Appendix A6.1.3.27 Validation Summary AA33664-01 (Nicotine, Cotinine and trans-3′-Hydroxycotinine in Plasma)

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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 – Nicotine in Plasma- AA33664-01

Information Requested		Data	
Analyte		Nicotine	
Matrix		Human Plasma	
Anticoagulant		K_2EDTA	
Bioanalytical Method (BAM) Number		BAM AA33664-01	
Assay Method		LC-MS/MS	
Detector		AB SCIEX API 5000™ and QTRAP® 5500	
Assay Volume Required		0.300 mL	
Standard Curve Range		0.200 – 10.0 ng/mL	
Regression Type		Linear (1/concentration^2)	
Quantitation Method		Peak Area Ratio	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-batch	LLOQ	12.1	-6.5
	Low	9.3	-0.5
	Medium	3.1	-4.7
	High	2.6	-3.3
Intra-batch (Batch 31)	LLOQ	14.0	-13.0
Aliquot Method: Manual	Low	12.9	5.0
Extraction Method: Automated	Medium	3.4	-4.0
	High	2.0	-3.7
Intra-batch (Batch 32)	LLOQ	9.6	-8.0
Aliquot Method: Manual	Low	5.4	-4.2
Extraction Method: Automated	Medium	2.1	-3.0
	High	2.4	-1.1
Intra-batch (Batch 34)	LLOQ	7.8	2.0
Aliquot Method: Manual	Low	5.2	-2.5
Extraction Method: Automated	Medium	1.7	-7.3
	High	1.6	-5.2

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Information Requested		Data
Recovery		Recovery (%)
Analyte	Low	97
	High	94
Sample Shipping Stability		19 days in polypropylene tubes at -80°C
Long-Term Storage Stability (Days)		Long-Term Stability: 1041 days in polypropylene tubes at -20°C
Short-term Stability		27 hours in polypropylene tubes at ambient temperature under white light
Freeze and Thaw Stability		6 cycles in polypropylene tubes at -20°C under white light 7 cycles in polypropylene tubes at -20°C under UV-shielded light
Post-preparative Stability		135 hours in a polypropylene 96 well plate at 5°C
Stock Solution Stability		574 days at 100 µg/mL in water in a polypropylene container at -20°C
Substock Solution Stability		299 days at 10.0 ng/mL in water in a polypropylene container at -20°C 323 days at 1.00 ng/mL in water in a polypropylene container at -20°C
Internal Standard Stability		refer to stock and substock solution stability data collected from unlabeled nicotine for labeled internal standard stock and substock stability
Hemolyzed Sample Integrity		No significant interference for nicotine was observed in any of the 3 hemolyzed human plasma (EDTA) lots (fortified with 5% whole blood) that were fortified at the concentration of the LLOQ (0.200 ng/mL) and in any of the 3 lots (fortified with 5% whole blood) that were fortified at the concentration of the high QC (7.50 ng/mL) sample No significant interference for nicotine was observed in any of the 3 hemolyzed human plasma (EDTA) lots (fortified with 2% whole blood) that were fortified at the concentration of the LLOQ (0.200 ng/mL) or in any of the 3 hemolyzed human plasma (EDTA) lots (fortified with 2% whole blood) that were fortified at the concentration of the high QC (7.50 ng/mL) samples
Turbid Sample Integrity		No significant interference for nicotine was observed in any of the 3 turbid human plasma (EDTA) lots that were fortified near the low QC concentration and in any of the 3 lots that were fortified near the high QC concentration
Sample Aliquot Frozen Storage Stability		samples aliquotted manually at a volume of 0.300 mL, stored for 307 hours in a polypropylene 96 well plate at -20°C prior to extraction

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Information Requested	Data
Stability of Analyte During Sample Collection and Handling	Up to 120 minutes in human whole blood (EDTA) in polypropylene tubes at ambient temperature under UV-shielded light Up to 180 minutes in human whole blood (EDTA) in polypropylene tubes at ambient temperature under white light Up to 48 hours in human whole blood (EDTA) in polypropylene tubes in an ice water bath under white light
Dilution Integrity	up to 60.0 ng/mL
Processed Sample Integrity	138 hours in a polypropylene 96 well plate at 5°C
Batch Size	192 injections

Table 2. Validation Summary – Cotinine in Plasma- AA33664-01

Information Requested		Data	
Analyte		Cotinine	
Matrix		Human Plasma	
Anticoagulant		K_2EDTA	
Bioanalytical Method (BAM) Number		BAM AA33664-01	
Assay Method		LC-MS/MS	
Detector		AB SCIEX API 5000™ and QTRAP® 5500	
Assay Volume Required		0.300 mL	
Standard Curve Range		1.00 – 100 ng/mL	
Regression Type		Linear (1/concentration^2)	
Quantitation Method		Peak Area Ratio	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-batch	LLOQ	4.2	-4.3
	Low	2.3	-0.3
	Medium	2.1	1.3
	High	2.4	2.1
Intra-batch (Batch 31)	LLOQ	1.6	-7.2
Aliquot Method: Manual	Low	2.2	-1.7
Extraction Method: Automated	Medium	1.5	-0.7
	High	2.4	0.3
Intra-batch (Batch 32)	LLOQ	2.2	-5.3
Aliquot Method: Manual	Low	1.1	-0.7
Extraction Method: Automated	Medium	1.8	2.7
	High	2.0	3.3
Intra-batch (Batch 34)	LLOQ	4.3	-0.5
Aliquot Method: Manual	Low	2.1	1.7
Extraction Method: Automated	Medium	1.7	2.0
	High	1.9	2.8
Recovery		Recovery (%)	
Analyte	Low	91	
	Medium	90	
	High	90	

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Information Requested	Data
Sample Shipping Stability	10 days in polypropylene tubes at -80°C
Long-term Stability	739 days in polypropylene tubes at -20°C
Short-term Stability	27 hours in polypropylene tubes at ambient temperature under white light
Freeze and Thaw Stability	6 cycles in polypropylene tubes at -20°C under white light 7 cycles in polypropylene tubes at -20°C under UV-shielded light
Post-preparative Stability	135 hours in a polypropylene 96 well plate at 5°C
Stock Solution Stability	39 days at 200 µg/mL in water in a polypropylene container at -20°C

Table 3. Validation Summary – trans-3′-Hydroxycotinine - AA33664-01

Information Requested		Data	
Analyte		*trans*-3′-Hydroxycotinine	
Matrix		Human Plasma	
Anticoagulant		K_2EDTA	
Bioanalytical Method (BAM) Number		BAM AA33664-01	
Assay Method		LC-MS/MS	
Detector		AB SCIEX API 5000™ and QTRAP® 5500	
Assay Volume Required		0.300 mL	
Standard Curve Range		1.00 – 100 ng/mL	
Regression Type		Linear (1/concentration^2)	
Quantitation Method		Peak Area Ratio	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-batch	LLOQ	3.7	-4.2
	Low	3.2	1.3
	Medium	2.3	-1.3
	High	3.0	-0.1
Intra-batch (Batch 31)	LLOQ	4.7	-2.5
Aliquot Method: Manual	Low	3.5	0.7
Extraction Method: Automated	Medium	1.7	-3.0
	High	2.6	-1.6
Intra-batch (Batch 32)	LLOQ	3.1	-6.6
Aliquot Method: Manual	Low	2.2	0.0
Extraction Method: Automated	Medium	1.7	-0.7
	High	2.8	0.1
Intra-batch (Batch 34)	LLOQ	1.7	-3.4
Aliquot Method: Manual	Low	2.8	4.0
Extraction Method: Automated	Medium	2.7	-0.7
	High	3.1	1.2
Recovery		Recovery (%)	
Analyte	Low	75	
	Medium	77	
	High	76	

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Information Requested	Data
Sample Shipping Stability	10 days in polypropylene tubes at -80°C
Long-term Stability	739 days in polypropylene tubes at -20°C
Short-term Stability	27 hours in polypropylene tubes at ambient temperature under white light
Freeze and Thaw Stability	6 cycles in polypropylene tubes at -20°C under white light 7 cycles in polypropylene tubes at -20°C under UV-shielded light
Post-preparative Stability	135 hours in a polypropylene 96 well plate at 5°C
Stock Solution Stability	572 days at approximately 1000 µg/mL in water in a polypropylene container at -20°C
Long-Term Substock Solution Stability	299 days at 10.0 ng/mL in water in a polypropylene container at -20°C 323 days at 5.00 ng/mL in water in a polypropylene container at -20°C

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