Chapter 14

Quantification of Docetaxel in Serum Using Turbulent Flow Liquid Chromatography Electrospray Tandem Mass Spectrometry (TFC-HPLC-ESI-MS/MS)

Christopher A. Crutchfield, Mark A. Marzinke, and William A. Clarke

Abstract

Docetaxel is a second-generation taxane and is used clinically as an anti-neoplastic agent in cancer chemotherapy via an anti-mitotic mechanism. Its efficacy is limited to a narrow therapeutic window. Inappropriately high concentrations may cause erythema, fluid retention, nausea, diarrhea, and neutropenia. As a result, dosing recommendations have changed from high dosage loading every 3 weeks to lower dosage loading weekly. We describe a method that can be used for therapeutic drug monitoring of docetaxel levels using turbulent flow liquid chromatography electrospray tandem mass spectrometry (TFC-HPLC-ESI-MS/MS). The method is rapid, requiring only 6.3 min per analytical run following a simple protein crash. The method requires only 100 μL of serum. Concentrations of docetaxel were quantified by a calibration curve relating the peak-area ratio of docetaxel to a deuterated internal standard (docetaxel-D9). The method was linear from 7.8 to 1000 ng/mL, with imprecision ≤6.2 %.

Key words Docetaxel, Anti-neoplastic, Turbulent flow liquid chromatography electrospray tandem mass spectrometry

1 Introduction

Docetaxel is a chemotherapeutic used to treat solid tumors, including breast, non-small-cell lung, and prostate cancer [1–3]. However, its use can cause side effects including erythema, nausea, diarrhea, fluid retention, and neutropenia [1]. Initially docetaxel had a standard dosage of 75–100 mg/m^2 over 1 h every 3 weeks [1], however later studies suggested a lower dose of 20–40 mg/m^2 every 1 week [4]. Docetaxel is highly protein bound [5] and its pharmacological effect is tied to its free form. With frequent dosing and high protein affinity, clinical teams may find utility in monitoring the pharmacokinetics of docetaxel in their patient population. This method [6] enables rapid analysis of docetaxel using turboflow on-line extraction prior to analytical separation and analysis using tandem mass spectrometry.

Uttam Garg (ed.), *Clinical Applications of Mass Spectrometry in Drug Analysis: Methods and Protocols*, Methods in Molecular Biology, vol. 1383, DOI 10.1007/978-1-4939-3252-8_14,

2 Materials

2.1 Sample

Human serum.

2.2 Solvents and Reagents

1. Mobile Phase A, 0.1 % (v/v) formic acid in HPLC-grade water, stable for 1 month at room temperature, 18–24 °C.
2. Mobile Phase B, 0.1 % (v/v) formic acid in HPLC-grade methanol, stable for 1 month at room temperature, 18–24 °C.
3. Mobile Phase C, 40:40:20 acetonitrile:isopropanol:acetone.
4. Human drug-free normal serum.

2.3 Standards and Internal Standard

1. Primary standard of docetaxel was prepared by dissolving docetaxel powder (Toronto Research Chemicals) in methanol at a final concentration of 5 mg/mL.
2. Primary internal standard was prepared by dissolving docetaxel-d9 powder (Toronto Research Chemicals) in methanol at a final concentration of 1 mg/mL.
3. Primary working solutions are prepared by diluting the primary standard solution to concentrations of 100, 10, and 1 μg/mL.
4. I.S. Working Solution/Extraction Solution (500 ng/mL docetaxel-d9) was prepared by adding 500 μL of the 1 mg/mL primary internal standard to a Class A 1000 mL volumetric flask, filling to the level, and mixing.

2.4 Calibrators and Controls

1. Calibrators: Prepare calibrators 1–9 by diluting working stock solutions with drug-free normal human serum in 10 mL class A volumetric flasks (Table 1).
2. Controls: Using independently prepared working stock solutions, prepare low, medium, and high QC levels at 50, 250, and 1000 ng/mL.

2.5 Analytical Equipment and Supplies

1. Aria TLX1 system equipped with a CTC HTC PAL Autosampler and two Agilent 1250 Pumps coupled to a Thermo TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific).
2. Pre-Analytical column: Thermo Cyclone-P 0.5 × 50 mm (Thermo Fisher Scientific).
3. Analytical column: Thermo Scientific Hypersil Gold C-18 2.1 × 50 mm, particle size 3 μm.
4. 1.8 mL glass HPLC vials.
5. 1.5 polypropylene microcentrifuge tubes.

Table 1
Preparation of calibrators

Calibrator	Working stock concentration (μg/mL)	Working stock volume (μL)	Final volume (mL)	Final concentration (ng/mL)
1	1	78	10	7.8
2	1	156	10	15.6
3	1	313	10	31.3
4	1	625	10	62.5
5	10	125	10	125
6	10	250	10	250
7	10	500	10	500
8	100	100	10	1000
9	100	200	10	2000

3 Methods

3.1 Stepwise Procedure

1. To a labeled 1.5 mL polypropylene centrifuge tube, pipette 100 μL of serum (calibrator, control, or unknown sample) (*see* **Note 1**).
2. Add 300 μL of extraction solution.
3. Cap and vortex for 20 s.
4. Centrifuge for 5 min at 18,000 × *g*.
5. Dilute 300 μL supernatant 1:1 with HPLC-grade water in a labeled 1.8 mL glass vial.
6. Cap and vortex briefly.
7. Please vials into autosampler.
8. Inject 25 μL and analyze.

3.2 Sample Analysis

1. Instrumental operating parameters are given in Table 2.
2. Data are analyzed using LCQuan (Thermo Scientific).
3. Standard curves are generated based on linear regression with $1/x^2$ weighting of the analyte/internal standard peak-area ratio relative to the nominal analyte concentration.
4. Imprecision is typically ≤6.2 % at all QC levels.

Table 2
HPLC-MS/MS operation conditions

A. HPLC										
Time (min)	Length (s)	TX flow rate (mL/min)	TX mobile phase A (%)	TX mobile phase B (%)	TX mobile phase C (%)	Tee	Loop	LX flow rate (mL/min)	LX mobile phase A (%)	LX mobile phase B (%)
0	30	1.50	100	0	0		Out	0.25	100	0
0.5	45	0.20	25	75	0	Tee	In	0.50	100	0
1.25	200	1.50	0	0	100		In	0.25	10	90
4.58	45	1.50	25	0	100		In	0.25	0	100
5.33	60	1.50	100	0	0		Out	0.25	100	0

B. MS/MS tune settings	
Parameter	Value
Spray voltage (V)	3500
Sheath gas	35
Aux gas	35
Capillary temperature (°C)	200

C. Precursor and product ions			
Compound	Precursor	Product	CE (eV)
Docetaxel	808.4	225.9	10
Docetaxel-D9	817.4	226.9	10

4 Notes

1. Matrix effects were evaluated using post-column infusion as well as comparison of spiked sera and spiked solvent. Matrix effects were <14 %.

References

1. Salminen E, Bergman M, Huhtala S, Ekholm E (1999) Docetaxel: standard recommended dose of 100 mg/m(2) is effective but not feasible for some metastatic breast cancer patients heavily pretreated with chemotherapy-A phase II single-center study. J Clin Oncol 17:1127
2. Saloustros E, Georgoulias V (2008) Docetaxel in the treatment of advanced non-small-cell lung cancer. Expert Rev Anticancer Ther 8:1207–1222
3. Gilbert DC, Parker C (2005) Docetaxel for the treatment of prostate cancer. Future Oncol 1:307–314
4. Burstein HJ, Manola J, Younger J et al (2000) Docetaxel administered on a weekly basis for metastatic breast cancer. J Clin Oncol 18:1212–1219
5. Clarke DSJ, Rivory LP (2012) Clinical pharmacokinetics of docetaxel. Clin Pharmacokinet 36:99–114
6. Marzinke MA, Breaud AR, Clarke W (2013) The development and clinical validation of a turbulent-flow liquid chromatography-tandem mass spectrometric method for the rapid quantitation of docetaxel in serum. Clin Chim Acta 417:12–18