Appendix A6.1.3.38 Validation Summary ZZ37540-01 (4-Aminobiphenyl in Urine, o-Toluidine in Urine, 2-Aminonaphthalene in Urine, and 1-Aminonaphthalene in Urine)

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TABLE OF TABLES

Table 1. Validation Summary – 4-Aminobiphenyl in Urine - ZZ37540-01 3
Table 2. Validation Summary – o-Toluidine in Urine - ZZ37540-01..... 6
Table 3. Validation Summary – 2-Aminonaphthalene in Urine - ZZ37540-01..... 10
Table 4. Validation Summary – 1-Aminonaphthalene in Urine - ZZ37540-01..... 14

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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 – 4-Aminobiphenyl in Urine - ZZ37540-01

Information Requested	Data
Validation Summary	(b) (4) Validation Study ZZ37540-01
Analyte	4-Aminobiphenyl
Internal Standard (IS)	[$^{13}C_6$]4-Aminobiphenyl
Method Description	Liquid-liquid extraction with analysis/detection by LC-MS/MS
Limit of Quantitation (pg/mL)	1.50 pg/mL
Average Recovery of Drug (% Mean)	From Non-Smoker Urine: 84% at 37.5 pg/mL 82% at 375 pg/mL From Smoker Urine: 87% at 37.5 pg/mL 92% at 375 pg/mL From 0.1% Bovine Serum Albumin: 80% at 1.50 pg/mL 84% at 3.75 pg/mL 84% at 30.0 pg/mL
Average Recovery of IS (% Mean)	From 0.1% Bovine Serum Albumin: 85%
Standard Curve Concentrations (pg/mL)	1.50, 2.25, 3.75, 7.50, 15.0, 30.0, 63.0, and 75.0 pg/mL
QC Concentrations (pg/mL)	LLOQ QC, 3.35, 5.60, 15.4, and 59.4 pg/mL
QC Intra-Batch Precision Range (% CV)	0.8 to 20.4%
QC Intra-Batch Accuracy Range (% Bias)	-11.3 to 11.3%
QC Inter-Batch Precision Range (% CV)	2.4 to 11.5%
QC Inter-Batch Accuracy Range (% Bias)	-2.7 to 0.3%
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours in brown polypropylene tubes at ambient temperature under UV-shielded light Cumulative Short-Term Stability: 54 hours in brown polypropylene tubes at ambient temperature under UV-shielded light (total of all thaw cycles)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 141 days at approximately 400 µg/mL in methanol in a polypropylene container at -20°C
Processed Stability (Hrs)	Post-Preparative Stability: 337 hours in a polypropylene 96 well plate at 5°C Processed Sample Integrity: 306 hours in a polypropylene

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	96 well plate at 5°C
Freeze-Thaw Stability (Cycles)	6 freeze (-20°C)-thaw (ambient temperature) cycles in brown polypropylene tubes under UV-shielded light
Long-Term Storage Stability (Days)	Long-Term Stability: 185 days in brown polypropylene tubes at -20°C
Dilution Integrity	Up to 130 pg/mL, diluted 2-fold
Selectivity	No significant interference at the retention time and mass transition of [$^{13}C_6$]4-aminobiphenyl (IS) was observed from endogenous components in any of the 7 human urine lots screened

Additional Information – 4-Aminobiphenyl in Urine - ZZ37540-01

Matrix		Human Urine	
Bioanalytical Method (BAM) SOP Number		BAM SOP ZZ37540-01	
Detector		AB SCIEX API 4000™	
Assay Volume Required		2.00 mL	
Regression Type		Weighted linear (1/concentration)	
Quantitation Method		Peak area ratio	
Multiple Analytes Measured by Assay		4-Aminobiphenyl at 3.35 pg/mL fortified with *o*-toluidine at 1000 pg/mL, 2-aminonaphthalene at 500 pg/mL, and 1-aminonaphthalene at 500 pg/mL	
Selectivity from Degradants or Isomers		3-Aminobiphenyl (75.0 pg/mL) 2-Aminobiphenyl (75.0 pg/mL) *m*-Toludine (1000 pg/mL) *p*-Toludine (1000 pg/mL)	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-Batch	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	11.5 5.6 4.9 2.6 2.4	0.0 -2.7 -2.5 0.0 0.3
Intra-Batch (Batch 170) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	6.4 5.2 7.8 2.5 0.9	2.7 0.3 -3.0 -0.6 -2.2
Intra-Batch (Batch 171) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	3.7 1.8 3.0 1.8 1.8	-4.0 -3.0 -2.0 -0.6 1.2
Intra-Batch (Batch 172) **Aliquot Method: Manual** **Extraction Method:**	**LLOQ** **Low** **Low-Medium**	8.9 5.4 2.6	3.3 2.7 2.5

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Additional Information – 4-Aminobiphenyl in Urine - ZZ37540-01			
Manual	**High-Medium High**	1.8 0.8	1.3 -0.7
Intra-Batch (Batch 173) Aliquot Method: Manual Extraction Method: Manual	**LLOQ Low Low-Medium High-Medium High**	5.2 4.9 4.4 1.9 2.6	-11.3 -6.0 -6.6 0.6 3.4
Intra-Batch (Batch 178)[1] Aliquot Method: Manual Extraction Method: Manual	**LLOQ Low Low-Medium High-Medium High**	20.4 3.9 2.3 1.8 1.6	11.3 -8.7 -4.8 0.6 0.8
Intra-Batch (Batch 177) Aliquot Method: Manual Extraction Method: Manual	**LLOQ Low Low-Medium High-Medium High**	2.9 4.1 3.0 3.8 2.3	0.7 -2.1 -2.1 -3.2 1.5
Intra-Batch (Batch 182) Aliquot Method: Manual Extraction Method: Manual	**LLOQ Low Low-Medium High-Medium High**	5.2 5.0 4.8 2.6 1.4	-2.7 -2.4 -1.3 0.0 -1.5
Matrix Effect		No significant matrix effect was observed in any of the 6 human urine lots that were fortified with 4-aminobiphenyl near the concentration of the LLOQ (1.50 pg/mL), in any of the 6 human urine lots that were fortified with 4-aminobiphenyl near the concentration of the low-medium QC (3.75 pg/mL), or in any of the 6 human urine lots that were fortified with 4-aminobiphenyl near the concentration of the high QC (30.0 pg/mL) samples	
Sample Shipping Stability		7 days in brown polypropylene tubes at -80°C	
Long-Term Stability for Stock Solutions (Substock)		66 days at 10.0 µg/mL in 30:70 methanol:water in a polypropylene container at -20°C 121 days at 0.0600 ng/mL in 30:70 methanol:water in a polypropylene container at -20°C	
Long-Term Stability for Stock Solutions (Internal Standard)		Refer to long-term stability for stock solutions data collected from unlabeled 4-aminobiphenyl for labeled internal standard stability	
Short-Term Stability for Stock Solutions		6 hours at approximately 400 µg/mL in methanol in a	

[1] Batch fails intra-day PA, but passes batch acceptance criteria.

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Additional Information – 4-Aminobiphenyl in Urine - ZZ37540-01	
(Stock)	polypropylene container at ambient temperature under UV-shielded light
Short-Term Stability for Stock Solutions (Substock)	6 hours at 10.0 µg/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light 6 hours at 0.0600 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light
Short-Term Stability for Stock Solutions (Internal Standard Stock)	Refer to short-term stability for stock solutions data collected from unlabeled 4-aminobiphenyl for labeled internal standard stability
Short-Term Stability for Stock Solutions (Internal Standard Substock)	6 hours at 15.0 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light
Stability of Analyte During Sample Collection and Handling	Up to 60 minutes in non-smoker human urine in white and brown lidded high-density polyethylene containers at ambient temperature under UV-shielded light Up to 24 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C in a refrigerator Up to 96 hours in smoker and non-smoker human urine in high density polyethylene containers at 5°C under UV-shielded light, up to 48 hours in smoker and non-smoker human urine in high density polyethylene containers at 20°C under UV-shielded light
Sample Aliquot Frozen Storage Stability	Samples aliquoted manually at a volume of 2.00 mL, stored for 168 hours in 13 x 100 mm washed glass screw-cap tubes at -20°C prior to extraction
Batch Size	120 injections

Table 2. Validation Summary – o-Toluidine in Urine - ZZ37540-01

Information Requested	Data
Validation Summary	(b) (4) Validation Study ZZ37540-01
Analyte	*o*-Toluidine
Internal Standard (IS)	[$^{13}C_6$]*o*-Toluidine
Method Description	Liquid-liquid extraction with analysis/detection by LC-MS/MS
Limit of Quantitation (pg/mL)	50.0 pg/mL
Average Recovery of Drug (% Mean)	From Non-Smoker Urine: 80% at 25.0 pg/mL 80% at 250 pg/mL From Smoker Urine: 76% at 25.0 pg/mL 89% at 250 pg/mL From 0.1% Bovine Serum Albumin:

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	84% at 50.0 pg/mL 85% at 150 pg/mL 91% at 600 pg/mL
Average Recovery of IS (% Mean)	From 0.1% Bovine Serum Albumin: 79%
Standard Curve Concentrations (pg/mL)	50.0, 100, 150, 200, 400, 600, 840, and 1000 pg/mL
QC Concentrations (pg/mL)	LLOQ QC, 113, 213, 593, and 830 pg/mL
QC Intra-Batch Precision Range (% CV)	2.1 to 26.7%
QC Intra-Batch Accuracy Range (% Bias)	-9.1 to 18.2%
QC Inter-Batch Precision Range (% CV)	3.7 to 13.4%
QC Inter-Batch Accuracy Range (% Bias)	-4.6 to 1.8%
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours in brown polypropylene tubes at ambient temperature under UV-shielded light Cumulative Short-Term Stability: 54 hours in brown polypropylene tubes at ambient temperature under UV-shielded light (total of all thaw cycles)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 129 days at approximately 400 µg/mL in methanol in a polypropylene container at -20°C
Processed Stability (Hrs)	Post-Preparative Stability: 337 hours in a polypropylene 96 well plate at 5°C Processed Sample Integrity: 101 hours in a polypropylene 96 well plate at 5°C
Freeze-Thaw Stability (Cycles)	6 freeze (-20°C)-thaw (ambient temperature) cycles in brown polypropylene tubes under UV-shielded light
Long-Term Storage Stability (Days)	Long-Term Stability: 187 days in brown polypropylene tubes at -20°C
Dilution Integrity	up to 2090 pg/mL, diluted 2-fold
Selectivity	No significant interference at the retention time and mass transition of [$^{13}C_6$]*o*-toluidine (IS) was observed from endogenous components in any of the 7 human urine lots screened

Additional Information – *o*-Toluidine in Urine - ZZ37540-01

Matrix	Human Urine
Bioanalytical Method (BAM) SOP Number	BAM SOP ZZ37540-01
Detector	AB SCIEX API 4000™
Assay Volume Required	2.00 mL
Regression Type	Weighted linear (1/concentration^2)
Quantitation Method	Peak area ratio
Multiple Analytes Measured by Assay	*o*-Toluidine at 113 pg/mL fortified with 4-aminobiphenyl at 75.0 pg/mL, 2-aminonaphthalene at 500 pg/mL, and 1-aminonaphthalene at 500 pg/mL

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Additional Information – *o*-Toluidine in Urine - ZZ37540-01

Selectivity from Degradants or Isomers		3-Aminobiphenyl (75.0 pg/mL) 2-Aminobiphenyl (75.0 pg/mL) *m*-Toludine (1000 pg/mL) *p*-Toludine (1000 pg/mL)	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-Batch	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	13.4 4.5 3.7 8.3 8.5	1.8 -0.9 -0.5 -4.6 -3.9
Intra-Batch (Batch 170) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	2.7 2.8 5.7 4.1 6.5	-3.0 1.8 -1.4 3.7 3.9
Intra-Batch (Batch 171)[2] **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	4.6 6.7 4.7 17.1 15.0	2.6 1.8 1.4 -3.5 0.5
Intra-Batch (Batch 172) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	5.8 2.9 2.5 5.1 7.4	-6.0 -4.4 2.3 0.0 -2.8
Intra-Batch (Batch 173) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	2.2 4.7 2.6 2.1 4.3	2.8 0.0 0.5 -7.1 -5.1
Intra-Batch (Batch 174) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	3.0 3.8 2.7 3.6 3.7	-1.8 -2.7 -0.9 -9.1 -8.8

[2] Batch fails intra-day PA, but passes batch acceptance criteria.

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Additional Information – *o*-Toluidine in Urine - ZZ37540-01			
Intra-Batch (Batch 175)[3] **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	26.7 2.1 2.6 2.1 3.5	18.2 -1.8 -0.5 -7.9 -7.3
Intra-Batch (Batch 182) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	3.0 4.5 3.5 3.7 5.2	-0.4 -4.4 -2.8 -8.1 -7.3
Matrix Effect		No significant matrix effect was observed in any of the 6 human urine lots that were fortified with *o*-toluidine near the concentration of the LLOQ (50.0 pg/mL), in any of the 6 human urine lots that were fortified with *o*-toluidine near the concentration of the low-medium QC (150 pg/mL), or in 5 of the 6 human urine lots that were fortified with *o*-toluidine near the concentration of the high QC (600 pg/mL) samples	
Sample Shipping Stability		7 days in brown polypropylene tubes at -80°C	
Long-Term Stability for Stock Solutions (Substock)		66 days at 2000 ng/mL in 30:70 methanol:water in a polypropylene container at -20°C 121 days at 2.00 ng/mL in 30:70 methanol:water in a polypropylene container at -20°C	
Long-Term Stability for Stock Solutions (Internal Standard)		Refer to long-term stability for stock solutions data collected from unlabeled *o*-toluidine for labeled internal standard stability	
Short-Term Stability for Stock Solutions (Stock)		6 hours at approximately 400 µg/mL in methanol in a polypropylene container at ambient temperature under UV-shielded light	
Short-Term Stability for Stock Solutions (Substock)		6 hours at 2000 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light 6 hours at 2.00 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light	
Short-Term Stability for Stock Solutions (Internal Standard Stock)		Refer to short-term stability for stock solutions data collected from unlabeled *o*-toluidine for labeled internal standard stability	
Short-Term Stability for Stock Solutions (Internal Standard Substock)		6 hours at 10.0 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light	

[3] Batch fails intra-day PA, but passes batch acceptance criteria.

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Additional Information – *o*-Toluidine in Urine - ZZ37540-01	
Stability of Analyte During Sample Collection and Handling	Up to 60 minutes in non-smoker human urine in white and brown lidded high-density polyethylene containers at ambient temperature under UV-shielded light Up to 24 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C in a refrigerator Up to 96 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C under UV-shielded light, up to 48 hours in smoker and non-smoker human urine in high-density polyethylene containers at 20°C under UV-shielded light, and up to 48 hours in smoker and non-smoker human urine in high-density polyethylene containers at 40°C under UV-shielded light
Sample Aliquot Frozen Storage Stability	Samples aliquoted manually at a volume of 2.00 mL, stored for 168 hours in 13 x 100 mm washed glass screw-cap tubes at -20°C prior to extraction
Batch Size	120 injections

Table 3. Validation Summary – 2-Aminonaphthalene in Urine - ZZ37540-01

Information Requested	Data
Validation Summary	(b) (4) Validation Study ZZ37540-01
Analyte	2-Aminonaphthalene
Internal Standard (IS)	[$^{13}C_4$]2-Aminonaphthalene
Method Description	Liquid-liquid extraction with analysis/detection by LC-MS/MS
Limit of Quantitation (pg/mL)	2.50 pg/mL
Average Recovery of Drug (% Mean)	From Non-Smoker Urine: 84% at 25.0 pg/mL 81% at 250 pg/mL From Smoker Urine: 81% at 25.0 pg/mL 92% at 250 pg/mL From 0.1% Bovine Serum Albumin: 87% at 2.50 pg/mL 91% at 10.0 pg/mL 100% at 250 pg/mL
Average Recovery of IS (% Mean)	From 0.1% Bovine Serum Albumin: 82%
Standard Curve Concentrations (pg/mL)	2.50, 4.00, 10.0, 32.0, 100, 250, 425, and 500 pg/mL
QC Concentrations (pg/mL)	LLOQ QC, 3.87, 7.87, 26.5, and 396 pg/mL
QC Intra-Batch Precision Range (% CV)	1.0 to 25.9%
QC Intra-Batch Accuracy Range (% Bias)	-10.9 to 11.6%
QC Inter-Batch Precision Range (% CV)	2.5 to 12.0%
QC Inter-Batch Accuracy Range (% Bias)	-3.9 to 5.6%
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours in brown polypropylene

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	tubes at ambient temperature under UV-shielded light Cumulative Short-Term Stability: 54 hours in brown polypropylene tubes at ambient temperature under UV-shielded light (total of all thaw cycles)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 133 days at approximately 400 µg/mL in methanol in a polypropylene container at -20°C
Processed Stability (Hrs)	Post-Preparative Stability: 337 hours in a polypropylene 96 well plate at 5°C Processed Sample Integrity: 306 hours in a polypropylene 96 well plate at 5°C
Freeze-Thaw Stability (Cycles)	6 freeze (-20°C)-thaw (ambient temperature) cycles in brown polypropylene tubes under UV-shielded light
Long-Term Storage Stability (Days)	Long-Term Stability: 185 days in brown polypropylene tubes at -20°C
Dilution Integrity	Up to 777 pg/mL, diluted 2-fold
Selectivity	No significant interference at the retention time and mass transition of [$^{13}C_4$]2-aminonaphthalene (IS) was observed from endogenous components in any of the 7 human urine lots screened

Additional Information – 2-Aminonaphthalene in Urine - ZZ37540-01

Matrix		Human Urine	
Bioanalytical Method (BAM) SOP Number		BAM SOP ZZ37540-01	
Detector		AB SCIEX API 4000™	
Assay Volume Required		2.00 mL	
Regression Type		Weighted linear (1/concentration^2)	
Quantitation Method		Peak area ratio	
Multiple Analytes Measured by Assay		2-Aminonaphthalene at 3.87 pg/mL fortified with 4-aminobiphenyl at 75.0 pg/mL, *o*-toluidine at 1000 pg/mL, and 1-aminonaphthalene at 500 pg/mL	
Selectivity from Degradants or Isomers		3-Aminobiphenyl (75.0 pg/mL) 2-Aminobiphenyl (75.0 pg/mL) *m*-Toludine (1000 pg/mL) *p*-Toludine (1000 pg/mL)	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-Batch	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	12.0 8.1 4.6 2.5 3.2	2.4 -3.9 -1.1 0.4 5.6

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Additional Information – 2-Aminonaphthalene in Urine - ZZ37540-01			
Intra-Batch (Batch 170)[4] Aliquot Method: Manual Extraction Method: Manual	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	7.3 5.7 5.8 2.0 1.0	5.6 4.4 -1.3 0.8 3.3
Intra-Batch (Batch 171)[4] Aliquot Method: Manual Extraction Method: Manual	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	3.4 4.4 5.6 3.9 3.0	-0.4 5.4 2.0 -0.4 5.3
Intra-Batch (Batch 172) Aliquot Method: Manual Extraction Method: Manual	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	4.6 4.2 1.1 1.4 1.6	-4.0 -9.6 -6.4 0.0 3.0
Intra-Batch (Batch 173) Aliquot Method: Manual Extraction Method: Manual	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	4.6 4.0 2.9 1.8 1.7	-1.2 -10.9 -1.8 0.4 5.8
Intra-Batch (Batch 174) Aliquot Method: Manual Extraction Method: Manual	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	9.0 5.4 2.3 1.6 2.5	3.6 -3.1 3.4 1.5 9.1
Intra-Batch (Batch 175)[5] Aliquot Method: Manual Extraction Method: Manual	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	25.9 7.0 3.5 2.4 2.0	11.6 -10.6 -1.0 2.3 9.8
Intra-Batch (Batch 182) Aliquot Method: Manual Extraction Method: Manual	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	2.2 1.2 2.7 3.1 1.0	0.8 -3.1 -2.7 -1.5 2.3

[4] Batch fails intra-day PA, but passes batch acceptance criteria.

[5] Batch fails intra-day PA, but passes batch acceptance criteria.

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Additional Information – 2-Aminonaphthalene in Urine - ZZ37540-01	
Matrix Effect	No significant matrix effect was observed in any of the 6 human urine lots that were fortified with 2-aminonaphthalene near the concentration of the LLOQ (2.50 pg/mL), in any of the 6 human urine lots that were fortified with 2-aminonaphthalene near the concentration of the low-medium QC (10.0 pg/mL), or in any of the 6 human urine lots that were fortified with 2-aminonaphthalene near the concentration of the high QC (250 pg/mL) samples
Sample Shipping Stability	7 days in brown polypropylene tubes at -80°C
Long-Term Stability for Stock Solutions (Substock)	67 days at 500 ng/mL in 30:70 methanol:water in a polypropylene container at -20°C 78 days at 0.100 ng/mL in 30:70 methanol:water in a polypropylene container at -20°C
Long-Term Stability for Stock Solutions (Internal Standard)	Refer to long-term stability for stock solutions data collected from unlabeled 2-aminonaphthalene for labeled internal standard stability
Short-Term Stability for Stock Solutions (Stock)	6 hours at approximately 400 µg/mL in methanol in a polypropylene container at ambient temperature under UV-shielded light
Short-Term Stability for Stock Solutions (Substock)	6 hours at 500 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light 6 hours at 0.100 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light
Short-Term Stability for Stock Solutions (Internal Standard Stock)	Refer to short-term stability for stock solutions data collected from unlabeled 2-aminonaphthalene for labeled internal standard stability
Short-Term Stability for Stock Solutions (Internal Standard Substock)	6 hours at 10.0 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light
Stability of Analyte During Sample Collection and Handling	Up to 60 minutes in non-smoker human urine in white and brown lidded high-density polyethylene containers at ambient temperature under UV-shielded light Up to 24 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C in a refrigerator Up to 96 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C under UV-shielded light, up to 48 hours in smoker and non-smoker human urine in high-density polyethylene containers at 20°C under UV-shielded light, and up to 96 hours in smoker and non-smoker human urine in high-density polyethylene containers at 40°C under UV-shielded light

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Additional Information – 2-Aminonaphthalene in Urine - ZZ37540-01	
Sample Aliquot Frozen Storage Stability	Samples aliquoted manually at a volume of 2.00 mL, stored for 168 hours in 13 x 100 mm washed glass screw-cap tubes at -20°C prior to extraction
Batch Size	120 injections

Table 4. Validation Summary – 1-Aminonaphthalene in Urine - ZZ37540-01

Information Requested	Data
Validation Summary	(b) (4) Validation Study ZZ37540-01
Analyte	1-Aminonaphthalene
Internal Standard (IS)	[$^{13}C_{10}$]1-Aminonaphthalene
Method Description	Liquid-liquid extraction with analysis/detection by LC-MS/MS
Limit of Quantitation (pg/mL)	2.50 pg/mL
Average Recovery of Drug (% Mean)	From Non-Smoker Urine: 55% at 25.0 pg/mL 53% at 250 pg/mL From Smoker Urine: 56% at 25.0 pg/mL 64% at 250 pg/mL From 0.1% Bovine Serum Albumin: 91% at 2.50 pg/mL 92% at 10.0 pg/mL 98% at 250 pg/mL
Average Recovery of IS (% Mean)	From 0.1% Bovine Serum Albumin: 79%
Standard Curve Concentrations (pg/mL)	2.50, 4.00, 10.0, 32.0, 100, 250, 425, and 500 pg/mL
QC Concentrations (pg/mL)	LLOQ QC, 6.05, 10.1, 57.6, and 398 pg/mL
QC Intra-Batch Precision Range (% CV)	0.7 to 25.1%
QC Intra-Batch Accuracy Range (% Bias)	-10.4 to 6.5%
QC Inter-Batch Precision Range (% CV)	3.1 to 11.7%
QC Inter-Batch Accuracy Range (% Bias)	-3.0 to 3.3%
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours in brown polypropylene tubes at ambient temperature under UV-shielded light Cumulative Short-Term Stability: 54 hours in brown polypropylene tubes at ambient temperature under UV-shielded light (total of all thaw cycles)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 136 days at approximately 400 µg/mL in methanol in a polypropylene container at -20°C
Processed Stability (Hrs)	Post-Preparative Stability: 337 hours in a polypropylene 96 well plate at 5°C Processed Sample Integrity: 306 hours in a polypropylene 96 well plate at 5°C

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Freeze-Thaw Stability (Cycles)	6 freeze (-20°C)-thaw (ambient temperature) cycles in brown polypropylene tubes under UV-shielded light
Long-Term Storage Stability (Days)	Long-Term Stability: 185 days in brown polypropylene tubes at -20°C
Dilution Integrity	Up to 808 pg/mL, diluted 2-fold
Selectivity	No significant interference at the retention time and mass transition of [$^{13}C_{10}$]1-aminonaphthalene (IS) was observed from endogenous components in any of the 7 human urine lots screened

Additional Information – 1-Aminonaphthalene in Urine - ZZ37540-01

Matrix		Human Urine	
Bioanalytical Method (BAM) SOP Number		BAM SOP ZZ37540-01	
Detector		AB SCIEX API 4000™	
Assay Volume Required		2.00 mL	
Regression Type		Weighted linear (1/concentration^2)	
Quantitation Method		Peak area ratio	
Multiple Analytes Measured by Assay		1-Aminonaphthalene at 6.05 pg/mL fortified with 4-aminobiphenyl at 75.0 pg/mL, *o*-toluidine at 1000 pg/mL, and 2-aminonaphthalene at 500 pg/mL	
Selectivity from Degradants or Isomers		3-Aminobiphenyl (75.0 pg/mL) 2-Aminobiphenyl (75.0 pg/mL) *m*-Toludine (1000 pg/mL) *p*-Toludine (1000 pg/mL)	
Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-Batch	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	11.7 9.4 7.9 3.6 3.1	-0.8 -3.0 -2.0 -0.5 3.3
Intra-Batch (Batch 170) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	5.9 4.5 8.2 2.5 1.9	-4.0 4.3 1.0 3.1 3.8
Intra-Batch (Batch 171) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	8.1 1.2 7.1 2.6 2.1	0.8 6.1 2.0 1.2 6.5

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Additional Information – 1-Aminonaphthalene in Urine - ZZ37540-01			
Intra-Batch (Batch 172) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	6.0 5.6 5.4 1.5 1.0	-8.0 -10.4 -7.3 -4.3 -1.0
Intra-Batch (Batch 173) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	7.4 6.0 2.5 1.9 1.5	4.8 1.0 1.0 2.6 6.0
Intra-Batch (Batch 174) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	2.9 6.8 3.5 1.4 1.2	-6.0 -5.1 -2.6 -1.6 2.5
Intra-Batch (Batch 175)[6] **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	25.1 2.6 13.3 2.8 0.7	5.2 -8.6 0.0 0.5 5.8
Intra-Batch (Batch 182) **Aliquot Method: Manual** **Extraction Method: Manual**	**LLOQ** **Low** **Low-Medium** **High-Medium** **High**	3.3 6.8 3.4 1.1 1.6	2.0 -9.8 -8.7 -4.7 0.0
Matrix Effect		No significant matrix effect was observed in 4 of the 6 human urine lots that were fortified with 1-aminonaphthalene near the concentration of the LLOQ (2.50 pg/mL), in any of the 6 human urine lots that were fortified with 1-aminonaphthalene near the concentration of the low-medium QC (10.0 pg/mL), or in any of the 6 human urine lots that were fortified with 1-aminonaphthalene near the concentration of the high QC (250 pg/mL) samples	
Sample Shipping Stability		7 days in brown polypropylene tubes at -80°C	
Long-Term Stability for Stock Solutions (Substock)		70 days at 500 ng/mL in 30:70 methanol:water in a polypropylene container at -20°C 83 days at 0.100 ng/mL in 30:70 methanol:water in a polypropylene container at -20°C	

[6] Batch fails intra-day PA, but passes batch acceptance criteria.

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Additional Information – 1-Aminonaphthalene in Urine - ZZ37540-01	
Long-Term Stability for Stock Solutions (Internal Standard)	Refer to long-term stability for stock solutions data collected from unlabeled 1-aminonaphthalene for labeled internal standard stability
Short-Term Stability for Stock Solutions (Stock)	6 hours at approximately 400 µg/mL in methanol in a polypropylene container at ambient temperature under UV-shielded light
Short-Term Stability for Stock Solutions (Substock)	6 hours at 500 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light 6 hours at 0.100 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light
Short-Term Stability for Stock Solutions (Internal Standard Stock)	Refer to short-term stability for stock solutions data collected from unlabeled 1-aminonaphthalene for labeled internal standard stability
Short-Term Stability for Stock Solutions (Internal Standard Substock)	6 hours at 10.0 ng/mL in 30:70 methanol:water in a polypropylene container at ambient temperature under UV-shielded light
Stability of Analyte During Sample Collection and Handling	Up to 60 minutes in non-smoker human urine in white and brown lidded high-density polyethylene containers at ambient temperature under UV-shielded light Up to 24 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C in a refrigerator Up to 96 hours in smoker and non-smoker human urine in high-density polyethylene containers at 5°C under UV-shielded light, up to 48 hours in smoker and non-smoker human urine in high-density polyethylene containers at 20°C under UV-shielded light, and up to 48 hours in smoker and non-smoker human urine in high-density polyethylene containers at 40°C under UV-shielded light
Sample Aliquot Frozen Storage Stability	Samples aliquoted manually at a volume of 2.00 mL, stored for 168 hours in 13 x 100 mm washed glass screw-cap tubes at -20°C prior to extraction
Batch Size	120 injections

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