Validated Multi-site GLP-compliant Analysis of Solithromycin (SOLI) in Monkey Plasma Samples Following Filtration to Remove Potentially Active Anthrax Spores

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ABSTRACT

A highly sensitive method was developed and validated to allow monkey plasma samples, potentially containing *Bacillus anthracis* (*B. anthracis*) spores, to be sterilized and shipped from the collection sites to the bioanalytical laboratory for analysis of Solithromycin (SOLI), a novel fluoroketolide antibiotic, and two of its metabolites. In the presence of plasma, the compounds were found to bind to the filtration devices typically used to remove *B. anthracis* spores and were unstable in the acetonitrile used for precipitating samples. A stabilizing solution was developed to both inhibit non-specific binding to the filter and preserve SOLI and its two metabolites during sample processing, storage and shipment. *B. anthracis* free samples can then be shipped to a bioanalytical laboratory for further processing. The entire process was validated between the sites to ensure accurate and precise bioanalytical data and safety from anthrax.

A collection of spore-free samples were first precipitated and diluted with a stabilizing solution containing deuterated analogs and semicarbazide hydrochloride as a preservative. They were subsequently filtered through a 0.2µm filter to mimic sample processing necessary for the removal of *B. anthracis* spores. These samples were then further diluted and processed using an Agilent SPEC C-18 SPE plate. The extracts were analyzed using a Waters HILIC column on a Waters Acquity/Xevo LC/MS/MS system.

Monkey plasma samples free of *B. anthracis* of known SOLI and metabolite concentrations were successfully diluted, filtered and shipped. Upon analysis we observed that the SOLI and metabolite concentrations detected in shipped samples corresponded to unfiltered samples at our site. Non-specific binding of SOLI and its metabolites to the cartridge was prevented and the samples were shown to be stable through the processing.

Validation of a method for analysis of SOLI and its metabolites across multiple sites, post filtration of *B. anthracis* spores. Harmonization between sites was necessary to ensure appropriate steps were taken to uniformly handle sample processing, as a portion of the sample extraction was performed at different sites. Successful integration of partially extracted samples from one site into a freshly prepared calibration curve at a second site.

MicroConstants
REGULATED BIOANALYSIS & DMPK

INTRODUCTION

A highly sensitive method was developed and validated to allow monkey plasma samples, potentially containing *Bacillus anthracis* (*B. anthracis*) spores, to be filtered and shipped from the testing site to the bioanalytical laboratory, MicroConstants, for analysis of Solithromycin (SOLI), a novel fluoroketolide antibiotic, and two of its metabolites. In the presence of plasma, the compounds were found to bind to the filtration devices typically used to remove *B. anthracis* spores. This was resolved by using acetonitrile to precipitate the samples prior to filtration. During development, SOLI was found to be unstable in acetonitrile when manipulated or stored in various container systems. A stabilizing solution was developed, allowing acetonitrile to be present while preventing non-specific binding to the filter and preserving SOLI and its two metabolites during sample processing, storage and shipment. *B. anthracis* free samples were then shipped to MicroConstants for further processing and quantitation. The entire process was validated between the sites to ensure safety from anthrax, as well as accurate and precise bioanalytical data.

METHOD

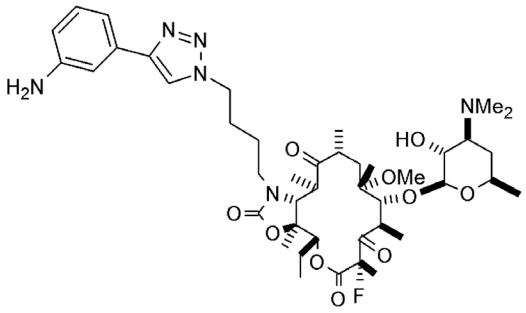
Plasma samples were first precipitated and diluted with a stabilizing solution containing 0.5M ammonium citrate:10% trifluoroacetic acid:5% semicarbazide HCl:isopropanol:water (12:4:9:60:15, v/v/v/v/v) and an acetonitrile solution containing deuterated analogs for two of the compounds. They were subsequently filtered through a 0.2µm filter to mimic sample processing necessary for the removal of B. anthracis spores. These samples were then stored at -70ºC and shipped on dry ice.

The samples were further diluted at MicroConstants with a solution containing NaCl:[0.5M ammonium citrate:10% trifluoroacetic acid:5% semicarbazide HCl:isopropanol:water (12:4:9:60:15, v/v/v/v/v)]:Water (1.8:20:180, w/v/v) and processed using an Agilent SPEC C-18 (15 mg) SPE plate. The samples were eluted with a solution containing [10% ammonium formate:water:1.25% citric acid:formic acid (2:2.4:1.6:4, v/v/v/v)]:methanol:acetonitrile (2:160:238, v/v/v).

Instrumentation:	Waters Acquity I-Class, FTN Autosampler coupled with a Waters XEVO TQ-S quadrupole mass spectrometer	
Column:	Waters HILIC, 150x2.1mm, 5µm	
Mobile Phase:	A: 20mM Ammonium Formate, 0.2% Formic Acid, 0.0002% Citric Acid in Water	
	B: 0.1% Formic Acid and 2.7 ppm Citric Acid in Methanol:Acetonitrile (50:50, v/v)	
Elution Mode:	Isocratic (A:B, 8:92)	
Flow Rate:	0.300 mL/min	
Mass Transitions:	Solithromycin:	423.38 > 158.00
	Solithromycin-d_3:	424.88 > 161.00
	Metabolite 1:	444.40 > 158.00
	Metabolite 1-d_3:	447.40 > 161.00
	Metabolite 2:	703.65 > 158.00
Quantitation: Analyte/I.S.	Solithromycin/Solithromycin-d_3	
	Metabolite 1/Metabolite 1-d_3	
	Metabolite 2/Metabolite 1-d_3	
Curve Range:	Solithromycin:	10.0 to 25,000 ng/mL
	Metabolite 1:	1.00 to 2,500 ng/mL
	Metabolite 2:	1.00 to 2,500 ng/mL

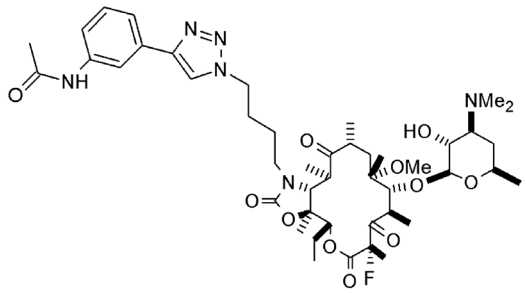
STRUCTURES

Solithromycin



$C_{43}H_{65}FN_6O_{10}$
Mol. Wt.: 845.01

Metabolite 1



Metabolite 2

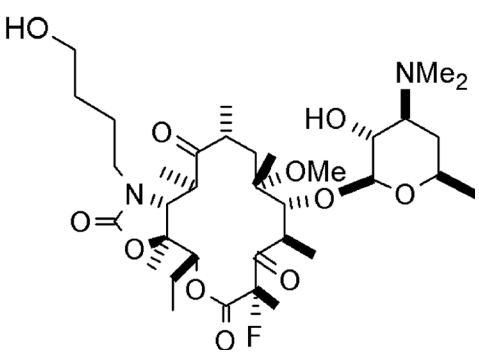
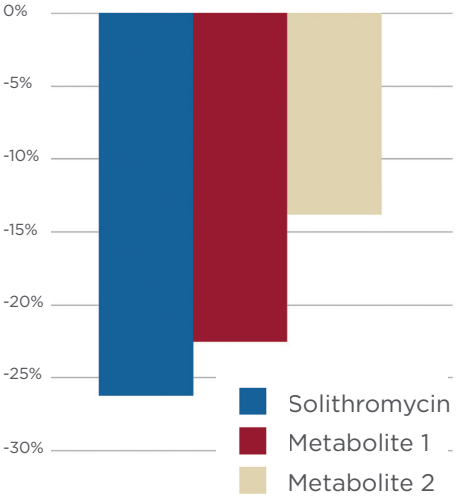
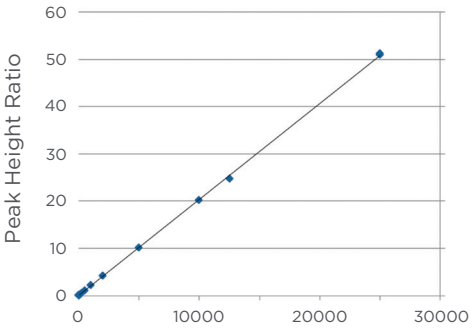


FIGURE 1
Observed Percent Loss Without Preservation and Rescue Reagent

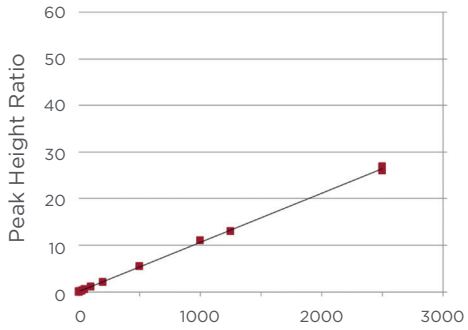


CALIBRATION CURVES

Solithromycin (10 - 25,000 ng/mL)



Metabolite 1 (1 - 2,500 ng/mL)



Metabolite 2 (1 - 2,500 ng/mL)

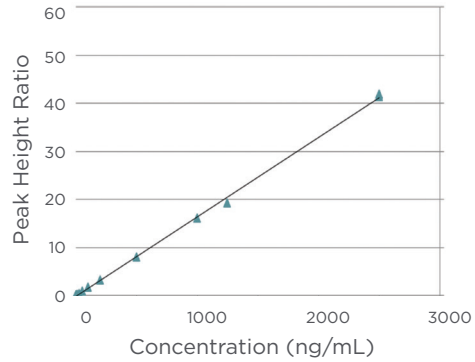


FIGURE 2
Representative Chromatograms at the LLOQ Level

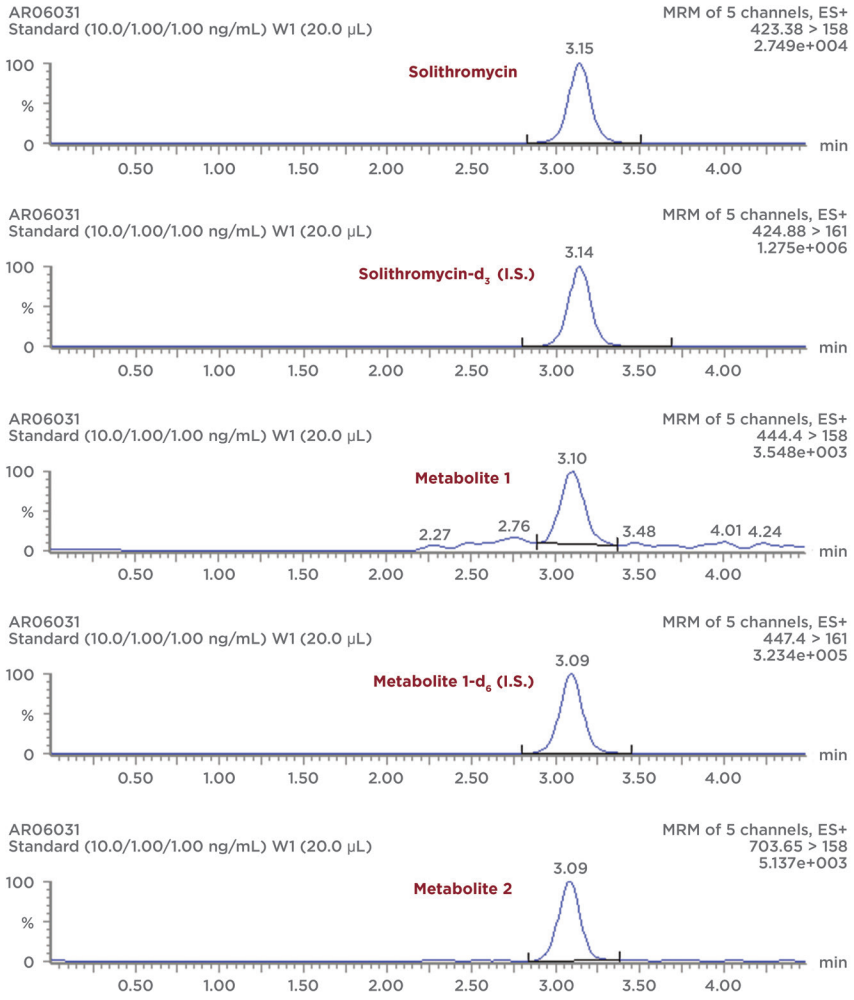


TABLE 1
Peak Heights of Solithromycin in QCs

Theoretical concentration (ng/mL)	Replicate	Peak Heights	
		Non-filtered	Processed off-site
30.0	1	85,552	92,613
	2	85,787	83,976
	3	89,590	91,819
	4	85,936	83,597
	5	91,919	82,107
	6	89,074	88,388
	Mean	88,000	87,100
	%CV	2.97	5.17
	%Diff	1.03	
20,000	1	48,995,960	51,749,864
	2	49,242,116	50,812,580
	3	49,327,336	52,261,380
	4	47,769,044	50,951,676
	5	49,162,152	49,314,444
	6	48,215,800	48,898,652
	Mean	48,800,000	50,700,000
	%CV	1.31	2.61
	%Diff	-3.82	

TABLE 2
Results of QCs Filtered/Processed Off-site

Analyte	Replicate	Concentration (ng/mL)			
		10.0	30.0	300	20,000
Solithromycin	1	10.6	31.6	328	22,100
	2	10.7	31.4	334	22,100
	3	9.49	33.3	317	22,900
	4	10.9	32.1	335	22,100
	5	10.5	31.6	323	20,900
	6	10.3	32.7	334	22,100
	Mean	10.4	32.1	329	22,000
	%CV	4.76	2.33	2.22	2.91
	%DEV	4.00	7.00	9.67	10.0

Analyte	Replicate	Concentration (ng/mL)			
		1.00	3.00	30.0	2,000
Metabolite 1	1	0.863	3.07	32.9	2,070
	2	1.02	3.16	33.0	2,190
	3	0.902	3.03	30.8	2,200
	4	1.12	3.02	32.6	2,200
	5	1.00	3.02	31.8	2,000
	6	1.03	3.58	32.4	2,160
	Mean	0.989	3.15	32.3	2,140
	%CV	9.42	6.96	2.57	3.89
	%DEV	-1.10	5.00	7.67	7.00

Analyte	Replicate	Concentration (ng/mL)			
		1.00	3.00	30.0	2,000
Metabolite 2	1	1.11	3.34	32.0	2,200
	2	1.12	3.11	32.7	2,300
	3	1.02	3.17	31.5	2,340
	4	1.18	3.08	33.9	2,280
	5	1.12	3.30	33.3	2,240
	6	0.928	3.54	33.4	2,370
	Mean	1.08	3.26	32.8	2,290
	%CV	8.37	5.31	2.78	2.74
	%DEV	8.00	8.67	9.33	14.5

RESULTS

Monkey plasma samples containing known SOLI and metabolite concentrations were successfully preserved, precipitated with acetonitrile, filtered to remove *B. anthracis* and shipped from the testing site. Upon analysis at MicroConstants, we observed that the SOLI and metabolite concentrations detected in shipped samples corresponded to unfiltered samples. Non-specific binding of SOLI and its metabolites to the filtration cartridge was prevented and all compounds were shown to be stable through the entire sample handling procedure. The validated process allowed safe and accurate quantitation of SOLI and metabolites in plasma from monkeys exposed to *B. anthracis* in subsequent studies.

NOVEL ASPECTS/ CONCLUSIONS

- Validation of a method for analysis of SOLI and its metabolites across multiple sites, post filtration to remove *B. anthracis*.
- Harmonization between sites was necessary to ensure appropriate steps were taken to uniformly handle sample processing, as a portion of the sample extraction was performed at different sites.
- Successful integration of partially extracted samples from one site into a freshly prepared calibration curve at a second site.
- Monkey plasma samples from potentially bacteremic animals may successfully be filtered to remove *B. anthracis* and analyzed using a validated LC/MS/MS method to provide accurate and precise concentrations for SOLI and its metabolites.

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