LC-MS/MS Method for the Determination of Paclitaxel in Human Serum

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Key Words
SPE, SOLA, Accucore RP-MS, paclitaxel

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

Introduction
Paclitaxel (Figure 1) is an anti-mitotic agent (inhibits the process of cell division) used in cancer chemotherapy. Paclitaxel (trade name TAXOL®) is used to treat ovarian, breast, head and neck cancer, and advanced forms of Kaposi's sarcoma. The extraction of paclitaxel from human plasma using SOLA solid phase extraction (SPE) products is demonstrated in this application.

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

These include:

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

SOLA SPE plates or cartridges have significant advantages when analyzing compounds in complex matrices, particularly in high-throughput bioanalytical and clinical research laboratories where reduced failure rates, higher analysis speed, and lower solvent requirements are critical. SOLA products' superior performance gives higher confidence in analytical results and lowers cost without compromising 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 instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The Accucore RP-MS column uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

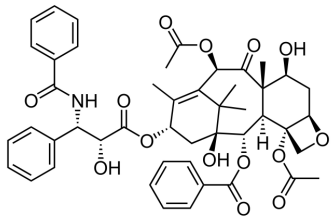

Figure 1: Paclitaxel

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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 Target DP Certified 2 mL clear vial with ID patch, blue DP cap with bonded PTFE/silicone septum	MSCERT4000-34W

Sample Handling Equipment	Part Number
Thermo Scientific 96 well plate vacuum manifold	60103-351
Thermo Scientific™ UltraVap™ high speed sample concentrator	CLS-229070

Sample Pretreatment

A standard spiking solution of paclitaxel was prepared in acetonitrile.
A working internal standard solution (docetaxel) was prepared in acetonitrile.
200 µL of blank human plasma was taken.
For standards and quality control (QC) samples, 10 µL of standard spiking solution was added, with 10 µL of acetonitrile added for blanks.
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):	Paclitaxel, docetaxel (IS)	
Matrix:	Human serum	
Plate type:	Thermo Scientific SOLA 10 mg/2 mL	60309-001
Conditioning stage:	Add 0.5 mL methanol and then 0.5 mL water to the SPE cartridge/well.	
Application stage:	Apply spiked sample at approximately 1 mL/min to the SPE cartridge/well.	
Washing stage:	Add 250 µL water / acetonitrile (70:30 v/v) to the SPE cartridge/well.	
Elution stage:	Apply 2 x 250 µL acetonitrile + 1% ammonia at approximately 1 mL/min to the SPE cartridge/well.	
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 × 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 volume:	2.5 µL	
Injection wash solvent 1:	Water / acetonitrile (80:20 v/v)	
Injection wash solvent 2:	IPA / 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
Compound transition details are provided in Table 1.	

Compound	Paclitaxel	Docetaxel (IS)
Parent (*m/z*)	876.3	830.3
Products (*m/z*)	308.3	549.2
Collision energy (V)	25	23
S-lens (V)	141	121

Table 1: Compound transition details

Data Processing	
Software:	Thermo Scientific™ LC QUAN™ software

Results

Paclitaxel standards, extracted from human serum, gave a linear calibration curve over the dynamic range of 0.1 to 10 ng/mL with an r^2 coefficient of 0.9982 (Figure 2 and Table 2).
The chromatography of the lower limit of quantitation (LLOQ) sample at 0.1 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 at each of the QC levels was ≤6.6 % CV (Table 3).

Overspikes (post extraction fortified blank samples) were analyzed at a concentration of 0.75 ng/mL and used to calculate recovery. The percentage recovery level of paclitaxel was 116% (Table 4.)

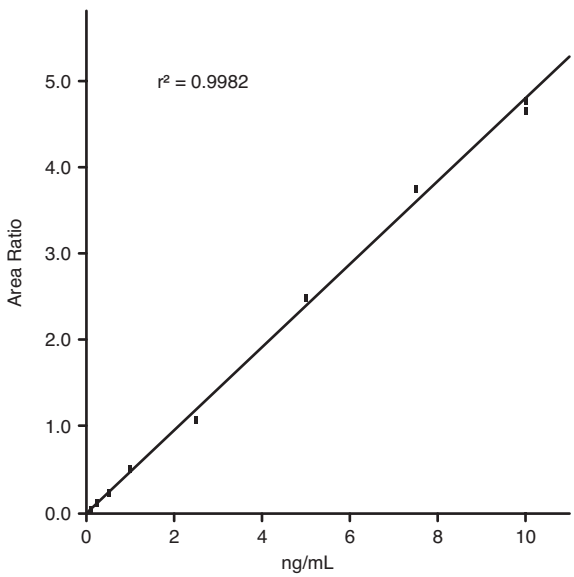

Figure 2: Paclitaxel linearity over the dynamic range 0.1-10 ng/mL

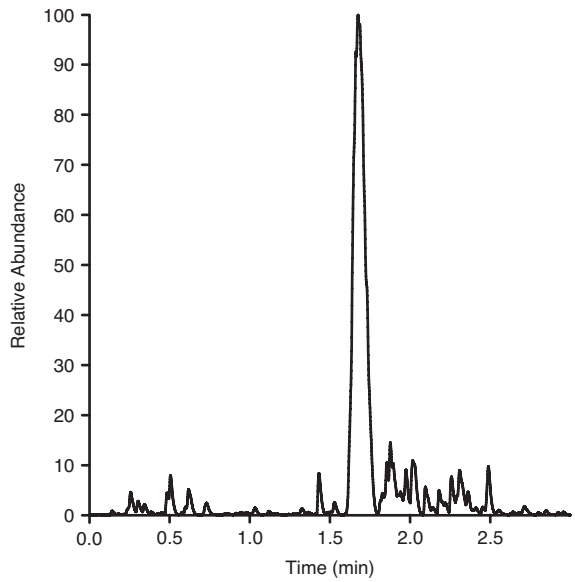

Figure 3: Representative chromatogram of paclitaxel SRM, extracted from human serum at 0.1 ng/mL

Accuracy and Precision

Standard	Specified concentration (ng/mL)	Calculated Concentration (ng/mL)	% Diff
S1	0.10	0.096	-3.71
S2	0.25	0.251	0.21
S3	0.50	0.506	1.22
S4	1.00	1.07	7.22
S5	2.50	2.26	-9.67
S6	5.00	5.20	4.01
S7	7.50	7.82	4.21
S8	10.0	9.94	-0.65
S8	10.0	9.72	-2.85

Table 2: Accuracy of back-calculated values for paclitaxel extracted standards over the linear range 0.1–10 ng/mL

Standard	Concentration (ng/mL)	Average Calculated Concentration (n=6)	Precision % CV
QCL	0.3	0.28	6.6
QCM	1.5	1.45	4.5
QCH	6	6.06	6.0

Table 3: Average precision data for six replicate QCs for paclitaxel

Recovery

Standard	Response	% Recovery
Average area response (n=6)	192366	116
Overspike area response	165330	

Table 4: Recovery data for paclitaxel

Conclusion

- SOLA SPE plates and Accucore RP-MS HPLC columns allow for a simple extraction and rapid quantification of paclitaxel from human serum using an internal standard.
- An LLOQ of 0.1 ng/mL was demonstrated.
- Extraction recovery was 116%.
- The method showed excellent precision with %CV (n=6) ≤6.6%.

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