Appendix A6.1.3.40 Validation Summary VAA98876-03 (Total 1-Hydroxypyrene in Urine)

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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 – Total 1-Hydroxypyrene (1-OHP) - VAA98876-03

Information Requested	Data
Validation Summary	(b) (4) Validation Study VAA98876-03
Analyte	Total 1-Hydroxypyrene (1-OHP)
Internal Standard (IS)	1-OHP-$^{13}C_6$
Method Description	Solid phase extraction with analysis/detection by LC-MS/MS
Standard Curve Concentration Range (pg/mL)	10.0 - 2000 pg/mL
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 216 days at -20°C at 1.00 mg/mL
Processed Stability (Hrs)	Processed Sample Integrity: 116 hours at 5°C
Long-Term Storage Stability (Days)	Long-Term Stability: 19 days at -80°C at QC low and QC high level in human urine; 36 days at -20°C at 50.0 pg/mL and 1750 pg/mL in Urisub®
Dilution Integrity	Up to 10000 pg/mL (dilution 1:10 with Urisub®)
Selectivity	No interference in 7 matrix lots for 1-OHP-$^{13}C_6$ (IS)

Additional Information – Total 1-Hydroxypyrene (1-OHP) - VAA98876-03			
Matrix		Human Urine	
SOP Numbers		SOP SM1-384A, SOP SM1-384B and SOP SM1-384C	
Detector		Applied Biosystems/MDS SCIEX TRIPLE QUAD 6500	
Assay Volume Required		0.5 mL	
Regression Type		Linear (1/concentration^2)	
Quantitation Method		Peak area ratio	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-Batch (Batches 12 – 16)	**LLOQ**	11.0	113.4
	Low	9.8	106.8
	Medium	4.3	101.5
	High	4.1	99.2

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Additional Information – Total 1-Hydroxypyrene (1-OHP) - VAA98876-03			
Intra-Batch (Batch 14)	**LLOQ**	8.7	123.8
	Low	4.0	110.3
	Medium	3.0	107.0
	High	1.5	103.8
Intra-Batch (Batch 16)	**LLOQ**	6.2	95.6
	Low	5.9	99.3
	Medium	2.9	98.4
	High	3.3	97.1
Matrix Effect		All 7 investigated lots within acceptance	
Short-Term Stability for Stock Solutions (Stock)		17 hours at ambient temperature at 1.00 mg/mL and 100 ng/mL	
Batch Size		96 injections	

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