Appendix A6.1.3.39 Validation Summary VAA98876-02 (MHBMA in Urine)

Confidentiality Statement

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TABLE OF TABLES

Table 1. Validation Summary – MHBMA in Urine - VAA98876-02 3

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The following validation summary has been prepared by (b) (4) Full method validation report and related SOPs are all available at (b) (4)

(b) (4)

Table 1. Validation Summary – MHBMA in Urine - VAA98876-02

Information Requested	Data
Validation Summary	(b) (4) Validation Study VAA98876-02
Analyte	Monohydroxy-3-butenyl-mercapturic acid (MHBMA)
Internal Standard (IS)	MHBMA-^{15}N-$^{13}C_3$
Method Description	Solid phase extraction with analysis/detection by LC-MS/MS using two analytical columns or using single analytical column
Standard Curve Concentration Range (ng/mL)	0.100 – 20.0 ng/mL
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours at room temperature at 500 µg/mL and 1.00 ng/mL
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 60 days at -20°C at 500 µg/mL and 10.0 µg/mL; 27 days at -20°C at 50.0 ng/mL
Processed Stability (Hrs)	Processed Sample Integrity: 122 hours at 5°C
Freeze-Thaw Stability (Cycles)	4 freeze (-20°C)-thaw (ambient temperature) cycles; 3 freeze (-80°C)-thaw (ambient temperature) cycles
Long-Term Storage Stability (Days)	Long-Term Stability: 8 days at -80°C (UV-shielded polypropylene tubes) in human urine; 95 days at -20°C (UV shielded polypropylene tubes) in Urisub®
Dilution Integrity	Up to 100 ng/mL (dilution 1:10 with Urisub®)
Selectivity	No interference in 6 matrix lots for MHBMA-^{15}N-$^{13}C_3$ (IS)

Additional Information – MHBMA in Urine - VAA98876-02		
Matrix	Human Urine	
SOP Number	SOP SM1-383A	
Detector	AB/MDS Sciex API QTRAP® 5500	
Assay Volume Required	0.250 mL	
Regression Type	Linear (1/concentration^2)	
Quantitation Method	Peak area ratio	
Quality Control Samples	Precision (% CV)	Accuracy (% Bias)

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Additional Information – MHBMA in Urine - VAA98876-02			
Inter-Batch (Batches 6 - 8)	**LLOQ**	2.1	98.0
	Low	2.1	97.9
	Medium	13.8	110.6
	High	2.4	100.4
Inter-Batch (Batches 29 - 31)	**LLOQ**	7.9	110.4
	Low	2.3	97.5
	Medium	1.6	93.7
	High	1.0	98.1
Intra-Batch (Batch 8)	**LLOQ**	2.4	98.2
	Low	1.6	96.5
	Medium	1.6	94.4
	High	1.2	100.1
Intra-Batch (Batch 29)	**LLOQ**	10.8	117.6
	Low	0.9	100.3
	Medium	1.4	94.5
	High	0.7	98.0
Matrix Effect		All 6 investigated lots were within acceptance	
Short-Term Stability for Stock Solutions (Stock)		60 days at -20°C at 500 µg/mL and 10.0 µg/mL	
Batch Size		96 injections	

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