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## Abstract

Tissue distribution studies provide essential information on distribution and accumulation of pharmaceutical compounds. Results can aid in the design and interpretation of future toxicology and pharmacology studies. Budgetary constraints often limit sponsors of animal studies to collecting only the tissues critical to their programs. However, sponsors could develop a more complete picture of compound distribution by quantifying additional tissue types. We demonstrate efficient methodologies of preparation and analysis that permit researchers to cost-effectively analyze more types of tissues.

The challenge was to analyze, using LC/MS/MS, nine different tissues from two species. Samples of femur bone marrow, heart, median lobe liver, whole eye, mesenteric lymph node, mandibular salivary gland, spleen, and thymus from rat and monkey were analyzed for CEM-101, a novel macrolide antibiotic. These analyses were to be performed in a timely, economical manner, without developing individual methods for each tissue.

## Introduction

Tissue samples have been traditionally homogenized by sonic probe or handheld blender. These techniques can be problematic due to limited throughput and risk of carryover. To solve these problems, our laboratory uses the FastPrep® system capable of simultaneously processing up to 48 tissue samples in 2mL sample tubes. The FastPrep® system uses individual sample tubes to eliminate carryover during preparation. We save additional time and resources by preparing standards in solvent and quality control samples in each respective matrix. The accuracy and precision of the solvent-prepared standards establish method performance across all tissue types, while quality control samples prepared in matrix indicate potential matrix effects.

Method development began by homogenizing commercially available tissue samples using the FastPrep® system. Optimization included adjusting the speed (m/s), run time (sec), number of cycles of the FastPrep®, and selecting the appropriate mass and type of materials used for homogenization. Crushed garnet and stainless steel ball bearings were added to each pre-weighted tissue sample with a known volume of solvent. In combination with the FastPrep® system's multidirectional motion, these materials create shearing forces to homogenize the tissue efficiently. Once method development was complete, several study samples of each type were homogenized, prepared for analysis using the Hamilton MicroLab Star liquid handling system, and analyzed by LC/MS/MS to establish the concentration range for all other samples in the study. After this initial sample screen, a dilution factor was determined for each tissue, and applied to all remaining study samples to minimize the number of samples requiring dilution and re-analysis. Using this dilution factor, we prepared quality control samples (QCs) from commercially available tissues at relevant concentrations for the planned quantitation range of CEM-101. The QCs were then frozen until the time of study sample analysis. The precision of the QCs provides an indication of acceptable method performance, while accuracy demonstrates stability and/or recovery of CEM-101 in frozen homogenate.

## Methods

### Sample Preparation

Analyte: CEM-101 Internal Standard: CEM-101-d<sub>3</sub>  
Standards were prepared in ACN to quantify all tissues from rat and monkey. Quality control samples were prepared in rat and monkey tissue homogenate, prepared using the FastPrep® system.

- \*Rat Tissue Analysis
    - Standard Concentrations: 25.0, 50.0, 100, 500, 1000, 5000, 10000, 25000 ng/mL
    - QC Concentrations: 250, 2500, 15000 ng/mL
  - \*Monkey Tissue Analysis
    - Standard Concentrations: 50.0, 100, 500, 1000, 5000, 10000, 50000, 100000 ng/mL
    - QC Concentrations: 400, 7500, 75000 ng/mL
- Using the Hamilton MicroLab Star, 25.0µL of standard solution or 25.0µL of tissue homogenate (study samples, QCs) was added to 250µL of internal standard solution (CEM-101-d<sub>3</sub> at 25.0 ng/mL in ACN) in a 96-well Whatman filtration plate, then capped, and mixed on a plate shaker.
- \*Using the Hamilton MicroLab Star, for rat tissue analysis 150µL of extract collected from vacuum filtration was added to 150µL of water, then capped, mixed on a plate shaker, and submitted for analysis. In monkey tissue analysis, 100µL of extract was added to 400µL of water.

### Sample Analysis

- \*Column: Varian MonoChrom CN, 50 x 2.0mm, 3µm
- \*Elution: Gradient flow, Mobile Phase A: 5mM Ammonium formate with 0.1% FA, Mobile Phase B: 50:50 MeOH:ACN with 0.1% FA
- \*Detector: Applied Biosystems/MDS Sciex API 4000 MS/MS

### Data Analysis

- \*Weighing: 1/(concentration)
- \*Chromatographic Peak Integrations: Applied Biosystems/MDS Sciex Analyst 1.4.1 or 1.4.2

## Laboratory Equipment

- FastPrep System
- Interchangeable tube adapters
- Materials aid in tissue destruction (garnet, BBs)



•Hamilton MicroLab Star Robotics

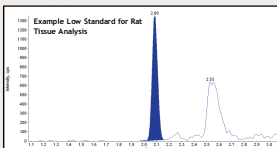
•12 independent pipette channels w/TADM

•96-well probe head

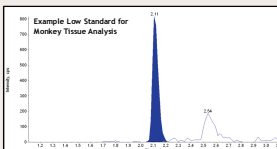
## Results

### Method Sensitivity

- Rat Tissue Analysis LLOQ 25.0 ng/mL



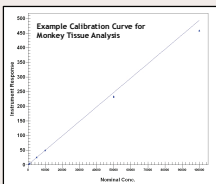
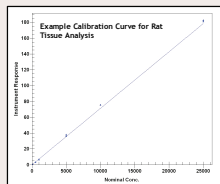
- Monkey Tissue Analysis LLOQ 50.0 ng/mL



- Standard working solutions were made in neat acetonitrile, and prepared in the same manner as tissue samples

### Linearity & Regression Statistics

	Number of Curves	Mean Slope	Mean Intercept	Mean R <sup>2</sup>	LLOQ (ng/mL)	ULOQ (ng/mL)
Rat Study	9	7.26E-03	6.40E-03	0.9989	25.0	25000
Monkey Study	20	4.28E-03	2.62E-02	0.9947	50.0	100000



## Calibration Standard Performance

Rat	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
	25.0 (ng/mL)	50.0 (ng/mL)	100 (ng/mL)	500 (ng/mL)	1000 (ng/mL)	5000 (ng/mL)	10000 (ng/mL)	25000 (ng/mL)
Intra-run Mean	25.6	48.7	97.2	477	973	5110	10400	26000
Intra-run SD	1.82	2.36	3.85	13.1	26.2	166	377	882
Intra-run %CV	7.1	4.9	4.0	2.8	2.7	3.2	3.6	3.3
Intra-run %Bias	-2.7	-2.8	-4.6	-2.7	2.2	2.2	4.0	4.0
n	18	18	18	18	18	18	18	18

Monkey	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
	50.0 (ng/mL)	100 (ng/mL)	500 (ng/mL)	1000 (ng/mL)	5000 (ng/mL)	10000 (ng/mL)	50000 (ng/mL)	100000 (ng/mL)
Intra-run Mean	50.2	98.3	508	1040	5130	10400	48300	92500
Intra-run SD	2.88	6.43	29.9	48.9	276	462	2340	4620
Intra-run %CV	5.8	6.5	5.9	4.7	5.4	4.4	4.8	5.0
Intra-run %Bias	0.4	-1.7	1.5	4.0	2.5	4.0	-3.4	-7.1
n	39	40	40	39	40	39	40	40

The accuracy of each replicate must be  $\pm 20\%$  of nominal concentration for standards to be acceptable. Standards were analyzed once before and once after unknown samples.

## Rat Tissue QC Performance

Rat	Tissue	Low QC	Mid QC	High QC
		250 (ng/mL)	1500 (ng/mL)	15000 (ng/mL)
Intra-run Mean	Bone	196	1940	13000
Intra-run SD	Bone	8.72	153	379
Intra-run %CV	Bone	4.4	7.9	2.9
Intra-run %Bias	Bone	-21.6	-22.4	-19.3
n	Bone	4	4	4
Intra-run Mean	Heart	238	2840	16600
Intra-run SD	Heart	8.66	173	574
Intra-run %CV	Heart	3.6	6.8	3.5
Intra-run %Bias	Heart	-4.8	1.6	10.7
n	Heart	4	4	4
Intra-run Mean	Liver	196	2070	13000
Intra-run SD	Liver	21.4	49.8	369
Intra-run %CV	Liver	10.9	2.4	2.8
Intra-run %Bias	Liver	-21.6	-17.2	-13.3
n	Liver	4	4	4
Intra-run Mean	Eye	187	2590	13400
Intra-run SD	Eye	12.4	180	942
Intra-run %CV	Eye	6.3	6.8	7.0
Intra-run %Bias	Eye	-21.2	-12.4	-10.7
n	Eye	4	4	4
Intra-run Mean	Lung	224	2460	14600
Intra-run SD	Lung	5.74	71.4	479
Intra-run %CV	Lung	2.6	2.9	3.3
Intra-run %Bias	Lung	-10.4	-2.0	-2.7
n	Lung	4	4	4
Intra-run Mean	Lymph	146	1380	9380
Intra-run SD	Lymph	6.78	27.1	289
Intra-run %CV	Lymph	4.6	2.0	3.1
Intra-run %Bias	Lymph	-41.6	-44.8	-37.5
n	Lymph	4	4	4
Intra-run Mean	Node	176	1760	11600
Intra-run SD	Node	8.66	98.5	492
Intra-run %CV	Node	4.9	3.3	3.9
Intra-run %Bias	Node	-29.4	-29.6	-23.3
n	Node	4	4	4
Intra-run Mean	Spleen	179	1760	12200
Intra-run SD	Spleen	10.8	142	642
Intra-run %CV	Spleen	6.0	8.1	4.4
Intra-run %Bias	Spleen	-29.4	-29.6	-19.7
n	Spleen	4	4	4
Intra-run Mean	Thymus	191	1820	12000
Intra-run SD	Thymus	7.72	34.0	465
Intra-run %CV	Thymus	4.0	1.9	3.8
Intra-run %Bias	Thymus	-23.6	-27.2	-18.0
n	Thymus	4	4	4

The percent coefficient of variation must be  $\leq 15\%$  for quality control samples to be acceptable. No accuracy requirement was used since the standards were in solution.

## Monkey Tissue QC Performance

Monkey	Tissue	Low QC	Mid QC	High QC
		250 (ng/mL)	2500 (ng/mL)	15000 (ng/mL)
Intra-run Mean	Bone	423	7880	70700
Intra-run SD	Bone	45.7	458	4201
Intra-run %CV	Bone	9.6	6.3	5.7
Intra-run %Bias	Bone	6.8	6.1	-5.7
n	Bone	4	4	4
Intra-run Mean	Heart	349	7470	71100
Intra-run SD	Heart	28.5	603	1780
Intra-run %CV	Heart	8.2	8.1	2.5
Intra-run %Bias	Heart	-12.8	-2.4	-5.2
n	Heart	4	4	4
Intra-run Mean	Liver	293	6190	64400
Intra-run SD	Liver	10.3	188	3490
Intra-run %CV	Liver	3.5	3.0	5.4
Intra-run %Bias	Liver	-26.8	-17.5	-14.1
n	Liver	4	4	4
Intra-run Mean	Eye	395	6770	63400
Intra-run SD	Eye	6.45	54	4330
Intra-run %CV	Eye	1.4	8.2	6.8
Intra-run %Bias	Eye	-3.8	-9.7	-16.5
n	Eye	4	4	4
Intra-run Mean	Lung	379	6760	74800
Intra-run SD	Lung	24.8	232	1500
Intra-run %CV	Lung	6.5	3.3	2.1
Intra-run %Bias	Lung	-5.3	-9.9	-0.1
n	Lung	4	4	4
Intra-run Mean	Lymph	291	6270	60600
Intra-run SD	Lymph	6.60	340	2290
Intra-run %CV	Lymph	2.3	5.4	3.8
Intra-run %Bias	Lymph	-27.3	-16.4	-19.2
n	Lymph	4	4	4
Intra-run Mean	Salivary Gland	387	6690	70900
Intra-run SD	Salivary Gland	21.2	358	2470
Intra-run %CV	Salivary Gland	5.9	5.4	3.5
Intra-run %Bias	Salivary Gland	-10.8	-10.8	-5.5
n	Salivary Gland	4	4	4
Intra-run Mean	Spleen	359	6270	65700
Intra-run SD	Spleen	26.2	177	1590
Intra-run %CV	Spleen	7.3	2.8	2.4
Intra-run %Bias	Spleen	-10.5	-16.4	-12.4
n	Spleen	4	4	4
Intra-run Mean	Thymus	321	6460	68000
Intra-run SD	Thymus	22.3	398	4750
Intra-run %CV	Thymus	6.7	6.2	7.0
Intra-run %Bias	Thymus	-17.3	-13.6	-9.3
n	Thymus	4	4	4

The percent coefficient of variation must be  $\leq 15\%$  for quality control samples to be acceptable. No accuracy requirement was used since the standards were in solution.

## Conclusions

- Tissue distribution studies are frequently limited by budget constraints. Here we demonstrate a cost-effective, high throughput approach that allows more types of tissues to be analyzed providing a more comprehensive picture of compound distribution.
- A single LC-MS/MS method was successfully used to measure CEM-101 in nine different rat and monkey tissues. Performance of the method was rugged and consistent across all tissue types, supported by the excellent precision (avg. < 5%) and accuracy (avg. < 5%) of all standards.
- Preparing standards in solution and quality control samples in each respective matrix realized additional efficiencies. While some QC samples indicate low bias, they provide tissue-specific information allowing a more accurate interpretation of the study data.
- Automating the method using the FastPrep® and Hamilton MicroLab Star reduced sample preparation time and eliminated carryover while improving precision, accuracy, and reliability.