Appendix A6.1.3.41 Validation Summary VAA98876-01 (SBMA and S-PMA in Urine)

Confidentiality Statement

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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 – SBMA in Urine - VAA98876-01

Information Requested	Data
Validation Summary	(b) (4) Validation Study VAA98876-01
Analyte	S-Benzyl Mercapturic Acid (SBMA)
Method Description	Solid phase extraction with analysis/detection by LC-MS/MS
Standard Curve Concentration Range (ng/mL)	0.100 – 25.0 ng/mL

Additional Information – SBMA in Urine - VAA98876-01			
Matrix		Human Urine	
SOP Numbers		SOP SM1-382A and SOP SM1-382B	
Detector		Applied Biosystems / MDS Sciex API 4000™	
Assay Volume Required		0.500 mL	
Regression Type		Linear (1/concentration^2)	
Quantitation Method		Peak area ratio	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-Batch (Batches 9 – 11 and 16)	**100 pg/mL**	6.0	1.0
	175 pg/mL	2.2	6.3[1]
	699 pg/mL	8.2	0.0[2]
	1500 pg/mL	8.0	-5.3
	5200 pg/mL	8.3	-6.5
	18,200 pg/mL	8.6	-9.9
Intra-Batch (Batch 11)	**100 pg/mL**	2.6	0.0
	175 pg/mL	2.7	5.7
	699 pg/mL	2.1	4.7
	1500 pg/mL	1.4	0.0
	5200 pg/mL	0.8	-2.7
	18,200 pg/mL	1.1	-4.9
Intra-Batch (Batch 16)	**276 pg/mL**	1.2	6.5
	1120 pg/mL	1.2	-12.3
	1920 pg/mL	2.1	-12.5
	5760 pg/mL	1.5	-12.2
	18,400 pg/mL	1.2	-14.1

[1] Inter-run P&A calculated from Batches 9 - 11 only, as a different QC A lot (with a different dilution factor of human urine diluted with UriSub®) was used in Batch 16.

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[2] The basal level (nominal value of QC BL) was determined as mean concentration determined for the QC BL samples analyzed in the P&A batches (Batches 9 – 11 and 16).

Table 2. Validation Summary – S-PMA in Urine - VAA98876-01

Information Requested	Data
Validation Summary	(b) (4) Validation Study VAA98876-01
Analyte	S-Phenyl Mercapturic Acid (SPMA)
Method Description	Solid phase extraction with analysis/detection by LC-MS/MS
Standard Curve Concentration Range (ng/mL)	0.0250 – 6.25 ng/mL

Additional Information – S-PMA in Urine - VAA98876-01			
Matrix		Human Urine	
SOP Numbers		SOP SM1-382A and SOP SM1-382B	
Detector		Applied Biosystems / MDS Sciex API 4000™	
Assay Volume Required		0.500 mL	
Regression Type		Linear (1/concentration[2])	
Quantitation Method		Peak area ratio	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-Batch (Batches 9 – 11 and 16)	**25.0 pg/mL**	12.8	10.0
	74.9 pg/mL	12.9	0.0[1]
	475 pg/mL	5.8	8.2
	1200 pg/mL	4.1	7.5
	4450 pg/mL	2.2	6.3
Intra-Batch (Batch 11)	**25.0 pg/mL**	4.4	18.0
	74.9 pg/mL	6.6	7.7
	475 pg/mL	1.4	13.9
	1200 pg/mL	1.4	9.2
	4450 pg/mL	2.6	7.2
Intra-Batch (Batch 16)	**71.9 pg/mL**	6.5	8.2
	427 pg/mL	2.3	9.8
	1160 pg/mL	1.7	5.2
	4340 pg/mL	0.8	2.8

[1] Inter-run P&A calculated from Batches 9 - 11 only, as a different QC A lot (with a different dilution factor of human urine diluted with UriSub®) was used in Batch 16.

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