Application Note 20581

LC-MS/MS Method for the Determination of Docetaxel in Human Serum for Clinical Research

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Key Words
SPE, SOLA, Accucore RP-MS, docetaxel, clinical research

Abstract
A liquid chromatography-tandem mass spectrometry method for the analysis of docetaxel in human serum for clinical research has been developed. Using Thermo Scientific™ SOLA™ cartridges or plates, sample preparation is fast and gives excellent reproducibility and recovery levels. The analysis was carried out on a Thermo Scientific Accucore™ RP-MS 2.6 µm, 50 x 2.1 mm column for a fast separation with a cycle time of 2 minutes and excellent peak shape.

Introduction
SOLA solid phase extraction (SPE) products introduce next-generation, innovative technological advancements, which give unparalleled performance characteristics compared to conventional SPE, phospholipid, and protein precipitation products.

This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA products have significant advantages for the analyst when processing compounds in complex matrices, particularly in high-throughput clinical research laboratories where reduced failure rate, higher analysis speed, and lower sample/solvent requirements are critical. The increased performance gives higher confidence in analytical results and lowers cost without compromising the ease of use or requiring complex method development.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous, but have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. Accucore RP-MS columns use an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of the Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Docetaxel is an anti-mitotic agent used in cancer chemotherapy (Figure 1). The extraction of docetaxel from human plasma is demonstrated in this application.

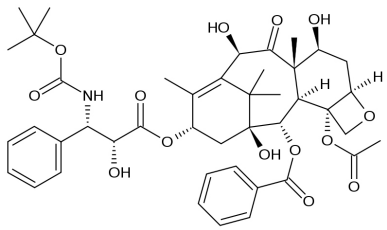

Figure 1: Docetaxel

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Experimental Details

Consumables	Part Number
Fisher Scientific™ LC/MS grade water	W/011217
Fisher Scientific LC/MS grade methanol	M/4062/17
Fisher Scientific LC/MS grade acetonitrile	A/0626/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08
Fisher Scientific Sodium acetate	S/2120/50
Thermo Scientific National™ Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample Handling Equipment	Part Number
Thermo Scientific 96 well plate vacuum manifold	60103-351
Thermo Scientific Ultra vap	CLS-229070

Sample Pretreatment

A standard spiking solution of docetaxel was prepared in acetonitrile. A working internal standard solution (paclitaxel) was prepared in acetonitrile. A sample of 200 µL of blank human plasma was taken. For standards and quality control (QC) samples, 10 µL of standard spiking solution was added to 200 µL of blank human plasma, and for blanks, 10 µL of acetonitrile was added.

For standards and QCs, 10 µL of working internal standard solution was added, and for blanks, 10 µL of acetonitrile was added. All samples were vortexed for 30 seconds and then centrifuged for 5 minutes at 5000 rpm.

Sample Preparation		Part Number
Compound(s):	Docetaxel, paclitaxel (IS)	
Matrix:	Human serum	
Plate type:	Thermo Scientific SOLA 10 mg/2 mL	60309-001
Conditioning stage:	0.5 mL methanol followed by 0.5 mL water	
Application stage:	Apply spiked sample.	
Washing stage:	250 µL water / acetonitrile (70:30 v/v)	
Elution stage:	2 x 250 µL acetonitrile + 1% ammonia	
Additional stage:	Dry down under nitrogen without heat and reconstitute in 200 µL water/ acetonitrile (50:50 v/v) + 0.5% 20 µM sodium acetate. Mix well. (Sodium acetate was required to ensure the reproducible formation of a sodium adduct, which was used for quantification. At this low concentration of sodium acetate no source contamination was observed.)	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Dionex™ UltiMate™ 3000 RSLC HPLC system	
Column:	Accucore RP-MS, 2.6 µm, 50 x 2.1 mm	17626-052130
Mobile phase A:	Water + 0.1% formic acid	
Mobile phase B:	Methanol + 0.1% formic acid	
Gradient:	60%–70% B in 2 minutes	
Flow rate:	0.6 mL/min	
Column temperature:	30 °C	
Injection details:	2.5 µL	
Injection wash solvent 1:	Water / acetonitrile (80:20 v/v)	
Injection wash solvent 2:	Propan-2-ol / acetonitrile / acetone (45:45:10 v/v/v)	

MS Conditions	
Instrumentation:	Thermo Scientific TSQ Vantage™ MS
Ionization conditions:	HESI
Polarity:	Positive
Spray voltage (V):	4000
Vaporizer temperature (°C):	450
Sheath gas pressure (Arb):	30
Aux gas pressure (Arb):	20
Capillary temp (°C):	365
Collision pressure (mTorr):	1.5
Scan time (s):	0.02
Q1 (FWHM):	0.7
Q3 (FWHM):	0.7

The compound transition information is shown in Table 1.

Compound	Paclitaxel (IS)	Docetaxel
Parent *(m/z)*	876.25	830.26
Products *(m/z)*	308.25	549.24
Collision energy (V)	25	23
S-lens (V)	141	121

Table 1: Compound transition details

Data Processing	
Software:	Thermo Scientific LCQUAN™

Results

Docetaxel standards extracted from human serum gave a linear calibration curve over the dynamic range of 0.25 to 10 ng/mL with an r^2 coefficient of 0.9974 (Figure 2 and Table 2). The chromatography of the LLOQ at 0.25 ng/mL is shown in Figure 3.

QC samples were run in replicates of six at concentrations of 0.3, 1.5, and 6 ng/mL. The precision of each of the QC levels was ≤ 8.8% CV at all three levels (Table 3).

Overspiked samples were analyzed at concentrations of 0.75 ng/mL and used to calculate recovery. The average percentage recovery level of docetaxel was 109% (Table 4.)

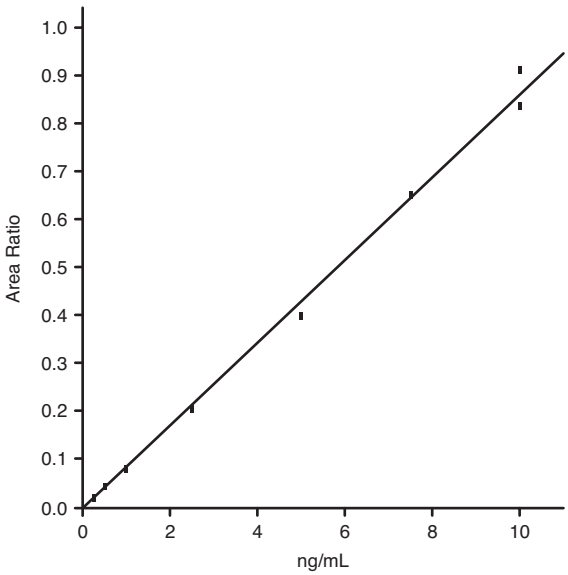

Figure 2: Docetaxel linearity over the dynamic range 0.25 to 10 ng/mL

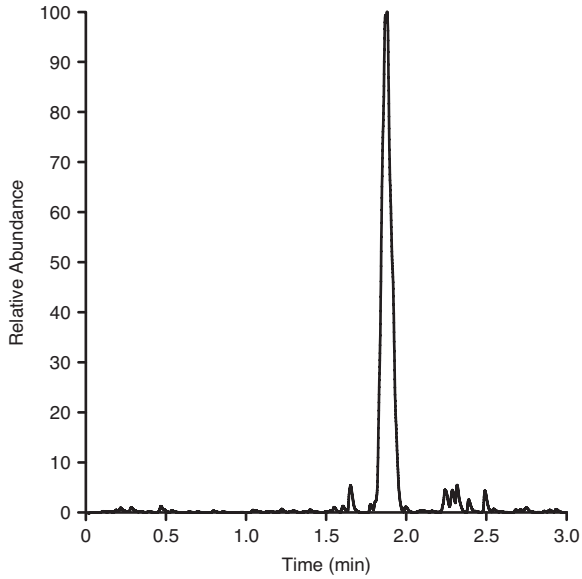

Figure 3: Representative chromatogram of docetaxel SRM, extracted from human plasma at 0.25 ng/mL

Accuracy and Precision

Standard	Specified Concentration (ng/mL)	Calculated Concentration (ng/mL)	%Diff
S1	0.250	0.261	4.52
S2	0.500	0.525	5.06
S3	1.00	0.959	-4.14
S4	2.50	2.39	-4.59
S5	5.00	4.59	-8.21
S6	7.00	7.48	6.87
S7	10.0	10.5	4.54
S7	10.0	9.60	-4.04

Table 3: Accuracy data for docetaxel extracted standards over the linear range 0.25 to 10 ng/mL

Quality Control	Concentration (ng/mL)	Average Calculated Concentration (n=6)	Precision (%CV)
QCL	0.300	0.313	4.5
QCM	1.50	1.53	8.8
QCH	6.00	6.02	5.9

Table 4: Average precision data for six replicate QCs for docetaxel

Recovery

	Response	% Recovery
Average area response (n=6)	260035	109
Overspiked area response	238397	

Table 5: Recovery data for docetaxel

Conclusion

- SOLA SPE plates and Accucore RP-MS HPLC columns allow for a simple extraction and rapid quantification of docetaxel from human serum for clinical research using an internal standard.
- An LLOQ of 0.25 ng/mL was demonstrated.
- Extraction recovery was 109%.
- The method showed excellent precision with %CV (n=6) ≤ 8.8%.

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