Validation of an LC-MS/MS Method for the Quantification of Paclitaxel in Porcine Artery, Kidney, Liver, Lung, Muscle, Myocardium, and Spleen Tissues

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Why Measure Tissue Concentrations?

During development of a drug eluting stent (DES) or drug eluting balloon (DEB) many questions relating to the drug arise that must be answered in order to bring a device to market.

- What is the local drug exposure?
- What is the systemic drug exposure?
- What is the biological response and safety of the drug?
- What are the local drug release characteristics?
- How long does the drug stay in the system?

To answer many of these questions, drug concentrations in blood and tissues need to be determined. Typically the target artery section and an artery section proximal and distal to the target site are assayed for drug concentration. In addition, non-target tissues are samples to determine systemic exposure including myocardial tissue sections distal to the implantation site, and sections of kidney, liver, lung, muscle, and spleen tissues.

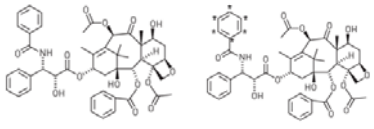
BASi developed a bioanalytical method for the analysis of paclitaxel in blood, plasma, and tissues to help answer these questions. The work presented herein relates only to the analysis of tissue.

Introduction and Purpose

Paclitaxel, originally approved for cancer chemotherapy, has proven effective as an anti-proliferative agent indicated for the prevention of restenosis (recurrent narrowing) of coronary stents. The purpose of this work was to develop and validate an LC-MS/MS method for the quantification of paclitaxel in porcine artery, kidney, liver, lung, muscle, myocardium, and spleen tissues. Tissues are sampled to determine impact of paclitaxel formulation on the concentration of paclitaxel in target tissue (artery) versus non-target tissues (kidney, liver, lung, muscle, myocardium, and spleen).

Typically multiple animals are sampled at time points such as 1 hour, 1 day, 7 days, 30 days, 90 days, 180 days, and 365 days. Time points may vary depending on the composition of the coating and how long the drug stays in the system. At minimum study duration should extend until there are no detectable drug levels.

Paclitaxel **$^{13}C_6$ Paclitaxel**



Methods

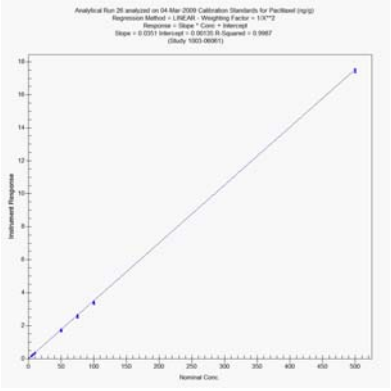
The method for the analysis of paclitaxel in porcine tissue involves homogenizing whole tissue with a buffer. Stable label internal standard solution is added to a 400 µL aliquot of the homogenate. Liquid-liquid extraction is performed using 1 mL of methyl tert-butyl ether (MTBE). Following evaporation and reconstitution with acetonitrile/water/TFA, the samples are separated by reversed-phase high performance liquid chromatography using a Zorbax C18 column with isocratic elution using a mobile phase consisting of acetonitrile/water/acetic acid. Detection by MS/MS incorporates an electrospray interface in positive ion mode.

Mass Spectrometric analysis is performed on either a Sciex API4000 or Micromass Quattro Ultima with MRM detection. The transitions monitored are 854 > 286 for paclitaxel and 860 > 292 for $^{13}C_6$ Paclitaxel.

Results

The overall accuracy (% deviation from nominal) and precision (%CV) of the method was acceptable with ± 7.6% deviation (±10.3 at the LOQ) and < 9.0% CV (15.7 at the LOQ) in porcine artery, kidney, liver, lung, muscle, myocardium, and spleen tissues. Paclitaxel is stable in tissue homogenate stored on the bench at least 22 hours, after 4 – 6 freeze-thaw cycles, in extracted tissue homogenate stored on the bench for 4 – 5 days, or frozen for 62 – 661 days. The method was found to be robust to 10 – 1000 fold dilution, autosampler re-injection reproducibility, and six different lots of tissue.

Example Standard Curve



Validation Results and Statistics

Limit of Quantification (LOQ) Concentration: 0.3 ng/g
Low Level (Low) Concentration: 0.75 – 0.9 ng/g
Mid Level (Mid) Concentration: 10 ng/g
High Level (High) Concentration: 375 – 500 ng/g

Validation Sample Results

Matrix	Statistics	Validation Sample Level			
		LOQ	Low	Mid	High
Artery	Average %Bias	3.9	2.7	2.3	-0.9
	CV	11.8	6.6	6.4	2.8
Kidney	Average %Bias	-7.0	3.3	1.0	-2.2
	CV	6.6	2.4	3.0	3.5
Liver	Average %Bias	-1.0	4.2	3.4	1.4
	CV	6.1	5.3	2.0	2.5
Lung	Average %Bias	3.3	4.4	1.0	-2.6
	CV	13.0	7.4	3.8	2.9
Muscle	Average %Bias	10.3	4.1	4.0	2.1
	CV	15.7	4.1	4.1	3.2
Myocardim	Average %Bias	6.6	5.4	0.3	1.8
	CV	5.5	4.5	2.3	2.6
Spleen	Average %Bias	3.7	3.1	4	1.1
	CV	9.6	2.7	2.7	3.7

Specificity (6 Lots) Testing Results

Matrix	Statistics	Specificity (6 Lots)
		Mid
Artery	Average %Bias	1.7
	CV	1.3
Kidney	Average %Bias	-0.6
	CV	3
Liver	Average %Bias	0.0
	CV	2.3
Lung	Average %Bias	-0.7
	CV	7.9
Muscle	Average %Bias	6.0
	CV	5.1
Myocardim	Average %Bias	0.0
	CV	3.2
Spleen	Average %Bias	4.0
	CV	4.4

Stability Testing Results

Matrix	Statistics	Freeze-Thaw		Room Temperature	
		Low	High	Low	High
Artery	Average %Bias	-9.2	-10.0	-3.0	-12.6
	CV	11.3	8.1	9.3	3.8
Kidney	Average %Bias	-13.2	-1.6	-10.5	-0.2
	CV	3.3	2.4	3.6	2.7
Liver	Average %Bias	6.1	-4.9	-4.6	-5.1
	CV	2.9	2.3	4.9	3.1
Lung	Average %Bias	7.1	-10.2	3.6	-8
	CV	4.4	3	6.4	3
Muscle	Average %Bias	1.3	-0.8	3.0	-1.3
	CV	5.9	2.4	12.9	2.5
Myocardim	Average %Bias	4.1	-7.7	-0.7	-5.6
	CV	13.5	2.1	9.5	2.2
Spleen	Average %Bias	4.4	0.8	6.7	-0.5
	CV	5.2	1.8	10.0	2.2

Extraction Efficiency Results

Matrix	Concentration Level	Paclitaxel Extraction Efficiency	$^{13}C_6$ Paclitaxel Extraction Efficiency
Artery	Low	53.5	48.7
	Mid	50.6	48.6
	High	48.0	47.5
Kidney	Low	60.9	61.0
	Mid	62.4	65.5
	High	74.0	67.6
Liver	Low	31.7	31.9
	Mid	28.8	28.5
	High	25.8	25.4
Lung	Low	68.1	62.8
	Mid	75.2	71.0
	High	63.5	60.4
Muscle	Low	71.8	70.5
	Mid	68.6	69.8
	High	69.6	67.0
Myocardim	Low	88.0	87.1
	Mid	89.1	89.8
	High	92.9	91.4
Spleen	Low	74.4	73.0
	Mid	67.8	66.9
	High	69.3	66.9

Robustness Testing Results

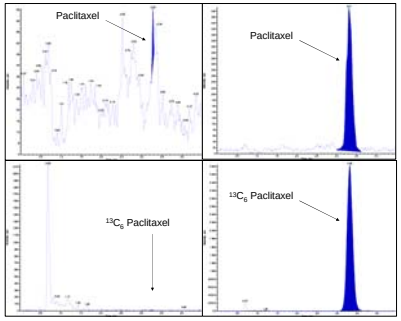
Matrix	Statistics	Re-injection Reproducibility			Processed Samples		Dilution
		Low	Mid	High	Low	High	Over-Range
Muscle	Average %Bias	5.3	6.0	0.8	4.4	-0.3	5.0
	CV	3.4	2.3	4.6	3.5	2.0	4.9
Myocardim	Average %Bias	5.9	2.9	4.2	-15.0	0.6	13.7
	CV	4.9	2.5	2.2	6.3	2.4	2.3
Spleen	Average %Bias	0.2	2	2.4	7.1	2.9	11.0
	CV	3.2	2.5	2.8	6.6	4.2	3.7

Matrix Factor Results

Matrix	Concentration Level	Paclitaxel Matrix Factor	$^{13}C_6$ Paclitaxel Matrix Factor
Muscle	Low	-23.8	-26.0
	Mid	-25.2	-25.7
	High	-23.2	-23.3
Spleen	Low	-28.6	-30.5
	Mid	-25.6	-24.4
	High	-24.0	-22.3

Example Chromatography

Double Blank **LLOQ**



Conclusions

A fast, simple, sensitive, specific, and robust method was validated with excellent linearity, precision, and accuracy for the quantification of paclitaxel in porcine artery, kidney, liver, lung, muscle, myocardium, and spleen tissues over the range of 0.3 – 500 ng/g. To accommodate higher concentration samples the method was also validated for a range of 0.5 – 500 µg/g in artery tissue. The method has been used successfully to assay samples from non-clinical trials.

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