

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 \times 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.

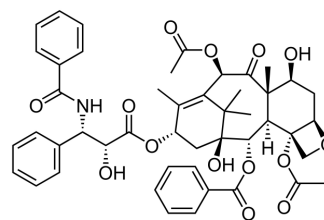


Figure 1: Paclitaxel

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. | |
| Application stage: | Apply spiked sample at approximately 1 mL/min | |
| Washing stage: | 250 µL water / acetonitrile (70:30 v/v) | |
| Elution stage: | 2 x 250 µL acetonitrile + 1% ammonia at approximately 1 mL/min | |
| 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 at all three levels (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.)

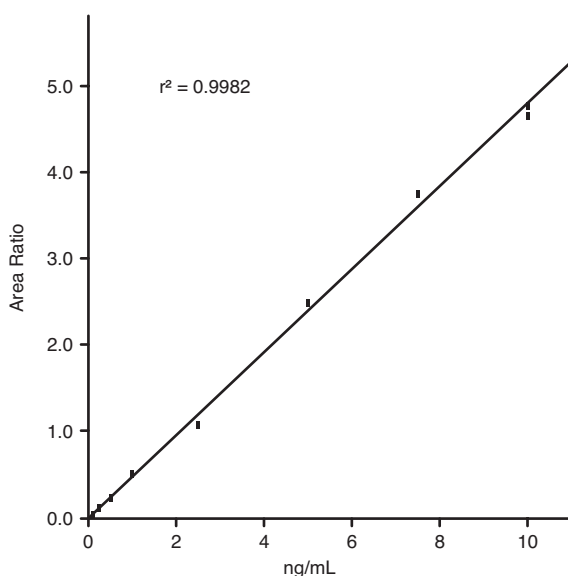


Figure 2: Paclitaxel linearity over the dynamic range 0.1-10 ng/mL

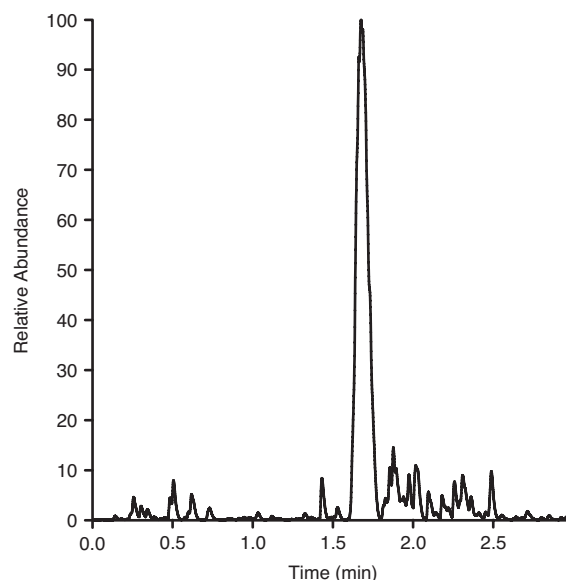


Figure 3: Representative chromatogram of paclitaxel SRM, extracted from human serum at 0.1 ng/mL

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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