



A New Liquid Chromatography-Tandem Mass Spectrometry Method (0.1-1,000 ng/mL) for Quantitation of Paclitaxel in Human and Rat Plasma

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Overview

A Liquid chromatography-tandem mass spectrometry (LC/MS/MS) method has been developed to improve sensitivity and to extend the calibration curve dynamic range for the quantitation of paclitaxel in rat and human plasma.

Monitoring of a sodium adduct ion of paclitaxel rather than its proton adduct provided greater sensitivity. Intense and consistent ion signals were achieved by addition of 0.05 mM of sodium acetate to the HPLC mobile phase. The calibration curve was extended to 0.1-1,000 ng/mL (10,000 fold). Ion source saturation was avoided by diluting the high concentration (≥ 50 ng/mL) standards and QC samples 20-fold with HPLC mobile phase. Excellent linearity, accuracy, and precision were obtained.

Both plasma methods were successfully validated.

Introduction

Paclitaxel (taxol), a natural product isolated from bark of the yew tree, *Taxus brevifolia*, is a highly potent and very important chemotherapeutic agent in the treatment of cancers. It is currently used clinically for treatment of ovarian and breast cancers and is under extensive investigation and development for treatment of non-small-cell lung cancer, small-cell lung cancer, and squamous cancers of the head and neck. Many studies involved with new applications are complex requiring more sensitive and rapid assays for the quantitation of paclitaxel in plasma samples from human and animals. We now report a rapid, specific, and highly sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS) method capable of quantifying paclitaxel from 0.2 mL of human and rat plasma at levels as low as 0.1 ng/mL.

Experimental

Liquid chromatography:

LC System: Waters 2690 Separations Module
Analytical Column: Ace C18 column, 2.1 x 50 mm, 3 μ m
Mobile Phase A: 1% HOAc and 0.05 mM NaOAc in H₂O
Mobile Phase B: 1% HOAc in CH₃OH
Mobile Phase C: CH₃CN
Gradient: A:B:C (38:62:0, 0.5 min), 3 min to A:B:C (30:70:0, 0.5 min), 0.5 min to A:B:C (10:40:50, 0.3 min), 0.2 min to initial.
Flow rate: 0.3 mL/min
Injection Volume: 15 μ L

Mass Spectrometry:

MS System: AB Sciex API-300 or API-4000 Mass Spectrometer using ESI interface with multiple reaction monitoring (MRM) detection in positive ionization mode.

The mass spectrometer was set up for the following ion transitions:

Paclitaxel (R_f 3.5 min): 876.5 \rightarrow 308.3
7-Epi-taxol (IS, R_f 3.5 min): 876.1 \rightarrow 591.4

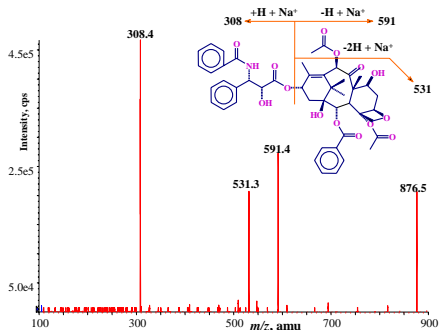


Figure 1. LC/ESI-MS/MS spectrum and fragmentation of paclitaxel. All of the major product ions are sodium adducts.

Extraction Procedure:

A mixture of 200 μ L of a human or rat plasma sample and 100 μ L of IS was partitioned with a mixture of 2 mL of *t*-butyl methyl ether (MTBE) and 2 mL of hexane. The MTBE-Hexane fraction was evaporated to dryness under a nitrogen stream. The resultant extract was reconstituted in 200 μ L of a 1:1 mixture of CH₃OH and 1% aqueous HOAc containing 0.05 mM NaOAc.

Ten μ L of the processed high concentration (≥ 50 ng/mL) standards and QC samples was combined with 190 μ L of a 1:1 mixture of CH₃OH and 1% aqueous HOAc containing 0.05 mM NaOAc to give 20-fold dilution samples.

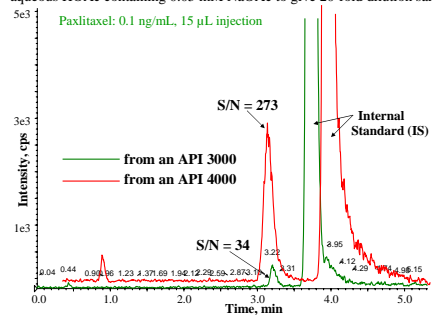


Figure 2. LC/MS/MS ion chromatogram of a 0.1 ng/mL (LLOQ) solution of paclitaxel (with IS) acquired by API-3000 or API-4000 instrument. The signal to noise ratio increased approximately 8 times from API-4000 vs. API-3000.

Results and Discussion

- Ion signal intensity of paclitaxel increased dramatically with an API-4000 mass spectrometer compared to an API-3000 system (Figure 2). The signal to noise ratio increased nearly 8 times. Intense and consistent ion signals were achieved by addition of 0.05 mM NaOAc to the HPLC mobile phase.
- The concentration of IS was carefully adjusted so that a nearly absolutely linear response was achieved after 20-fold dilution. Excellent linearity was obtained even with plotting non-dilution standards (0.1-50 ng/mL) and dilution standards (50-1,000 ng/mL) together. The slope values of both calibration curves ranged from 0.1-50 and 0.1-1,000 ng/mL are very similar (Figure 3) and the calculated concentrations for unknowns varies within $\pm 2\%$ using either linear regression equation (Table I).
- Good accuracy and precision were also obtained for the 20-fold dilution samples using three different diluents, i.e., control samples (with IS), blank plasma extracts (without IS), and HPLC mobile phase. The method was fully validated for both human plasma (Table I) and rat plasma.

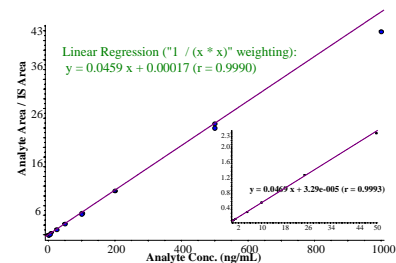


Figure 3. Typical calibration curves of paclitaxel extracted from human plasma at concentration ranges of 0.1-50 and 0.1-1,000 ng/mL.

Table I. Validation data summary of paclitaxel in human plasma

Calibration Curve Range		0.1 to 1000 ng/mL 0.9990 to 0.9995		
Correlation coefficient (n = 5)		0.9990 to 0.9995		
Accuracy & Precision		Conc. (ng/mL)	Accuracy Bias%	Precision CV%
Inter-Batch (n=18)	LLOQ	0.1	1.00	4.95
	Low	0.3	2.00	3.92
	Medium	9	-1.11	3.80
	High 1	45	-1.56	3.30
	High 2	900	-5.56	3.47
Storage Stability		Condition	% Freshly Prepared QC	
Room temperature		~ 24 hrs	95.96	to 97.37
Freeze and thaw		-20°C, 3 cycles	98.03	to 98.21
HPLC autosampler conditions		~ 4°C, 6 days	95.00	to 107
Method Recovery		%		
Paclitaxel		91.07	to	95.06
IS		87.93	to	90.46
Concentrations of QC Samples Calculated from Both Calibration Curves (n=6)				
	0-50 ng/mL	0-1,000 ng/mL	Mean (ng/mL)	% Variation
Low QC (0.3 ng/mL)	0.311	0.315	0.313	± 0.64
Medium QC (9 ng/mL)	8.77	8.97	8.87	± 1.13
High QC (45 ng/mL)	43.2	44.2	43.7	± 1.14

Conclusion

- The newly developed liquid chromatography-tandem mass spectrometry assay using an API-4000 mass spectrometer substantially improves speed, sensitivity, accuracy, and precision for the quantitation of paclitaxel in rat and human plasma at a concentration range of 0.1-1,000 ng/mL.