel concentration in mouse plasma is over the linear calibration curve range, approprite dilution of mouse plasma with blank mouse plasma is needed. The blank plasma for the dilution was the same lot used to prepare calibration standards.

3. Results and discussions

3.1. Selectivity and specificity

Docetaxel and paclitaxel formed predominantly protonated molecules ($[M + H]^+$) in the mobile phase containing formic acid using the electrospray ion source. The most sensitive daughter ions for docetaxel and paclitaxel were found at *m/z* 527.05 and 285.9, respectively. The MS/MS parameters were optimized to maximize the response for the docetaxel parent/daughter ion combination of *m/z* 808.2 > 527.05 and for the paclitaxel of 854.2 > 285.9 in the positive ion mode.

The formic acid modifier in mobile phase was shown to increase the signal response. Because of the wide pH range, the Waters XTerra MS C18 analytical HPLC column was used. In the course of development of this assay, the isocratic mobile phase from a previously reported method [19] was studied. When the mobile phase was 80% acetonitrile containing 0.1% formic acid, the retention times for docetaxel and paclitaxel were about 1.16 min and the peaks were close to the void volume peak. The peaks were wide and sensitivity was low. We therefore used gradient elution and much better peak shape and sensitivity were obtained.

Fig. 1 illustrates mass chromatograms of plasma blank extract and docetaxel and paclitaxel spiked plasma extract. With the chromatography conditions described, the retention times of docetaxel and internal standard paclitaxel were <5 min. The overall run length lasted 10 min. The retention times for docetaxel and paclitaxel were 4.4 and 4.5 min, respectively. Both docetaxel and paclitaxel eluted as sharp symmetrical peaks and plasma matrix components did not interfere with the analysis, whereas the interferences from plasma were the most troublesome problems when HPLC/UV assays were used. Peak shape and retention time was the same for injection of pure standards in mobile phase as for extracted plasma standards and samples. This LC/MS/MS method is selective for the detection of docetaxel in mouse plasma.

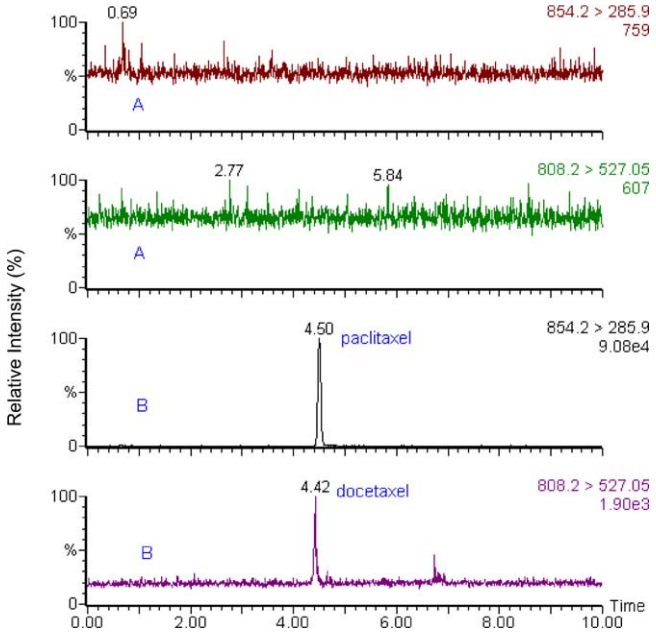

Fig. 1. Multiple reaction monitoring chromatograms for (A) mouse plasma blank, (B) mouse plasma spiked with 25 nM docetaxel and 300 nM paclitaxel internal standard.

3.2. Mouse plasma sample preparation

In this paper, we develop a simple and rapid plasma sample preparation method by single step protein precipitation with 1:1 acetonitrile/methanol. To optimize preparation procedure, different volumes of 1:1 acetonitrile/methanol (100, 150 and 200 μl) were added to 40 μl mouse plasma to precipitate protein. The results demonstrate that 100 μl 1:1 acetonitrile/methanol give the best sensitivity and least sample dilution.

3.3. Recovery

The extraction recovery of docetaxel and internal standard (paclitaxel) was determined by comparing peak areas obtained after whole extraction with those obtained from direct injections of standard solutions. The mean recovery of docetaxel was 100% (CV 8.54%, $n = 3$) at 50 nM, 92.8% (CV 0.54%, $n = 3$) at 400 nM and 97.0% (CV 1.17%, $n = 3$) at 2000 nM, respectively. The mean recovery of paclitaxel was 91.2% (CV 0.60%, $n = 3$) at 300 nM, which is the concentration we use for the assay.

3.4. Calibration and quantification

A standard curve was prepared by injecting the extracts from five known concentrations of docetaxel in plasma with internal standard. The calibration curve was obtained by weighted *1/x* least-squares linear regression analysis of known drug concentration ratios versus peak area ratios. Plasma samples and quality control samples concentrations were calculated by using the regressed equation of the straight-line $y = ax + b$ where y is the peak area ratio of docetaxel to paclitaxel, a the slope, b the y-intercept and x the ratio of unknown docetaxel concentration to paclitaxel internal standard concentration. The method demonstrated excellent linearity over the range of 25–2500 nM with a reproducible correlation coefficient of $r^2 > 0.997$. All standards needed to meet the criteria of <15% deviation from nominal concentration. The limit of detection for docetaxel in plasma, defined as a minimum signal-to-noise of 3, was 8 nM. The lower limit of quantitation was 25 nM. At this concentration, the intra- and inter-day precisions (CV) were <15%. The accuracy was within 15%.

Table 1
Precision and accuracy of docetaxel determination in mouse plasma during intra-day assay

	Quality control		
	Low (50 nM)	Medium (400 nM)	High (2000 nM)
Average (nM)	52.6	391.1	2232.8
Precision (CV, %)	9.5	5.5	3.9
Accuracy average (%)	105.2	97.8	111.6
Accuracy range (%)	88.8–122.8	89.2–106.8	104.8–116.3

3.5. Precision and accuracy

Analytical runs were performed on 3 days, and each day included a calibration curve processed in duplicate and a set of QC samples. LQC, MQC and HQC were mouse plasma spiked with 50, 400 and 2000 nM of docetaxel. For day 1, QC samples were 10 LQC, 5 MQC and 5 HQC. For day 2, QC samples were 5 LQC, 10 MQC and 5 HQC. For day 3, QC samples were 5 LQC, 5 MQC and 10 HQC. The intra-assay validation (Table 1) was obtained from ten replicate QC samples on the same day, and the inter-assay validation (Table 2) was obtained from 20 measurement of QC from 3 days. The intra- and inter-day precisions (CV) of analysis were 9.5 and 9.7% for LQC, 5.5 and 4.9% for MQC and 3.9 and 6.3% for HQC, respectively. The accuracy range were 86.2–122.8% for LQC, 88–106.8% for MQC and 93.6–116.5% for HQC.

3.6. Application of the method

For most chemotherapy agents it is likely that drug response is a complex trait, with multiple polymorphic genes contributing with various strengths to overall treatment outcome. Therefore, in order to more fully understand the genetic basis of chemotherapy response, genome-wide approaches are needed in which no a priori assumptions are made about genes or genomic regions associated with the drug effect under investigation. Thus, the laboratory mouse provides an ideal model system for the study of pharmacogenomics.

We determined mouse plasma docetaxel concentration for two females from each strain of 129/SvImJ, FVB/nJ and NZB/B1nJ by generating one standard curve, duplicates of each of LQC, MQC and HQC, and duplicates of each mouse plasma sample. The average docetaxel concentrations were 5.4 μM for strain of 129/SvImJ, 2.8 μM for strain of FVB/nJ, and 1.85 μM for strain of NZB/B1nJ. Here we demonstrate that docetaxel metabolism between inbred strains exhibits large variability. This phenotypic difference can be used in genetic mapping studies to identify genomic regions influencing docetaxel metabolism. Additionally, these data suggest that inbred mouse strains are a useful model system in which to identify genetic modifiers of drug metabolism and response in a way not currently feasible in human patients.

Table 2
Precision and accuracy of docetaxel determination in mouse plasma during inter-day assay

	Quality control		
	Low (50 nM)	Medium (400 nM)	High (2000 nM)
Average (nM)	51.3	391.7	2176.9
Precision (CV, %)	9.7	4.9	6.3
Accuracy average (%)	102.5	97.9	108.8
Accuracy range (%)	86.2–122.8	88.0–106.8	93.6–116.5

4. Conclusions

In conclusion, a simple and rapid assay for the quantitative determination of docetaxed in mouse plasma has been described. The method is accurate, precise, sensitive and selective. Plasma matrix components do not interfere with the analysis. As sample preparation consists of a simple, rapid one-step protein precipitation, it avoids the use of the complicated and expensive manual solid phase extraction techniques and time-consuming liquid–liquid solvent extraction with evaporation that were used in previously reported procedures. This method only needs a small volume plasma sample (40 μl) and is suitable for use in small animals, such as in mouse pre-clinical studies.

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