Validation of Quantitative Methods

Analyte	Amphetamines (amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxy-N-ethylamphetamine (MDEA))
Unit of Measure	ng/mL
Analyst Performing Validation Studies	Sara Dempsey, Pucheng Ke, and Corissa L. Rodgers
Responsible Supervisor	Dayong Lee
Start Date	August 14, 2020
Completion Date	September 24, 2020
Primary Matrix	Blood
Secondary Matrix	N/A
Low Calibrator Concentration	10 ng/mL
Highest Calibrator Concentration	500 ng/mL for amphetamine, MDA, and MDEA; 1000 ng/mL for methamphetamine and MDMA
Equipment/Instrument	LCMS-1
Instrument Serial Number	SG1939G104
Method	AMP.M

Validation Approval

Analyst: Sara Dempsey — Digitally signed by Sara Dempsey Date: 2020.11.20 11:58:45 -06'00' — 11/20/2020 (Date)

Analyst: Pucheng Ke — Digitally signed by Pucheng Ke Date: 2020.11.20 16:51:15 -06'00' — 11/20/2020 (Date)

Analyst: Corissa L. Rodgers, M.S. — Digitally signed by Corissa L. Rodgers, M.S. Date: 2020.11.20 13:03:19 -06'00' — 11/20/2020 (Date)

Responsible Supervisor: Dayong Lee — Digitally signed by Dayong Lee Date: 2020.11.23 12:22:01 -06'00' — 11/23/2020 (Date)

Quality Director: Erika Ziemak — Digitally signed by Erika Ziemak Date: 2020.12.01 14:40:14 -06'00' — 12/1/2020 (Date)

This method validation study, even though it may depict an approval date by both the Toxicology section and the Quality Division, will not be used in casework until ANAB grants an expansion in our scope of accreditation to include this test method and instrument technology.

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Amphetamine
Units: ng/mL
Method: AMP.M
Instrument: LCMS-1
SOP Reference: Toxicology Analytical Manual v3.5

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes Σ|%RE|	%RE = 91.51 ($1/x^2$)	1/x2 weighting will be used. %RE for unweighted and 1/x weighting are 209.09 and 112.01, respectively. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.
1	Validation Calibration	%RE Calibrators ±20% of target	Max %RE = 8.91	N/A
1	Case Work Calibration	%RE Calibrators ±20% of target	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
1	Comparison of Validation Calibration to Casework Calibration	95% CI of slope includes 1 95% CI of intercept includes 0	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
2	Limit of Detection (LOD)	Signal to Noise ≥3.3 Acceptable detection and identification criteria	LOD = 10 ng/mL S:N= 1403	The lowest non-zero calibrator was defined as the LOD.
3	Limit of Quantitation (LOQ)	Bias: ≤20% Within-Run Precision: CV ≤20% Between-Run Precision: CV ≤20%	Bias = 2.76% Within-Run Precision= 10.56% Between-Run Precision = 5.97%	Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Number in red was > 20% target value. Numbers with * were > 20% target value due to insufficient IS.
4	Bias & Precision	%Bias ≤20% Within-Run %CV ≤20% Between-Run %CV ≤20%	Max Bias = -10.15% Max Within-Run Precision = 18.13% Max Between-Run Precision = 12.48%	Number in red was not within 20% of the target value.
5	Carryover	No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.	No significant carryover observed following samples containing analyte at up to 2000 ng/mL.	N/A
6	Matrix Interference	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020
6	Interference from stable isotope internal standard	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	
6	Exogenous Substances Interferences	Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies	No significant interference observed.	N/A
7	Dilution Integrity	Average %Bias must be less than 20%	It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.	N/A
8	Processed Sample Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	N/A	Study not performed. Sample preparation will be completed once started without prolonged interruptions.
8	Autosampler Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	Punctured and unpunctured samples were shown to be stable for 48 hours.	AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for amphetamine were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.
9	Matrix Effects	Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%	Average percent suppression values for LQC and HQC were -6% and -5%, respectively.	N/A

*** Alternate criteria is any deviation from that described in the SOP as determined by the Supervisor**

ADDITIONAL STUDIES (Describe any additional studies required for this validation.)

Validation Study 1

LINEARITY

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Study Date	Target (x)	Target $(x)^2$	Calculated (y)	Predicted	Residual	%RE
AMP_20200814B_SD	10	100	10.00	10.40	-0.41	0.02
	20	400	19.86	20.18	-0.33	0.71
	50	2500	50.74	49.60	1.13	1.47
	100	10000	102.93	98.90	4.03	2.93
	250	62500	231.20	248.80	-17.60	7.52
	500	250000	528.94	505.28	23.65	5.79
AMP_20200814B_PK	10	100	9.83	10.40	-0.57	1.70
	20	400	21.31	20.18	1.12	6.53
	50	2500	45.54	49.60	-4.06	8.91
	100	10000	99.96	98.90	1.06	0.04
	250	62500	267.96	248.80	19.17	7.19
	500	250000	484.74	505.28	-20.54	3.05
AMP_20200828B_PK	10	100	9.83	10.40	-0.58	1.72
	20	400	20.90	20.18	0.71	4.49
	50	2500	49.18	49.60	-0.42	1.64
	100	10000	97.24	98.90	-1.66	2.76
	250	62500	255.38	248.80	6.58	2.15
	500	250000	497.35	505.28	-7.94	0.53
AMP_20200828B_SD	10	100	9.92	10.40	-0.48	0.77
	20	400	20.10	20.18	-0.09	0.48
	50	2500	52.35	49.60	2.75	4.70
	100	10000	96.83	98.90	-2.07	3.17
	250	62500	241.70	248.80	-7.10	3.32
	500	250000	511.06	505.28	5.78	2.21
AMP_20200901B_CLR	10	100	9.81	10.40	-0.59	1.89
	20	400	21.08	20.18	0.90	5.42
	50	2500	47.46	49.60	-2.14	5.07
	100	10000	102.53	98.90	3.62	2.53
	250	62500	245.40	248.80	-3.40	1.84
	500	250000	504.74	505.28	-0.54	0.95

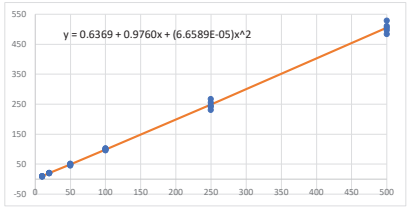
Σ|%RE| 91.51

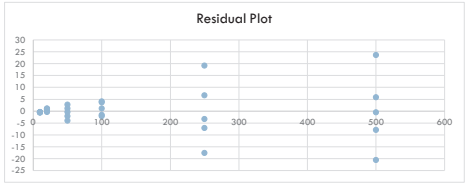
Results: The reported %RE is using $1/x^2$ weighting. Max %RE 8.91

Regression Statistics	
Multiple R	0.998956279
R Square	0.997913647
Adjusted R Square	0.997759102
Standard Error	8.459442209
Observations	30

ANOVA	df	SS	MS	F	Significance F
Regression	2	924170.9047	462085.4523	6457.119744	6.48241E-37
Residual	27	1932.178387	71.56216249		
Total	29	926103.0831			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.636927298	2.662898457	0.239185725	0.81276791	-4.826889018	6.100743613	-4.826889018	6.100743613
target x	0.97599925	0.036569563	26.68884094	6.08314E-21	0.900964705	1.051033796	0.900964705	1.051033796
x^2	6.6589E-05	7.04575E-05	0.94509442	0.352992162	-7.79778E-05	0.000211156	-7.79778E-05	0.000211156





Comments: $1/x^2$ weighting will be used. %RE for unweighted and 1/x weighting are 209.09 and 112.01, respectively. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.

Acceptance Criteria: The least complex weighting scheme that minimizes Σ|%RE|
%RE Calibrators ±20% of target

Validation Study 2

Sensitivity (LOD)

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Signal to Noise Ratio			
Concentration (ng/mL)	*10*			
AMP_20200814B_SD	1273			
	732			
	667			
	2315			
	1199			
	2541			
	2679			
	2447			
	1504			
AMP_20200828B_PK	338			
	1197			
	505			
	953			
	820			
	1742			
	1275			
	2773			
	704			
AMP_20200901B_PK	2098			
	709			
	238			
	1944			
	1338			
	1933			
	1187			
	688			
	2072			
AMP_20200901B_CLR	1935			
	630			
	1360			
	1617			
	2223			
	562			
	326			
	1204			
	2501			
Average Signal to Noise:	**1403**			

Established LOD: 10 ng/mL
Signal to Noise: 1403

Comments: The lowest non-zero calibrator was defined as the LOD.

Acceptance Criteria:
Signal to Noise ≥3.3
Acceptable detection and identification criteria

Validation Study 3 — SENSITIVITY (LOQ)

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LOQ
	Target Concentration (ng/mL):	*10*
AMP_20200814B_SD	1-1	10.32
	1-2	10.54
	1-3	9.76
	1-4	10.05
	1-5	10.03
	1-6	11.02
	1-7	10.44
	1-8	10.17
	1-9	9.95
Within Run	**Mean**	**10.25**
	SD	**0.38**
	%CV	**3.69%**
	% Bias	**2.53%**
AMP_20200901B_PK	2-1	10.52
	2-2	10.41
	2-3	10.16
	2-4	10.25
	2-5	10.65
	2-6	11.09
	2-7	10.72
	2-8	10.79
	2-9	10.71
Within Run	**Mean**	**10.59**
	SD	**0.29**
	%CV	**2.70%**
	% Bias	**5.87%**
AMP_20200901B_CLR	3-1	57.75*
	3-2	10.04
	3-3	9.77
	3-4	10.00
	3-5	9.29
	3-6	10.51
	3-7	12.90
	3-8	10.15
	3-9	10.05
Within Run	**Mean**	**10.34**
	SD	**1.09**
	%CV	**10.56%**
	% Bias	**3.38%**
AMP_20200828B_PK	4-1	10.28
	4-2	9.81
	4-3	10.18
	4-4	9.54
	4-5	9.76
	4-6	3.93*
	4-7	9.92
	4-8	10.10
	4-9	9.82
Within Run	**Mean**	**9.93**
	SD	**0.24**
	%CV	**2.45%**
	% Bias	**-0.73%**

Mean		**10.28**
SD		**0.61**
Precision (%CV)	***Max Within-Run***	**10.56%**
	Between-Run	**5.97%**
% Bias		**2.76%**

Comments: Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Number in red was > 20% target value. Numbers with * were > 20% target value due to insufficient IS.

Acceptance Criteria:
Bias: ≤20%
Within-Run Precision: CV ≤20%
Between-Run Precision: CV ≤20%

Validation Study 4

BIAS AND PRECISION

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LQC	MQC	UTAK	HQC
	Target Concentration (ng/mL):	*25*	*100*	*73.97*	*400*
AMP_20200814B_SD	1-1	25.18	92.86	63.75	356.77
	1-2	23.61	88.67	68.46	347.61
	1-3	24.71	91.58	65.43	347.62
	1-4			69.28	
Within Run	**Mean**	**24.50**	**91.04**	**66.73**	**350.67**
	SD	**0.80**	**2.15**	**2.59**	**5.28**
	%CV	**3.28%**	**2.36%**	**3.87%**	**1.51%**
	% Bias	**-1.99%**	**-8.96%**	**-9.79%**	**-12.33%**
AMP_20200814B_PK	2-1	25.15	81.17	79.66	392.65
	2-2	28.47	83.01	80.62	403.58
	2-3	27.54	89.47	92.96	390.02
	2-4			95.46	
Within Run	**Mean**	**27.05**	**84.55**	**87.18**	**395.42**
	SD	**1.71**	**4.36**	**8.20**	**7.19**
	%CV	**6.34%**	**5.16%**	**9.40%**	**1.82%**
	% Bias	**8.21%**	**-15.45%**	**17.85%**	**-1.14%**
AMP_20200828B_PK	3-1	25.51	90.29	67.88	365.48
	3-2	27.62	97.78	70.78	363.46
	3-3	24.80	86.74	71.94	370.54
	3-4			66.47	
Within Run	**Mean**	**25.98**	**91.61**	**69.27**	**366.49**
	SD	**1.47**	**5.64**	**2.53**	**3.65**
	%CV	**5.66%**	**6.15%**	**3.65%**	**0.99%**
	% Bias	**3.76%**	**-9.16%**	**-6.79%**	**-9.14%**
AMP_20200828B_SD	4-1	23.00	98.12	73.63	360.25
	4-2	24.10	93.39	77.05	355.84
	4-3	24.22	95.83	69.30	354.35
	4-4			70.85	
Within Run	**Mean**	**23.77**	**95.78**	**72.71**	**356.81**
	SD	**0.67**	**2.37**	**3.40**	**3.07**
	%CV	**2.83%**	**2.47%**	**4.68%**	**0.86%**
	% Bias	**-4.91%**	**-4.22%**	**-1.71%**	**-10.80%**
AMP_20200901B_CLR	5-1	27.25	109.59		356.50
	5-2	29.13	76.21		367.53
	5-3	25.16	90.95		362.03
Within Run	**Mean**	**27.18**	**92.25**		**362.02**
	SD	**1.98**	**16.73**		**5.52**
	%CV	**7.30%**	**18.13%**		**1.52%**
	% Bias	**8.73%**	**-7.75%**		**-9.49%**
AMP_20200901B_PK	6-1	24.67	80.30		370.86
	6-2	24.64	87.92		361.30
	6-3	24.32	83.34		360.92
Within Run	**Mean**	**24.54**	**83.85**		**364.36**
	SD	**0.19**	**3.83**		**5.63**
	%CV	**0.79%**	**4.57%**		**1.55%**
	% Bias	**-1.82%**	**-16.15%**		**-8.91%**
Mean		**25.51**	**89.85**	**73.97**	**365.96**
SD		**1.74**	**7.82**	**9.23**	**15.22**
Precision	***Max Within-Run***	**7.30%**	**18.13%**	**9.40%**	**1.82%**
(%CV)	***Between-Run***	**6.82%**	**8.70%**	**12.48%**	**4.16%**
% Bias		**2.02%**	**-10.15%**	**0.00%**	**-8.51%**

Comments: Number in red was not within 20% of the target value.

Acceptance Criteria:

%Bias ≤20%
Within-Run %CV ≤20%
Between-Run %CV ≤20%

Validation Study 5

CARRYOVER

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Average LOQ Response*: 136552

Study Date:	Response		
	AMP_20200814B_SD	AMP_20200828B_PK	AMP_20200901B_PK
Concentrated Sample (2000 ng/mL)	11401848	14582622	10479640
Blank	3916	2620	7013
%LOD Response	**2.87%**	**1.92%**	**5.14%**
		Maximum Response in Blank:	**5.1%**

Results: No significant carryover observed following samples containing analyte at up to 2000 ng/mL.
Comments: N/A

Acceptance Criteria: **No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.**

Validation Study 6

MATRIX & IS INTERFERENCE

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

LOQ Response

	Analyte	IS
Run 1	169346	627849
Run 2	92912	413897
Run 3	182678	700000
Run 4	106895	466605
Run 5	130930	502265
Average	**136552**	**542123**

Matrix Interference Study Date: AMP_20200814B_PK

Matrix Source	Peak at Analyte RT	
	Peak Response	Percent of LOQ Response
4	920	0.67%
5	1067	0.78%
7	1404	1.03%
8	1171	0.86%
9	1737	1.27%
10	1776	1.30%
11	1587	1.16%
12	1041	0.76%
13	1307	0.96%
14	1657	1.21%

Interference from Stable-Isotope Internal Standards Study Date: AMP_20200828B_SD

Experiment	Peak at Analyte RT		Peak at IS RT	
	Peak Response	Percent of LOQ Response	Peak Response	Percent of LOQ Response
Matrix with IS but no D0 (IS = 50 ng/mL)	2822.47	2.07%	N/A	N/A
Matrix with D0 but no IS (D0 = 2000 ng/mL)	N/A	N/A	213.67	0.04%

Matrix Interference: No significant interference observed.
IS Interference: No significant interference observed.
Comments: LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020

Acceptance Criteria: **Response of blank matrix is less than 20% the average response of LOQ**

Validation Study 6 — **EXOGENOUS SUBSTANCE INTERFERENCE**

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target LQC Concentration (µg/mL): 25.51
Control Acceptance: 20%
Run Date: AMP_20200828B_SD

Group	Compound	Compound Concentration (µg/mL)	Calculated LQC Concentration (ng/mL)	% Difference from Target	Comment
Benzodiazepines	Alprazolam	1	24.39	-4%	No significant interference
	α-Hydroxyalprazolam				
	Clonazepam				
	7-Aminoclonazepam				
	Diazepam				
	Nordiazepam				
	Temazepam				
	Lorazepam				
	Oxazepam				
	Zolpidem				
Phencyclidine	Phencyclidine	1	26.05	2%	No significant interference
Opioids	Morphine	2	26.33	3%	No significant interference
	Hydrocodone	1			
	Buprenorphine	0.5			
	Norbuprenorphine				
	Codeine				
	Fentanyl				
	Norfentanyl oxalate				
	Hydromorphone				
	Methadone				
	EDDP				
	Oxycodone				
	Oxymorphone				
	Tramadol				
	o-Desmethyltramadol				
Cocaine and Metabolites	Benzoylecgonine	2	24.62	-3%	No significant interference
	Cocaine	0.5			
	Cocaethylene				
Carisoprodol/Meprobamate	Carisoprodol	1	25.07	-2%	No significant interference
	Meprobamate				
Basic and Neutral Mix	Amitriptyline	5	24.96	-2%	No significant interference
	Benzylpiperizine				
	Chlorpheniramine				
	Cyclobenzaprine				
	Dextromethorphan				
	Diphenhydramine				
	Doxylamine				
	Fluoxetine				
	Imipramine				
	Ketamine				
	Norketamine				
	Meperidine				
	Nortriptyline				
	Propoxyphene				
	Sertraline				
	Trazodone				
	Venlafaxine				
	Zopiclone				
Over-the-Counter Drugs	Acetaminophen	10	23.62	-7%	No significant interference
	Caffeine				
	Ibuprofen				
	Naproxen				
	Pseudoephedrine				
	Theobromine	1			
Cannabinoids	Δ9-THC	0.5	25.23	-1%	No significant interference
	11-Hydroxy-Δ9-THC				
	Δ9-THC-COOH				
	Δ8-THC				
	Cannabinol				
	Cannabinolic acid				

Conclusions: No significant interference observed.

Comments: N/A

Acceptance Criteria: Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies

Validation Study 7

DILUTION INTEGRITY

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target Concentration (ng/mL): 347.84

Run Date	Dilution Factor	Diluted Result (ng/mL)	Calculated Result (µg/mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
AMP_20200814B_SD	2	174.76	349.52	334.78	19.21	0.48	0.48	5.74
	2	170.88	341.77			-1.75	1.75	
	2	156.53	313.05			-10.00	10.00	
	5	71.21	356.04	341.60	16.67	2.36	2.36	4.88
	5	69.08	345.41			-0.70	0.70	
	5	64.67	323.35			-7.04	7.04	
	10	37.78	377.81	350.07	33.83	8.62	8.62	9.66
	10	36.00	360.02			3.50	3.50	
	10	31.24	312.38			-10.19	10.19	
AMP_20200828B_PK	2	185.55	371.09	397.18	24.63	6.69	6.69	6.20
	2	200.20	400.40			15.11	15.11	
	2	210.02	420.04			20.76	20.76	
	5	75.29	376.43	403.67	24.71	8.22	8.22	6.12
	5	84.93	424.66			22.09	22.09	
	5	81.98	409.92			17.85	17.85	
	10	36.02	360.17	397.91	32.97	3.55	3.55	8.29
	10	42.12	421.15			21.08	21.08	
	10	41.24	412.40			18.56	18.56	
AMP_20200901B_PK	2	191.73	383.47	396.07	11.64	0.74	0.74	2.94
	2	203.21	406.42			6.77	6.77	
	2	199.16	398.32			4.64	4.64	
	5	72.88	364.40	389.94	22.53	-4.27	4.27	5.78
	5	79.69	398.44			4.67	4.67	
	5	81.40	407.00			6.92	6.92	
	10	39.89	398.92	385.87	22.15	4.80	4.80	5.74
	10	36.03	360.30			-5.35	5.35	
	10	39.84	398.38			4.65	4.65	
AMP_20200828B_SD	2	182.29	364.58	361.56	9.25	4.81	4.81	2.56
	2	184.47	368.93			6.06	6.06	
	2	175.59	351.18			0.96	0.96	
	5	74.11	370.55	364.92	12.53	6.53	6.53	3.43
	5	74.73	373.66			7.42	7.42	
	5	70.11	350.56			0.78	0.78	
	10	32.51	325.06	342.41	19.55	-6.55	6.55	5.71
	10	36.36	363.59			4.53	4.53	
	10	33.86	338.57			-2.66	2.66	
AMP_20200901B_CLR	2	151.91	303.81	343.13	37.67	-12.66	12.66	10.98
	2	189.45	378.91			8.93	8.93	
	2	173.33	346.67			-0.34	0.34	
	5	73.11	365.53	379.82	12.44	5.09	5.09	3.27
	5	77.14	385.72			10.89	10.89	
	5	77.64	388.21			11.61	11.61	
	10	37.12	371.17	361.88	9.15	6.71	6.71	2.53
	10	35.29	352.87			1.45	1.45	
	10	36.16	361.60			3.96	3.96	

Dilution Factor	2
Avg %Bias	6.71
Max Within-Run Precision (%CV)	10.98

Dilution Factor	5
Avg %Bias	7.76
Max Within-Run Precision (%CV)	6.12

Dilution Factor	10
Avg %Bias	7.08
Max Within-Run Precision (%CV)	9.66

Results: It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.
Comments: N/A

Acceptance Criteria: Avg %Bias must be less than 20%

Validation Study 8

PROCESSED SAMPLE STABILITY

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Sample	Time Zero Response	24H Response	48H Response	72H Response	Acceptable Range		Pass/Unstable
						Low	High	
	LQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			
	HQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			

Results: N/A
Comments: Study not performed. Sample preparation will be completed once started without prolonged interruptions.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 8 — **AUTOSAMPLER STABILITY**

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Stability of Punctured Calibrators

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	Calibrator 1	10.07	0.29	10.03	9.97	0.26	-8%
	Calibrator 2	19.41	0.53	19.42	18.87	0.48	-9%
	Calibrator 3	52.51	1.36	52.21	50.77	1.24	-9%
	Calibrator 4	98.57	2.47	99.06	96.56	2.28	-8%
	Calibrator 5	242.13	5.54	243.33	232.58	5.04	-9%
	Calibrator 6	511.64	9.68	517.12	495.73	8.97	-7%

Stability of Punctured Calibrators

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	Calibrator 1	9.99	0.27	N/A	N/A	N/A	N/A	9.89	10.15	0.27	-1%
	Calibrator 2	20.26	0.50	N/A	N/A	N/A	N/A	20.08	19.05	0.50	-1%
	Calibrator 3	47.76	1.10	N/A	N/A	N/A	N/A	47.58	43.29	1.10	0%
	Calibrator 4	103.18	2.28	N/A	N/A	N/A	N/A	104.10	94.05	2.30	1%
	Calibrator 5	252.38	5.22	N/A	N/A	N/A	N/A	257.84	241.25	5.32	2%
	Calibrator 6	495.41	9.26	N/A	N/A	N/A	N/A	502.15	545.95	9.36	1%

Stability of Punctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	LQC	24.01	0.65	23.94	23.43	0.59	-9%
		24.04	0.65	23.97	23.52	0.59	-8%
		24.09	0.65	24.06	23.50	0.59	-9%
	HQC	355.37	7.53	358.66	343.70	6.93	-8%
		353.53	7.50	362.93	343.36	6.92	-8%
		354.29	7.52	358.23	343.16	6.92	-8%

Stability of Punctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	LQC	24.59	0.60	N/A	N/A	N/A	N/A	24.56	22.98	0.60	0%
		24.58	0.60	N/A	N/A	N/A	N/A	24.83	23.22	0.60	1%
		24.86	0.60	N/A	N/A	N/A	N/A	24.87	23.25	0.60	0%
	HQC	350.17	6.95	N/A	N/A	N/A	N/A	358.81	349.91	7.10	2%
		350.32	6.96	N/A	N/A	N/A	N/A	355.78	346.44	7.05	1%
		352.84	7.00	N/A	N/A	N/A	N/A	355.84	346.50	7.05	1%

Stability of Unpunctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	LQC	24.01	0.65	23.89	23.37	0.59	-9%
		24.04	0.65	23.92	23.62	0.59	-8%
		24.09	0.65	23.83	23.38	0.59	-9%
	HQC	355.37	7.53	362.06	346.22	6.97	-8%
		353.53	7.50	359.17	339.87	6.87	-8%
		354.29	7.52	359.49	342.61	6.91	-8%

Stability of Unpunctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	LQC	24.59	0.60	N/A	N/A	N/A	N/A	24.60	23.01	0.60	0%
		24.58	0.60	N/A	N/A	N/A	N/A	24.71	23.11	0.60	0%
		24.86	0.60	N/A	N/A	N/A	N/A	24.57	22.99	0.60	-1%
	HQC	350.17	6.95	N/A	N/A	N/A	N/A	355.33	345.92	7.04	1%
		350.32	6.96	N/A	N/A	N/A	N/A	358.10	349.10	7.09	2%
		352.84	7.00	N/A	N/A	N/A	N/A	357.76	348.71	7.08	1%

Results: Punctured and unpunctured samples were shown to be stable for 48 hours.

Comments: AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for amphetamine were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 9 **MATRIX EFFECTS**

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Neat Response at Analyte RT

	LQC	HQC
1	263201	2841207
2	243472	2860394
3	254761	2885998
4	258382	2862797
5	243900	2743578
6	245218	2879888
Mean	**251489**	**2845644**
SD	8445	52441
%CV	3%	2%

Neat Response at IS RT

LQC	HQC
408972	433611
378283	433593
435809	441189
422956	421527
388212	448823
399309	412403
405590	**431858**
21510	13162
5%	3%

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at Analyte RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	241477	-4%	2557616	-10%
5	231617	-8%	2823217	-1%
7	229002	-9%	2796095	-2%
8	242140	-4%	2676641	-6%
9	206062	-18%	2639858	-7%
10	236577	-6%	2524344	-11%
11	245566	-2%	2679573	-6%
12	248017	-1%	2754737	-3%
13	234754	-7%	2679574	-6%
14	244456	-3%	2891628	2%
Mean	235967		2702328	
SD	12209		115569	
%CV	5%		4%	
Avgerage % suppression/enhancement	-6%		-5%	

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at IS RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	426963	5%	397656	-8%
5	407552	0%	446633	3%
7	413441	2%	442097	2%
8	436401	8%	422638	-2%
9	365055	-10%	416398	-4%
10	416819	3%	395819	-8%
11	425983	5%	420782	-3%
12	434760	7%	434337	1%
13	414119	2%	414211	-4%
14	433702	7%	455849	6%
Mean	417480		424642	
SD	20968		20030	
%CV	5%		5%	
Avgerage % suppression/enhancement	3%		-2%	

Results: Average percent suppression values for LQC and HQC were -6% and -5%, respectively.

Comments: N/A

Acceptance Criteria: **Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%**

SUMMARY OF VALIDATION PERFORMANCE

Analyte: Amphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

Date	Reason
N/A	N/A

Deviations from SOP: N/A

Other Observations:
Working Standards Verified in Validation:
Calibrators: 200805C-C-10, 200805C-C-2.5, 200805C-C-0.2, 200923C-C-10, 200923C-C-2.5, 200923C-C-0.2
Controls:200807L-Q-Mix, 200813L-Q-0.5, 200813L-St-5
Internal Standard: 200805C-IS-1, 200903C-IS-1, 200915C-IS-1

Sample Preparation Steps: Refer to Toxicology Analytical Manual v3.5, "Amphetamines Confirmation by Liquid Chromatography-Tandem Mass Spectrometry" section titled "Extraction Procedure".

Location of Raw Data: Toxicology section shared electronic storage.

Recommended Maximum Run Length (Unknown Samples): 30

Conclusion: **This method is fit for use on casework for amphetamine confirmation analysis in blood.**

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Methamphetamine
Units: ng/mL
Method: AMP.M
Instrument: LCMS-1
SOP Reference: Toxicology Analytical Manual v3.5

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes Σ|%RE|	%RE = 130.99 (1/x)	1/x weighting will be used. %RE for unweighted and 1/x2 weighting are 235.48 and 131.99, respectively. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.
1	Validation Calibration	%RE Calibrators ±20% of target	Max %RE = 11.66	N/A
1	Case Work Calibration	%RE Calibrators ±20% of target	N/A	Not performed. All 7 calibrators used in validation will be used for casework.
1	Comparison of Validation Calibration to Casework Calibration	95% CI of slope includes 1 95% CI of intercept includes 0	N/A	Not performed. All 7 calibrators used in validation will be used for casework.
2	Limit of Detection (LOD)	Signal to Noise ≥3.3 Acceptable detection and identification criteria	LOD = 10 ng/mL S:N= 1373	The lowest non-zero calibrator was defined as the LOD.
3	Limit of Quantitation (LOQ)	Bias: ≤20% Within-Run Precision: CV ≤20% Between-Run Precision: CV ≤20%	Bias = 5.43% Within-Run Precision= 21.57% Between-Run Precision = 17.25%	Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Numbers in red were > 20% target value. Numbers with * were > 20% target value due to insufficient IS. Max within-run precision is greater than 20% due to the values of 15.15 ng/mL and 18.38 ng/mL being greater than 20% of the target value in AMP_20200901B_CLR and AMP_202008014B_SD respectively. LOQ data are deemed acceptable because a minimum of three values passing acceptance criteria were obtained for each source used over the four runs as defined in the SOP.
4	Bias & Precision	%Bias ≤20% Within-Run %CV ≤20% Between-Run %CV ≤20%	Max Bias = -6.64% Max Within-Run Precision = 18.28% Max Between-Run Precision = 17.09%	N/A
5	Carryover	No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.	No significant carryover observed following samples containing analyte at up to 2000 ng/mL.	N/A
6	Matrix Interference	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.
6	Interference from stable isotope internal standard	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	
6	Exogenous Substances Interferences	Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies	No significant interference observed.	N/A
7	Dilution Integrity	Average %Bias must be less than 20%	It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.	N/A
8	Processed Sample Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	N/A	Study not performed. Sample preparation will be completed once started without prolonged interruptions.
8	Autosampler Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	Punctured and unpunctured samples were shown to be stable for 48 hours.	Cal 7 relative response was beyond the range of the calibration curve and therefore was not quantified. The samples were deemed stable at 48 hrs since response ratio of analyte to IS was within 20% compared to time 0. AMP_20200904B_SD showed the punctured and unpunctured samples at 72 hr were not stable. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.
9	Matrix Effects	Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%	Average percent suppression values for LQC and HQC were -11% and -8%, respectively.	N/A

Validation Study 1

LINEARITY

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Study Date	Target (x)	Target (x)^2	Calculated (y)	Predicted	Residual	%RE
AMP_20200814B_PK	10	100	10.53	12.52	-1.99	5.31
	20	400	20.76	22.14	-1.38	3.80
	50	2500	44.41	51.04	-6.63	11.17
	100	10000	97.78	99.38	-1.61	2.22
	250	62500	266.82	245.73	21.09	6.73
	500	250000	486.15	494.01	-7.86	2.77
	1000	1000000	1004.44	1007.00	-2.56	0.44
AMP_20200828B_PK	10	100	9.56	12.52	-2.96	4.37
	20	400	20.71	22.14	-1.43	3.55
	50	2500	50.05	51.04	-0.99	0.10
	100	10000	99.08	99.38	-0.30	0.92
	250	62500	260.98	245.73	15.25	4.39
	500	250000	482.13	494.01	-11.88	3.57
	1000	1000000	1009.04	1007.00	2.04	0.90
AMP_20200828B_SD	10	100	9.24	12.52	-3.28	7.58
	20	400	20.45	22.14	-1.69	2.23
	50	2500	53.83	51.04	2.80	7.67
	100	10000	99.19	99.38	-0.20	0.81
	250	62500	245.62	245.73	-0.11	1.75
	500	250000	500.71	494.01	6.69	0.14
	1000	1000000	1001.58	1007.00	-5.41	0.16
AMP_20200901B_PK	10	100	9.89	12.52	-2.63	1.13
	20	400	20.48	22.14	-1.65	2.41
	50	2500	46.62	51.04	-4.41	6.75
	100	10000	104.84	99.38	5.45	4.84
	250.0	62500	259.08	245.73	13.35	3.63
	500	250000	480.41	494.01	-13.61	3.92
	1000	1000000	1010.75	1007.00	3.75	1.07
AMP_20200901B_CLR	10	100	8.83	12.52	-3.69	11.66
	20	400	21.48	22.14	-0.66	7.41
	50	2500	49.06	51.04	-1.98	1.87
	100	10000	108.14	99.38	8.76	8.14
	250	62500	253.17	245.73	7.44	1.27
	500	250000	476.79	494.01	-17.23	4.64
	1000	1000000	1016.49	1007.00	9.50	1.65

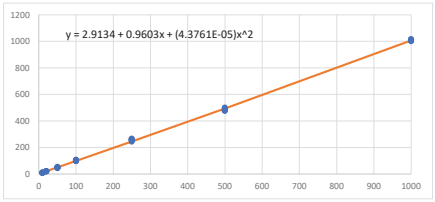
Σ|%RE| 130.99
Max %RE 11.66

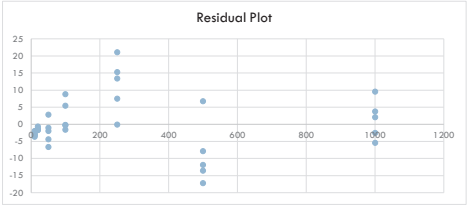
Regression Statistics	
Multiple R	0.999746586
R Square	0.999493236
Adjusted R Square	0.999461563
Standard Error	7.953397165
Observations	35

ANOVA

	df	SS	MS	F	Significance F
Regression	2	3992357.738	1996178.869	31556.88402	1.89187E-53
Residual	32	2024.208847	63.25652646		
Total	34	3994381.947			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	2.913355172	2.117251758	1.376007912	0.17837367	-1.399345531	7.226055875	-1.399345531	7.226055875
target x	0.9603227	0.015249158	62.9754582	3.98739E-35	0.929261182	0.991384218	0.929261182	0.991384218
x^2	4.37607E-05	1.50407E-05	2.909494521	0.006535296	1.31239E-05	7.43975E-05	1.31239E-05	7.43975E-05





Results: The reported %RE is for 1/x weighting.

Comments: 1/x weighting will be used. %RE for unweighted and 1/x^2 weighting are 235.48 and 131.99, respectively. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.

Acceptance Criteria: The least complex weighting scheme that minimizes Σ|%RE|
%RE Calibrators ±20% of target

Validation Study 2

Sensitivity (LOD)

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Signal to Noise Ratio			
Concentration (ng/mL)	*10*			
AMP_20200814B_SD	1812			
	1673			
	1245			
	2995			
	3785			
	2549			
	3191			
	704			
	2057			
AMP_20200828_PK	1276			
	765			
	1453			
	381			
	341			
	981			
	684			
	1223			
	1392			
AMP_20200901_PK	803			
	1326			
	206			
	1546			
	843			
	1640			
	1767			
	858			
	1745			
AMP_20200901_CLR	897			
	1369			
	2178			
	991			
	789			
	1618			
	1700			
	328			
	305			
Average Signal to Noise:	**1373**			

Established LOD: 10 ng/mL
Signal to Noise: 1373

Comments: The lowest non-zero calibrator was defined as the LOD.

Acceptance Criteria:
Signal to Noise ≥3.3
Acceptable detection and identification criteria

Validation Study 3 — SENSITIVITY (LOQ)

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LOQ
	Target Concentration (ng/mL):	*10*
AMP_202008014B_SD	1-1	10.56
	1-2	10.82
	1-3	12.49
	1-4	11.08
	1-5	12.27
	1-6	11.33
	1-7	11.17
	1-8	10.56
	1-9	18.38
Within Run	**Mean**	**12.07**
	SD	**2.46**
	%CV	**20.39%**
	% Bias	**20.74%**
AMP_20200828_PK	2-1	9.83
	2-2	10.22
	2-3	9.59
	2-4	9.88
	2-5	3.86*
	2-6	9.85
	2-7	10.30
	2-8	9.81
	2-9	10.19
Within Run	**Mean**	**9.96**
	SD	**0.25**
	%CV	**2.50%**
	% Bias	**-0.41%**
AMP 20200901B PK	3-1	9.83
	3-2	9.78
	3-3	9.96
	3-4	10.22
	3-5	10.29
	3-6	10.34
	3-7	10.37
	3-8	10.18
	3-9	9.90
Within Run	**Mean**	**10.10**
	SD	**0.23**
	%CV	**2.27%**
	% Bias	**0.96%**
AMP_20200901B_CLR	4-1	58.58*
	4-2	8.79
	4-3	9.51
	4-4	9.47
	4-5	8.05
	4-6	10.16
	4-7	15.15
	4-8	9.95
	4-9	9.26
Within Run	**Mean**	**10.04**
	SD	**2.17**
	%CV	**21.57%**
	% Bias	**0.41%**

Mean		**10.57**
SD		**1.82**
Precision	***Max Within-Run***	**21.57%**
(%CV)	***Between-Run***	**17.25%**
% Bias		**5.43%**

Comments: Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Numbers in red were > 20% target value. Numbers with * were > 20% target value due to insufficient IS. Max within-run precision is greater than 20% due to the values of 15.15 ng/mL and 18.38 ng/mL being greater than 20% of the target value in AMP_20200901B_CLR and AMP_202008014B_SD respectively. LOQ data are deemed acceptable because a minimum of three values passing acceptance criteria were obtained for each source used over the four runs as defined in the SOP.

Acceptance Criteria:
Bias: ≤20%
Within-Run Precision: CV ≤20%
Between-Run Precision: CV ≤20%

Validation Study 4

BIAS AND PRECISION

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LQC	MQC	UTAK	HQC
	Target Concentration (ng/mL):	*25*	*100*	*84.03*	*800*
AMP_202008014B_SD	1-1	25.20	94.11	66.64	NA
	1-2	23.42	89.34	72.17	NA
	1-3	24.54	90.71	67.83	NA
	1-4			78.13	
Within Run	**Mean**	**24.38**	**91.39**	**71.19**	
	SD	**0.90**	**2.46**	**5.20**	
	%CV	**3.69%**	**2.69%**	**7.30%**	
	% Bias	**-2.47%**	**-8.61%**	**-15.28%**	
AMP_202008014B_PK	2-1	25.22	82.88	104.41	889.93
	2-2	29.26	84.44	102.64	842.23
	2-3	28.41	91.83	108.70	816.73
	2-4			110.71	
Within Run	**Mean**	**27.63**	**86.39**	**106.62**	**849.63**
	SD	**2.13**	**4.78**	**3.73**	**37.15**
	%CV	**7.72%**	**5.54%**	**3.50%**	**4.37%**
	% Bias	**10.52%**	**-13.61%**	**26.88%**	**6.20%**
AMP_20200828B_PK	3-1	26.86	NA	82.66	757.03
	3-2	29.00	NA	78.04	768.73
	3-3	25.95	NA	76.71	771.34
	3-4			77.58	
Within Run	**Mean**	**27.27**		**78.75**	**765.70**
	SD	**1.57**		**2.67**	**7.62**
	%CV	**5.75%**		**3.39%**	**1.00%**
	% Bias	**9.08%**		**-6.29%**	**-4.29%**
AMP_20200828B_SD	4-1	23.30	103.46	81.53	806.91
	4-2	26.00	99.80	84.69	775.76
	4-3	25.04	101.17	74.99	815.42
	4-4			77.06	
Within Run	**Mean**	**24.78**	**101.48**	**79.57**	**799.36**
	SD	**1.37**	**1.85**	**4.37**	**20.88**
	%CV	**5.52%**	**1.82%**	**5.49%**	**2.61%**
	% Bias	**-0.88%**	**1.48%**	**-5.31%**	**-0.08%**
AMP_20200901B_PK	5-1	24.95	83.71		779.76
	5-2	25.24	91.55		766.31
	5-3	25.42	87.62		801.08
Within Run	**Mean**	**25.20**	**87.63**		**782.38**
	SD	**0.24**	**3.92**		**17.53**
	%CV	**0.94%**	**4.47%**		**2.24%**
	% Bias	**0.81%**	**-12.37%**		**-2.20%**
AMP_20200901B_CLR	6-1	27.84	118.49		758.56
	6-2	29.55	81.98		794.22
	6-3	26.01	99.24		778.26
Within Run	**Mean**	**27.80**	**99.90**		**777.01**
	SD	**1.77**	**18.26**		**17.86**
	%CV	**6.38%**	**18.28%**		**2.30%**
	% Bias	**11.20%**	**-0.10%**		**-2.87%**

Mean		**26.18**	**93.36**	**84.03**	**794.82**
SD		**1.91**	**9.80**	**14.36**	**35.89**
Precision (%CV)	***Max Within-Run***	**7.72%**	**18.28%**	**7.30%**	**4.37%**
	Between-Run	**7.29%**	**10.50%**	**17.09%**	**4.52%**
% Bias		**4.71%**	**-6.64%**	**0.00%**	**-0.65%**

Comments: N/A

Acceptance Criteria:

%Bias ≤20%
Within-Run %CV ≤20%
Between-Run %CV ≤20%

Validation Study 5

CARRYOVER

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Average LOQ Response*: 77171

Study Date:	Response		
	AMP_202008014B_SD	AMP_202008014B_PK	AMP_20200828B_PK
Concentrated Sample (2000 ng/mL)	6244867	6192835	8224432
Blank	4855	4455	2071
%LOD Response	**6.29%**	**5.77%**	**2.68%**
		Maximum Response in Blank:	**6.3%**

Results: No significant carryover observed following samples containing analyte at up to 2000 ng/mL.
Comments: N/A

Acceptance Criteria: **No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.**

Validation Study 6

MATRIX & IS INTERFERENCE

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

LOQ Response

	Analyte	IS
Run 1	65958	270023
Run 2	88777	300000
Run 3	86390	372619
Run 4	55281	242518
Run 5	89447	327379
Average	**77171**	**302508**

Matrix Interference Study Date: AMP_20200814B_PK

Matrix Source	Peak at Analyte RT	
	Peak Response	Percent of LOQ Response
4	2497	3.24%
5	3193	4.14%
7	4038	5.23%
8	2723	3.53%
9	3404	4.41%
10	2909	3.77%
11	3246	4.21%
12	2972	3.85%
13	3924	5.08%
14	4387	5.68%

Interference from Stable-Isotope Internal Standards Study Date: AMP_20200828B_SD

Experiment	Peak at Analyte RT		Peak at IS RT	
	Peak Response	Percent of LOQ Response	Peak Response	Percent of LOQ Response
Matrix with IS but no D0 (IS = 50 ng/mL)	2095	2.71%	N/A	N/A
Matrix with D0 but no IS (D0 = 2000 ng/mL)	N/A	N/A	139	0.05%

Matrix Interference: No significant interference observed.
IS Interference: No significant interference observed.
Comments: LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.

Acceptance Criteria: **Response of blank matrix is less than 20% the average response of LOQ**

Validation Study 6 — **EXOGENOUS SUBSTANCE INTERFERENCE**

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target LQC Concentration (µg/mL): 26.18
Control Acceptance: 20%
Run Date: AMP_20200828B_SD

Group	Compound	Compound Concentration (µg/mL)	Calculated LQC Concentration (ng/mL)	% Difference from Target	Comment
Benzodiazepines	Alprazolam	1	25.01	-4%	No significant interference
	α-Hydroxyalprazolam				
	Clonazepam				
	7-Aminoclonazepam				
	Diazepam				
	Nordiazepam				
	Temazepam				
	Lorazepam				
	Oxazepam				
	Zolpidem				
Phencyclidine	Phencyclidine	1	27.06	3%	No significant interference
Opioids	Morphine	2	25.61	-2%	No significant interference
	Hydrocodone	1			
	Buprenorphine	0.5			
	Norbuprenorphine				
	Codeine				
	Fentanyl				
	Norfentanyl oxalate				
	Hydromorphone				
	Methadone				
	EDDP				
	Oxycodone				
	Oxymorphone				
	Tramadol				
	o-Desmethyltramadol				
Cocaine and Metabolites	Benzoylecgonine	2	26.10	0%	No significant interference
	Cocaine	0.5			
	Cocaethylene				
Carisoprodol/Meprobamate	Carisoprodol	1	25.94	-1%	No significant interference
	Meprobamate				
Basic and Neutral Mix	Amitriptyline	5	25.77	-2%	No significant interference
	Benzylpiperizine				
	Chlorpheniramine				
	Cyclobenzaprine				
	Dextromethorphan				
	Diphenhydramine				
	Doxylamine				
	Fluoxetine				
	Imipramine				
	Ketamine				
	Norketamine				
	Meperidine				
	Nortriptyline				
	Propoxyphene				
	Sertraline				
	Trazodone				
	Venlafaxine				
	Zopiclone				
Over-the-Counter Drugs	Acetaminophen	10	24.00	-8%	No significant interference
	Caffeine				
	Ibuprofen				
	Naproxen				
	Pseudoephedrine				
	Theobromine	1			
Cannabinoids	Δ9-THC	0.5	25.93	-1%	No significant interference
	11-Hydroxy-Δ9-THC				
	Δ9-THC-COOH				
	Δ8-THC				
	Cannabinol				
	Cannabinolic acid				

Conclusions: No significant interference observed.

Comments: N/A

Acceptance Criteria: Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies

Validation Study 7

DILUTION INTEGRITY

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target Concentration (ng/mL): 359.36

Run Date	Dilution Factor	Diluted Result (ng/mL)	Calculated Result (µg/mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
AMP_202008014B_SD	2	173.96	347.92	334.63	20.02	-3.18	3.18	5.98
	2	172.19	344.39			-4.17	4.17	
	2	155.80	311.60			-13.29	13.29	
	5	68.41	342.05	326.11	19.01	-4.82	4.82	5.83
	5	66.24	331.21			-7.83	7.83	
	5	61.01	305.07			-15.11	15.11	
	10	35.64	356.45	331.55	30.01	-0.81	0.81	9.05
	10	34.00	339.96			-5.40	5.40	
	10	29.82	298.23			-17.01	17.01	
AMP_20200828B_PK	2	188.76	377.52	402.21	22.52	5.05	5.05	5.60
	2	203.75	407.50			13.40	13.40	
	2	210.81	421.62			17.33	17.33	
	5	75.64	378.21	407.81	26.47	5.24	5.24	6.49
	5	85.83	429.17			19.43	19.43	
	5	83.21	416.06			15.78	15.78	
	10	36.26	362.60	402.48	34.54	0.90	0.90	8.58
	10	42.28	422.83			17.66	17.66	
	10	42.20	422.02			17.44	17.44	
AMP_20200828B_SD	2	184.47	368.93	367.95	8.90	2.66	2.66	2.42
	2	188.16	376.32			4.72	4.72	
	2	179.30	358.60			-0.21	0.21	
	5	75.14	375.68	374.16	14.25	4.54	4.54	3.81
	5	77.52	387.60			7.86	7.86	
	5	71.84	359.21			-0.04	0.04	
	10	32.23	322.25	352.71	26.49	-10.33	10.33	7.51
	10	36.55	365.47			1.70	1.70	
	10	37.04	370.40			3.07	3.07	
AMP_202000901B_PK	2	197.77	395.53	409.19	12.16	10.07	10.07	2.97
	2	209.41	418.83			16.55	16.55	
	2	206.61	413.22			14.99	14.99	
	5	74.36	371.82	402.81	27.22	3.47	3.47	6.76
	5	82.75	413.77			15.14	15.14	
	5	84.57	422.83			17.66	17.66	
	10	40.81	408.11	394.19	25.89	13.57	13.57	6.57
	10	36.43	364.32			1.38	1.38	
	10	41.02	410.15			14.13	14.13	
AMP_202000901B_CLR	2	155.15	310.30	354.05	40.75	-13.65	13.65	11.51
	2	195.47	390.93			8.79	8.79	
	2	180.46	360.91			0.43	0.43	
	5	76.84	384.22	395.01	11.25	6.92	6.92	2.85
	5	78.83	394.15			9.68	9.68	
	5	81.33	406.67			13.17	13.17	
	10	37.90	379.04	367.68	9.91	5.48	5.48	2.70
	10	36.08	360.79			0.40	0.40	
	10	36.32	363.22			1.07	1.07	

Dilution Factor	2
Avg %Bias	8.57
Max Within-Run Precision (%CV)	11.51

Dilution Factor	5
Avg %Bias	9.78
Max Within-Run Precision (%CV)	6.76

Dilution Factor	10
Avg %Bias	7.36
Max Within-Run Precision (%CV)	9.05

Results: It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.
Comments: N/A

Acceptance Criteria: Avg %Bias must be less than 20%

Validation Study 8

PROCESSED SAMPLE STABILITY

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Sample	Time Zero Response	24H Response	48H Response	72H Response	Acceptable Range		Pass/Unstable
						Low	High	
	LQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			
	HQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			

Results:

Comments: Study not performed. Sample preparation will be completed once started without prolonged interruptions.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 8
Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

AUTOSAMPLER STABILITY
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Stability of Punctured Calibrators

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	Calibrator 1	9.14	0.28	8.97	8.61	0.28	-1%
	Calibrator 2	19.15	0.51	19.28	18.83	0.51	1%
	Calibrator 3	55.63	1.33	55.12	54.28	1.32	-1%
	Calibrator 4	104.71	2.39	105.17	103.60	2.40	0%
	Calibrator 5	248.82	5.25	247.96	242.65	5.24	0%
	Calibrator 6	477.88	8.99	474.66	456.28	8.94	-1%
	Calibrator 7	1037.02	13.93	1084.70	893.14	14.08	1%

Stability of Punctured Calibrators

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)	Concentration (Time Zero Curve)	Concentration (48 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20201019B_SD & AMP_20201021B_SD	Calibrator 1	9.86	0.30	N/A	N/A	N/A	N/A	9.79	8.80	0.30	-1%
	Calibrator 2	19.62	0.52	N/A	N/A	N/A	N/A	19.69	18.44	0.52	0%
	Calibrator 3	49.51	1.18	N/A	N/A	N/A	N/A	48.85	46.86	1.17	-1%
	Calibrator 4	105.60	2.38	N/A	N/A	N/A	N/A	106.44	102.97	2.40	1%
	Calibrator 5	252.11	5.24	N/A	N/A	N/A	N/A	257.46	250.01	5.34	2%
	Calibrator 6	483.64	8.96	N/A	N/A	N/A	N/A	499.49	485.29	9.17	2%
	Calibrator 7	1022.04	13.77	N/A	N/A	N/A	N/A	N/A*	N/A*	14.55	6%

Stability of Punctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	LQC	25.51	0.66	25.69	25.17	0.66	1%
		25.57	0.66	25.18	24.67	0.65	-1%
		25.48	0.65	25.74	25.23	0.66	1%
	HQC	771.64	12.32	781.48	717.47	12.41	1%
		771.52	12.32	791.01	724.69	12.49	1%
		784.15	12.43	789.84	723.81	12.48	0%

Stability of Punctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)	Concentration (Time Zero Curve)	Concentration (48 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20201019B_SD & AMP_20201021B_SD	LQC	25.53	0.65	N/A	N/A	N/A	N/A	25.43	24.04	0.65	0%
		25.38	0.65	N/A	N/A	N/A	N/A	25.55	24.16	0.65	1%
		25.48	0.65	N/A	N/A	N/A	N/A	25.59	24.20	0.65	0%
	HQC	771.25	12.19	N/A	N/A	N/A	N/A	818.65	793.63	12.58	3%
		773.44	12.21	N/A	N/A	N/A	N/A	815.76	790.86	12.56	3%
		760.24	12.10	N/A	N/A	N/A	N/A	817.24	792.28	12.57	4%

Stability of Unpunctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	LQC	25.51	0.66	25.71	25.20	0.66	1%
		25.57	0.66	25.47	24.95	0.65	0%
		25.48	0.65	25.59	25.07	0.66	0%
	HQC	771.64	12.32	794.41	727.24	12.51	2%
		771.52	12.32	772.44	710.56	12.33	0%
		784.15	12.43	772.80	710.84	12.33	-1%

Stability of Unpunctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)	Concentration (Time Zero Curve)	Concentration (48 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20201019B_SD & AMP_20201021B_SD	LQC	25.53	0.65	N/A	N/A	N/A	N/A	25.42	24.03	0.65	0%
		25.38	0.65	N/A	N/A	N/A	N/A	25.53	24.14	0.65	1%
		25.48	0.65	N/A	N/A	N/A	N/A	25.56	24.17	0.65	0%
	HQC	771.25	12.19	N/A	N/A	N/A	N/A	825.83	800.51	12.63	4%
		773.44	12.21	N/A	N/A	N/A	N/A	834.36	808.68	12.70	4%
		760.24	12.10	N/A	N/A	N/A	N/A	819.64	794.58	12.59	4%

Results: Punctured and unpunctured samples were shown to be stable for 48 hours.

Comments: Cal 7 relative response was beyond the range of the calibration curve and therefore was not quantified. The samples were deemed stable at 48 hrs since response ratio of analyte to IS was within 20% compared to time 0. AMP_20200904B_SD showed the punctured and unpunctured samples at 72 hr were not stable. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 9 **MATRIX EFFECTS**

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Neat Response at Analyte RT

	LQC	HQC
1	186790	3120037
2	175901	3140257
3	183088	3149629
4	186966	3139183
5	173831	3023923
6	174046	3166044
Mean	**180104**	**3123179**
SD	6236	50885
%CV	3%	2%

Neat Response at IS RT

	LQC	HQC
	280576	300386
	257202	301385
	303981	307923
	293909	297130
	265101	311246
	272994	280962
Mean	**278961**	**299839**
SD	17625	10600
%CV	6%	4%

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at Analyte RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	168063	-7%	2829211	-9%
5	147048	-18%	3000414	-4%
7	146034	-19%	2944539	-6%
8	163598	-9%	2804180	-10%
9	144713	-20%	2851479	-9%
10	159546	-11%	2600611	-17%
11	167479	-7%	2891832	-7%
12	183269	2%	2897534	-7%
13	151469	-16%	2836859	-9%
14	167610	-7%	3093322	-1%
Mean	159883		2874998	
SD	12484		130567	
%CV	8%		5%	
Avgerage % suppression/enhancement	-11%		-8%	

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at IS RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	284611	2%	274368	-8%
5	256822	-8%	302497	1%
7	256976	-8%	298344	0%
8	279249	0%	276282	-8%
9	248414	-11%	285105	-5%
10	273596	-2%	251192	-16%
11	280138	0%	288037	-4%
12	296923	6%	297712	-1%
13	256768	-8%	278564	-7%
14	286837	3%	313916	5%
Mean	272033		286602	
SD	16214		17761	
%CV	6%		6%	
Avgerage % suppression/enhancement	-2%		-4%	

Results: Average percent suppression values for LQC and HQC were -11% and -8%, respectively.

Comments: N/A

Acceptance Criteria: **Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%**

SUMMARY OF VALIDATION PERFORMANCE

Analyte: Methamphetamine
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

Date	Reason
N/A	N/A

Deviations from SOP: N/A

Other Observations:
Working Standards Verified in Validation:
Calibrators: 200805C-C-10, 200805C-C-2.5, 200805C-C-0.2, 200923C-C-10, 200923C-C-2.5, 200923C-C-0.2
Controls:200807L-Q-Mix, 200813L-Q-0.5, 200813L-St-5
Internal Standard: 200805C-IS-1, 200903C-IS-1, 200915C-IS-1

Sample Preparation Steps: Refer to Toxicology Analytical Manual v3.5, "Amphetamines Confirmation by Liquid Chromatography-Tandem Mass Spectrometry" section titled "Extraction Procedure".

Location of Raw Data: Toxicology section shared electronic storage.

Recommended Maximum Run Length (Unknown Samples): 30

Conclusion: **This method is fit for use on casework for methamphetamine confirmation analysis in blood.**

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: MDA
Units: ng/mL
Method: AMP.M
Instrument: LCMS-1
SOP Reference: Toxicology Analytical Manual v3.5

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes Σ|%RE|	Unweighted: = 560.76 1/x Weighting: = 332.63 1/x2 Weighting: = 329.77	Data were processed using 1/x2 weighting.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 1.0477 - 1.0937 95% CI of Intercept = -11.0805 - -0.3719	The linearity of the method is acceptable because the individual calibration curves on all five days met the acceptance criteria. Also, other analytical data including the ion ratios and quantification values of the controls and calibrators met acceptance criteria.
1	Validation Calibration	%RE Calibrators ±20% of target	Max %RE = 15.38	N/A
1	Case Work Calibration	%RE Calibrators ±20% of target	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
1	Comparison of Validation Calibration to Casework Calibration	95% CI of slope includes 1 95% CI of intercept includes 0	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
2	Limit of Detection (LOD)	Signal to Noise ≥3.3 Acceptable detection and identification criteria	LOD = 10 ng/mL S:N= 2622	Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Blood source 5 had an interference with the 77.1 m/z qualifier ion. This ion was removed from the acquistion method on 9/10/2020.
3	Limit of Quantitation (LOQ)	Bias: ≤20% Within-Run Precision: CV ≤20% Between-Run Precision: CV ≤20%	Bias = 4.62% Within-Run Precision= 10.05% Between-Run Precision = 5.64%	Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Blood source 5 had an interference with the 77.1 m/z qualifier ion. This ion was removed from the acquistion method on 9/10/2020. Number with * was > 20% target value due to insufficient IS. Number in red was > 20% target value.
4	Bias & Precision	%Bias ≤20% Within-Run %CV ≤20% Between-Run %CV ≤20%	Max Bias = -10.09% Max Within-Run Precision = 18.75% Max Between-Run Precision = 16.27%	Numbers in red were not within 20% of the target value.
5	Carryover	No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.	No significant carryover observed following samples containing analyte at up to 2000 ng/mL.	N/A
6	Matrix Interference	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.
6	Interference from stable isotope internal standard	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	
6	Exogenous Substances Interferences	Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies	No significant interference observed.	N/A
7	Dilution Integrity	Average %Bias must be less than 20%	It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.	N/A
8	Processed Sample Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	N/A	Study not performed. Sample preparation will be completed once started without prolonged interruptions.
8	Autosampler Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	Punctured and unpunctured samples were shown to be stable for 48 hours.	AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for MDA were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.
9	Matrix Effects	Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%	Average percent suppression values for LQC and HQC were -8% and -4%, respectively.	N/A
10	Additional Experiment	%RE Calibrators ±20% of target Bias: ≤20% Within-Run Precision: CV ≤20%	All acceptance criteria were met.	The purpose of this experiment was to verify the change in qualifier ions from 105.1 m/z and 77.1 m/z to 133.1 m/z and 79.1 m/z. Blank blood sources 4 (lot 310355), 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study.

Validation Study 1 **STANDARD CURVE WEIGHT VERIFICATION**

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Σ|%RE|
Unweighted:	560.76
1/x Weighting:	332.63
1/x² Weighting:	329.77

	C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
AMP_20200814B_SD	10	0.24036	1	2.40355	10	0.240355	100	0.057771	14.89155	48.9155	48.9155
	20	0.47392	1	9.4784	20	0.47392	400	0.2246	24.5532	22.76602	22.76602
	50	1.22437	1	61.21835	50	1.224367	2500	1.499075	55.59621	11.19242	11.19242
	100	2.58049	1	258.0485	100	2.580485	10000	6.658903	111.6934	11.69341	11.69341
	250	6.06747	1	1516.868	250	6.067473	62500	36.81423	255.9362	2.374489	2.374489
	500	13.39617	1	6698.085	500	13.39617	250000	179.4574	559.0952	11.81905	11.81905
AMP_20200814B_PK	10	0.21536	1	2.153614	10	0.21536141	100	0.046381	13.85767	38.57665	38.57665
	20	0.39552	1	7.910335	20	0.39551676	400	0.156434	21.30997	6.549872	6.549872
	50	0.80632	1	40.31583	50	0.80631669	2500	0.650147	38.30313	-23.3937	23.39374
	100	1.72844	1	172.8443	100	1.72844332	10000	2.987516	76.44784	-23.5522	23.55216
	250	5.00909	1	1252.272	250	5.00908786	62500	25.09096	212.155	-15.138	15.13798
	500	10.34035	1	5170.173	500	10.3403466	250000	106.9228	432.688	-13.4624	13.4624
AMP_20200828B_SD	10	0.25424	1	2.54236	10	0.254236	100	0.064636	15.46575	54.65752	54.65752
	20	0.48059	1	9.6118	20	0.48059	400	0.230967	24.82912	24.14558	24.14558
	50	1.22095	1	61.04725	50	1.220945	2500	1.490707	55.45465	10.90931	10.90931
	100	2.27145	1	227.1448	100	2.271448	10000	5.159476	98.90978	-1.09022	1.090219
	250	5.79295	1	1448.238	250	5.79295	62500	33.55827	244.5803	-2.16788	2.167881
	500	13.02508	1	6512.54	500	13.02508	250000	169.6527	543.7447	8.748944	8.748944
AMP_20200828B_PK	10	0.21531	1	2.153097	10	0.21530971	100	0.046358	13.85553	38.55526	38.55526
	20	0.41191	1	8.238193	20	0.41190967	400	0.16967	21.98808	9.940421	9.940421
	50	0.92830	1	46.41484	50	0.92829689	2500	0.861735	43.34897	-13.3021	13.30207
	100	1.82790	1	182.7904	100	1.82790406	10000	3.341233	80.56213	-19.4379	19.43787
	250	5.16230	1	1290.575	250	5.16229927	62500	26.64933	218.4928	-12.6029	12.60288
	500	10.53041	1	5265.204	500	10.5304083	250000	110.8895	440.5501	-11.89	11.88998
AMP_20200901B_CLR	10	0.24454	1	2.445443	10	0.2445443	100	0.059802	15.06485	50.64845	50.64845
	20	0.51138	1	10.22766	20	0.51138323	400	0.261513	26.10291	30.51455	30.51455
	50	1.19108	1	59.55381	50	1.19107613	2500	1.418662	54.2191	8.438197	8.438197
	100	2.70390	1	270.3896	100	2.70389566	10000	7.311052	116.7984	16.79842	16.79842
	250	6.35689	1	1589.222	250	6.35688939	62500	40.41004	267.9082	7.16329	7.16329
	500	13.21491	1	6607.454	500	13.2149071	250000	174.6338	551.5971	10.31942	10.31942
Sum	4650.0	108.8219	5	38787.56	4650	108.821915	1627500	936.7756			560.764
Slope	0.02417443										
Intercept	-0.11963977										
R^2	0.97762560										

Comments: Data were processed using $1/x^2$ weighting.

Acceptance Criteria: **The least complex weighting scheme that minimizes Σ|%RE|**

Validation Study 1

STANDARD CURVE WEIGHT VERIFICATION

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
	10	0.24036	0.1	0.240355	1	0.0240355	10	0.005777	11.25622	12.56217	12.56217
	20	0.47392	0.05	0.47392	1	0.023696	20	0.01123	21.16856	5.842787	5.842787
	50	1.22437	0.02	1.224367	1	0.02448734	50	0.029981	53.01702	6.034043	6.034043
	100	2.58049	0.01	2.580485	1	0.02580485	100	0.066589	110.5698	10.56975	10.56975
	250	6.06747	0.004	6.067473	1	0.02426989	250	0.147257	258.5552	3.422068	3.422068
	500	13.39617	0.002	13.39617	1	0.02679234	500	0.358915	569.5801	13.91602	13.91602
	10	0.21536	0.1	0.215361	1	0.02153614	10	0.004638	10.19551	1.955055	1.955055
	20	0.39552	0.05	0.395517	1	0.01977584	20	0.007822	17.84118	-10.7941	10.79412
	50	0.80632	0.02	0.806317	1	0.01612633	50	0.013003	35.27525	-29.4495	29.4495
	100	1.72844	0.01	1.728443	1	0.01728443	100	0.029875	74.40968	-25.5903	25.59032
	250	5.00909	0.004	5.009088	1	0.02003635	250	0.100364	213.638	-14.5448	14.54479
	500	10.34035	0.002	10.34035	1	0.02068069	500	0.213846	439.893	-12.0214	12.02139
	10	0.25424	0.1	0.254236	1	0.0254236	10	0.006464	11.84532	18.45317	18.45317
	20	0.48059	0.05	0.48059	1	0.0240295	20	0.011548	21.45163	7.258139	7.258139
	50	1.22095	0.02	1.220945	1	0.0244189	50	0.029814	52.87179	5.743588	5.743588
	100	2.27145	0.01	2.271448	1	0.02271448	100	0.051595	97.45443	-2.54557	2.545568
	250	5.79295	0.004	5.79295	1	0.0231718	250	0.134233	246.9046	-1.23816	1.23816
	500	13.02508	0.002	13.02508	1	0.02605016	500	0.339305	553.8313	10.76626	10.76626
	10	0.21531	0.1	0.21531	1	0.02153097	10	0.004636	10.19331	1.933113	1.933113
	20	0.41191	0.05	0.41191	1	0.02059548	20	0.008483	18.53688	-7.31559	7.315595
	50	0.92830	0.02	0.928297	1	0.01856594	50	0.017235	40.45201	-19.096	19.09599
	100	1.82790	0.01	1.827904	1	0.01827904	100	0.033412	78.63073	-21.3693	21.36927
	250	5.16230	0.004	5.162299	1	0.0206492	250	0.106597	220.1402	-11.9439	11.94392
	500	10.53041	0.002	10.53041	1	0.02106082	500	0.221779	447.9591	-10.4082	10.40817
	10	0.24454	0.1	0.244544	1	0.02445443	10	0.00598	11.43401	14.34008	14.34008
	20	0.51138	0.05	0.511383	1	0.02556916	20	0.013076	22.75847	13.79236	13.79236
	50	1.19108	0.02	1.191076	1	0.02382152	50	0.028373	51.60418	3.208359	3.208359
	100	2.70390	0.01	2.703896	1	0.02703896	100	0.073111	115.8072	15.80722	15.80722
	250	6.35689	0.004	6.356889	1	0.02542756	250	0.16164	270.8378	8.335123	8.335123
	500	13.21491	0.002	13.21491	1	0.02642981	500	0.349268	561.8874	12.37749	12.37749
Sum	4650	108.82192	0.93	108.8219	30	0.68375704	4650	2.585846			332.6336
Slope	0.02356305										
Intercept	-0.02487581										
R^2	0.99067280										

	C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
	10	0.24036	0.01	0.0240355	0.1	0.002404	1	0.000578	10.43041	4.304138	4.3041378
	20	0.47392	0.0025	0.023696	0.05	0.001185	1	0.000562	20.73251	3.662564	3.662563626
	50	1.22437	0.0004	0.02448734	0.02	0.00049	1	0.0006	53.83327	7.666547	7.666547487
	100	2.58049	0.0001	0.02580485	0.01	0.000258	1	0.000666	113.649	13.64901	13.64900734
	250	6.06747	0.000016	0.02426989	0.004	9.71E-05	1	0.000589	267.4533	6.981313	6.981312776
	500	13.39617	0.000004	0.02679234	0.002	5.36E-05	1	0.000718	590.7079	18.14158	18.14157723
	10	0.21536	0.01	0.02153614	0.1	0.002154	1	0.000464	9.327995	-6.72005	6.720049351
	20	0.39552	0.0025	0.01977584	0.05	0.000989	1	0.000391	17.2743	-13.6285	13.62851111
	50	0.80632	0.0004	0.01612633	0.02	0.000323	1	0.00026	35.39389	-29.2122	29.21222864
	100	1.72844	0.0001	0.01728443	0.01	0.000173	1	0.000299	76.0671	-23.9329	23.93289776
	250	5.00909	0.000016	0.02003635	0.004	8.01E-05	1	0.000401	220.77	-11.692	11.69201308
	500	10.34035	0.000004	0.02068069	0.002	4.14E-05	1	0.000428	455.9214	-8.81571	8.815710675
	10	0.25424	0.01	0.0254236	0.1	0.002542	1	0.000646	11.04268	10.42678	10.42677756
	20	0.48059	0.0025	0.0240295	0.05	0.001201	1	0.000577	21.02671	5.133567	5.133567415
	50	1.22095	0.0004	0.0244189	0.02	0.000488	1	0.000596	53.68234	7.364672	7.364671927
	100	2.27145	0.0001	0.02271448	0.01	0.000227	1	0.000516	100.018	0.017985	0.017984919
	250	5.79295	0.000016	0.0231718	0.004	9.27E-05	1	0.000537	255.3446	2.137842	2.137842036
	500	13.02508	0.000004	0.02605016	0.002	5.21E-05	1	0.000679	574.3398	14.86797	14.86796526
	10	0.21531	0.01	0.02153097	0.1	0.002153	1	0.000464	9.325715	-6.74285	6.742854411
	20	0.41191	0.0025	0.02059548	0.05	0.00103	1	0.000424	17.99736	-10.0132	10.01321251
	50	0.92830	0.0004	0.01856594	0.02	0.000371	1	0.000345	40.7742	-18.4516	18.45160837
	100	1.82790	0.0001	0.01827904	0.01	0.000183	1	0.000334	80.45412	-19.5459	19.5458776
	250	5.16230	0.000016	0.0206492	0.004	8.26E-05	1	0.000426	227.5278	-8.98887	8.988869882
	500	10.53041	0.000004	0.02106082	0.002	4.21E-05	1	0.000444	464.3047	-7.13906	7.13906059
	10	0.24454	0.01	0.02445443	0.1	0.002445	1	0.000598	10.6152	6.151957	6.151956886
	20	0.51138	0.0025	0.02556916	0.05	0.001278	1	0.000654	22.38494	11.92472	11.92471581
	50	1.19108	0.0004	0.02382152	0.02	0.000476	1	0.000567	52.36488	4.729756	4.72975621
	100	2.70390	0.0001	0.02703896	0.01	0.00027	1	0.000731	119.0924	19.09241	19.09241227
	250	6.35689	0.000016	0.02542756	0.004	0.000102	1	0.000647	280.2189	12.08755	12.08755086
	500	13.21491	0.000004	0.02642981	0.002	5.29E-05	1	0.000699	582.7127	16.54255	16.54254657
	4650	108.82192	0.0651	0.68375704	0.93	0.021337	30	0.015838			329.765788
Slope	0.02267159										
Intercept	0.00388090										
R^2	0.98557642										

Validation Study 1

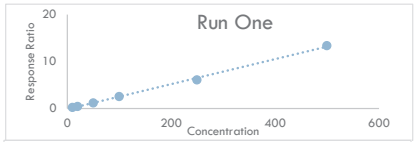
VALIDATION CURVE CALIBRATION

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

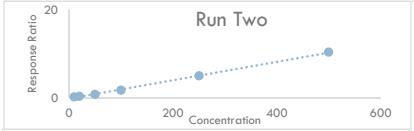
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
AMP_20200814B_SD	10	10.19	1.85	0.240
	20	19.37	3.14	0.474
	50	48.89	2.22	1.224
	100	102.23	2.23	2.580
	250	239.38	4.25	6.067
	500	527.64	5.53	13.396
AMP_20200814B_PK	10	10.31	3.13	0.215
	20	20.07	0.34	0.396
	50	42.31	15.38	0.806
	100	92.24	7.76	1.728
	250	269.88	7.95	5.009
	500	558.56	11.71	10.340
AMP_20200828B_PK	10	10.14	1.38	0.215
	20	20.17	0.85	0.412
	50	46.52	6.96	0.928
	100	92.42	7.58	1.828
	250	262.56	5.02	5.162
	500	536.46	7.29	10.530
AMP_20200828B_SD	10	10.13	1.35	0.254
	20	19.59	2.06	0.481
	50	50.51	1.02	1.221
	100	94.39	5.61	2.271
	250	241.47	3.41	5.793
	500	543.54	8.71	13.025
AMP_20200901B_CLR	10	10.02	0.16	0.2445
	20	20.31	1.54	0.5114
	50	46.53	6.95	1.1911
	100	104.88	4.88	2.7039
	250	245.78	1.69	6.3569
	500	510.31	2.06	13.2149

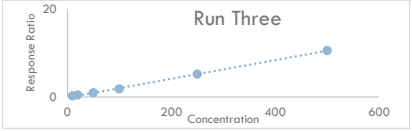
Max %RE = 15.38



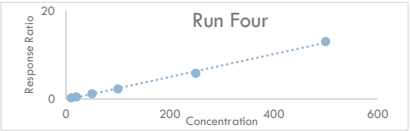
Slope	0.02663
Intercept	-0.13003
R^2	0.99893



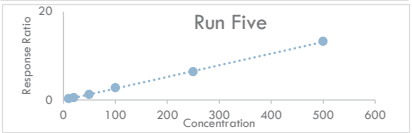
Slope	0.02084
Intercept	-0.14735
R^2	0.99938



Slope	0.02117
Intercept	-0.10148
R^2	0.99964



Slope	0.02586
Intercept	-0.16691
R^2	0.99836



Slope	0.02638
Intercept	-0.05243
R^2	0.99975

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

LINEARITY

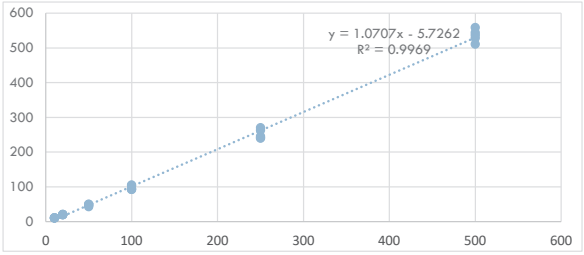
Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

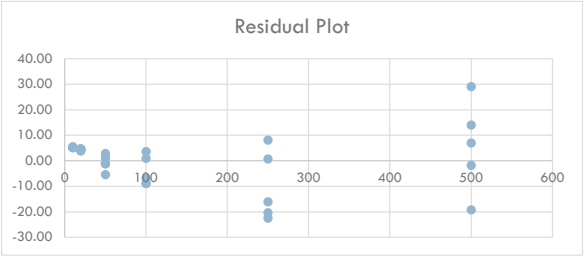
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Study Date	Target (x)	Calculated (y)	Predicted	Residual
AMP_20200814B_SD	10	10.19	4.98	5.20
	20	19.37	15.69	3.68
	50	48.89	47.81	1.08
	100	102.23	101.34	0.89
	250	239.38	261.95	-22.57
	500	527.64	529.62	-1.98
AMP_20200814B_PK	10	10.31	4.98	5.33
	20	20.07	15.69	4.38
	50	42.31	47.81	-5.50
	100	92.24	101.34	-9.10
	250	269.88	261.95	7.93
	500	558.56	529.62	28.94
AMP_20200828B_PK	10	10.14	4.98	5.16
	20	20.17	15.69	4.48
	50	46.52	47.81	-1.29
	100	92.42	101.34	-8.92
	250	262.56	261.95	0.61
	500	536.46	529.62	6.84
AMP_20200828B_SD	10	10.13	4.98	5.15
	20	19.59	15.69	3.90
	50	50.51	47.81	2.70
	100	94.39	101.34	-6.96
	250	241.47	261.95	-20.48
	500	543.54	529.62	13.92
AMP_20200901B_CLR	10	10.02	4.98	5.03
	20	20.31	15.69	4.62
	50	46.53	47.81	-1.28
	100	104.88	101.34	3.53
	250	245.78	261.95	-16.17
	500	510.31	529.62	-19.32

Slope	1.0707
Std err in slope, S_b	0.0112
Degrees freedom	28
Confidence level	95%
Student t	2.0484
Confidence interval	0.023
Slope	1.0707 ± 0.023
Range	1.0477 - 1.0937
	NO

Intercept	-5.7262
Std err in Intercept	2.6139
Degrees freedom	28
Confidence Level	95%
Student t	2.0484
Confidence interval	5.354
Intercept	-5.7262 ± 5.3543
Lower	-11.0805 - -0.3719
	NO





Comments: The linearity of the method is acceptable because the individual calibration curves on all five days met the acceptance criteria. Also, other analytical data including the ion ratios and quantification values of the controls and calibrators met acceptance criteria.

Acceptance Criteria:

95% CI of slope includes 1
95% CI of intercept includes 0

Validation Study 2

Sensitivity (LOD)

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Signal to Noise Ratio			
Concentration (ng/mL)	*10*			
AMP_20200814B_SD	NA			
	1619			
	2831			
	1029			
	5118			
	2699			
	4250			
	6302			
	894			
AMP_20200828B_PK	2547			
	NA			
	1988			
	3799			
	1398			
	1719			
	4532			
	5331			
	2724			
AMP_20200901B_CLR	NA			
	1103			
	3718			
	1141			
	2061			
	1917			
	2087			
	3133			
	3765			
AMP_20200901B_PK	797			
	NA			
	1541			
	1455			
	2836			
	3285			
	3781			
	838			
	1682			
Average Signal to Noise:	**2622**			

Established LOD: 10 ng/mL
Signal to Noise: 2622

Comments: Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Blood source 5 had an interference with the 77.1 m/z qualifier ion. This ion was removed from the acquistion method on 9/10/2020.

Acceptance Criteria: Signal to Noise ≥3.3
Acceptable detection and identification criteria

Validation Study 3

SENSITIVITY (LOQ)

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LOQ
	Target Concentration (ng/mL):	*10*
AMP_20200814B_SD	1-1	NA
	1-2	10.85
	1-3	10.22
	1-4	10.55
	1-5	10.86
	1-6	11.44
	1-7	10.53
	1-8	10.64
	1-9	10.47
Within Run	**Mean**	**10.69**
	SD	**0.36**
	%CV	**3.41%**
	% Bias	**6.94%**
	2-1	NA
AMP_20200828B_PK	2-2	10.56
	2-3	10.16
	2-4	10.35
	2-5	5.00*
	2-6	10.29
	2-7	10.63
	2-8	10.39
	2-9	10.52
Within Run	**Mean**	**10.41**
	SD	**0.17**
	%CV	**1.60%**
	% Bias	**4.13%**
	3-1	NA
AMP_20200901B_CLR	3-2	10.42
	3-3	9.82
	3-4	10.11
	3-5	9.36
	3-6	10.53
	3-7	12.89
	3-8	10.35
	3-9	10.18
Within Run	**Mean**	**10.46**
	SD	**1.05**
	%CV	**10.05%**
	% Bias	**4.57%**
	4-1	NA
AMP_20200901B_PK	4-2	9.88
	4-3	9.91
	4-4	10.34
	4-5	11.05
	4-6	10.34
	4-7	10.38
	4-8	10.25
	4-9	10.12
Within Run	**Mean**	**10.28**
	SD	**0.36**
	%CV	**3.54%**
	% Bias	**2.85%**

Mean		**10.46**
SD		**0.59**
Precision	***Max Within-Run***	**10.05%**
(%CV)	***Between-Run***	**5.64%**
% Bias		**4.62%**

Comments: Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Blood source 5 had an interference with the 77.1 m/z qualifier ion. This ion was removed from the acquistion method on 9/10/2020. Number with * was > 20% target value due to insufficient IS. Number in red was > 20% target value.

Acceptance Criteria:
Bias: ≤20%
Within-Run Precision: CV ≤20%
Between-Run Precision: CV ≤20%

Validation Study 4

BIAS AND PRECISION

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LQC	MQC	UTAK	HQC
	Target Concentration (ng/mL):	*25*	*100*	*78.71*	*400*
AMP_20200814B_SD	1-1	25.02	94.30	66.97	402.83
	1-2	23.79	89.77	71.87	404.40
	1-3	24.61	93.02	68.31	394.66
	1-4			72.49	
Within Run	Mean	24.47	92.36	69.91	400.63
	SD	0.63	2.33	2.69	5.23
	%CV	2.56%	2.53%	3.85%	1.31%
	% Bias	-2.10%	-7.64%	-11.18%	0.16%
AMP_20200814B_PK	2-1	22.89	76.35	94.69	457.18
	2-2	27.26	79.08	94.61	504.72
	2-3	26.06	85.16	101.84	470.01
	2-4			105.71	
Within Run	Mean	25.40	80.19	99.21	477.30
	SD	2.26	4.51	5.50	24.60
	%CV	8.90%	5.62%	5.54%	5.15%
	% Bias	1.62%	-19.81%	26.05%	19.33%
AMP_20200828B_PK	3-1	25.39	89.73	73.81	420.92
	3-2	27.46	97.57	72.36	400.50
	3-3	24.56	86.10	71.12	398.53
	3-4			70.42	
Within Run	Mean	25.80	91.13	71.93	406.65
	SD	1.49	5.86	1.49	12.39
	%CV	5.79%	6.43%	2.07%	3.05%
	% Bias	3.21%	-8.87%	-8.61%	1.66%
AMP_20200828B_SD	4-1	23.08	98.05	75.43	390.83
	4-2	24.14	92.95	79.57	386.11
	4-3	24.20	95.35	68.89	374.02
	4-4			71.21	
Within Run	Mean	23.81	95.45	73.77	383.65
	SD	0.63	2.55	4.72	8.67
	%CV	2.65%	2.67%	6.40%	2.26%
	% Bias	-4.77%	-4.55%	-6.27%	-4.09%
AMP_20200901B_CLR	5-1	28.14	114.14		371.26
	5-2	29.60	78.77		394.29
	5-3	25.39	92.47		390.31
Within Run	Mean	27.71	95.13		385.29
	SD	2.14	17.84		12.31
	%CV	7.71%	18.75%		3.19%
	% Bias	10.84%	-4.87%		-3.68%
AMP_20200901B_PK	6-1	24.17	80.84		404.33
	6-2	24.74	89.34		388.53
	6-3	23.89	85.38		380.12
Within Run	Mean	24.27	85.19		390.99
	SD	0.43	4.26		12.29
	%CV	1.79%	5.00%		3.14%
	% Bias	-2.92%	-14.81%		-2.25%

Mean		25.24	89.91	78.71	407.42
SD		1.81	8.92	12.80	35.22
Precision (%CV)	*Max Within-Run*	8.90%	18.75%	6.40%	5.15%
	Between-Run	7.18%	9.92%	16.27%	8.65%
% Bias		0.98%	-10.09%	0.00%	1.85%

Comments: Numbers in red were not within 20% of the target value.

Acceptance Criteria:

%Bias ≤20%
Within-Run %CV ≤20%
Between-Run %CV ≤20%

Validation Study 5

CARRYOVER

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Average LOQ Response*: 202648

Study Date:	Response		
	AMP_20200814B_SD	AMP_20200814B_PK	AMP_20200828B_PK
Concentrated Sample (2000 ng/mL)	14714024	13840992	16918292
Blank	3987	3572	4366
%LOD Response	**1.97%**	**1.76%**	**2.15%**
		Maximum Response in Blank:	**2.2%**

Results: No significant carryover observed following samples containing analyte at up to 2000 ng/mL.
Comments: N/A

Acceptance Criteria: **No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.**

Validation Study 6

MATRIX & IS INTERFERENCE

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

LOQ Response

	Analyte	IS
Run 1	222811	927008
Run 2	146270	679186
Run 3	246190	1000000
Run 4	211291	981337
Run 5	186675	763357
Average	**202648**	**870178**

Matrix Interference Study Date: AMP_20200814B_PK

Matrix Source	Peak at Analyte RT	
	Peak Response	Percent of LOQ Response
4	551	0.27%
5	774	0.38%
7	848	0.42%
8	1167	0.58%
9	1189	0.59%
10	981	0.48%
11	1029	0.51%
12	500	0.25%
13	651	0.32%
14	1334	0.66%

Interference from Stable-Isotope Internal Standards Study Date: AMP_20200828B_SD

Experiment	Peak at Analyte RT		Peak at IS RT	
	Peak Response	Percent of LOQ Response	Peak Response	Percent of LOQ Response
Matrix with IS but no D0 (IS = 50 ng/mL)	5476	2.70%	N/A	N/A
Matrix with D0 but no IS (D0 = 2000 ng/mL)	N/A	N/A	352	0.04%

Matrix Interference: No significant interference observed.
IS Interference: No significant interference observed.
Comments: LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.

Acceptance Criteria: **Response of blank matrix is less than 20% the average response of LOQ**

Validation Study 6 — **EXOGENOUS SUBSTANCE INTERFERENCE**

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target LQC Concentration (µg/mL): 25.24
Control Acceptance: 20%
Run Date: AMP_20200828B_SD

Group	Compound	Compound Concentration (µg/mL)	Calculated LQC Concentration (ng/mL)	% Difference from Target	Comment
Benzodiazepines	Alprazolam	1	24.66	-2%	No significant interference
	α-Hydroxyalprazolam				
	Clonazepam				
	7-Aminoclonazepam				
	Diazepam				
	Nordiazepam				
	Temazepam				
	Lorazepam				
	Oxazepam				
	Zolpidem				
Phencyclidine	Phencyclidine	1	25.75	2%	No significant interference
Opioids	Morphine	2	25.63	2%	No significant interference
	Hydrocodone	1			
	Buprenorphine	0.5			
	Norbuprenorphine				
	Codeine				
	Fentanyl				
	Norfentanyl oxalate				
	Hydromorphone				
	Methadone				
	EDDP				
	Oxycodone				
	Oxymorphone				
	Tramadol				
	o-Desmethyltramadol				
Cocaine and Metabolites	Benzoylecgonine	2	24.61	-3%	No significant interference
	Cocaine	0.5			
	Cocaethylene				
Carisoprodol/Meprobamate	Carisoprodol	1	24.63	-2%	No significant interference
	Meprobamate				
Basic and Neutral Mix	Amitriptyline	5	24.43	-3%	No significant interference
	Benzylpiperizine				
	Chlorpheniramine				
	Cyclobenzaprine				
	Dextromethorphan				
	Diphenhydramine				
	Doxylamine				
	Fluoxetine				
	Imipramine				
	Ketamine				
	Norketamine				
	Meperidine				
	Nortriptyline				
	Propoxyphene				
	Sertraline				
	Trazodone				
	Venlafaxine				
	Zopiclone				
Over-the-Counter Drugs	Acetaminophen	10	23.24	-8%	No significant interference
	Caffeine				
	Ibuprofen				
	Naproxen				
	Pseudoephedrine				
	Theobromine	1			
Cannabinoids	Δ9-THC	0.5	25.25	0%	No significant interference
	11-Hydroxy-Δ9-THC				
	Δ9-THC-COOH				
	Δ8-THC				
	Cannabinol				
	Cannabinolic acid				

Conclusions: No significant interference observed.

Comments: N/A

Acceptance Criteria: Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies

Validation Study 7

DILUTION INTEGRITY

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target Concentration (ng/mL): 366.55

Run Date	Dilution Factor	Diluted Result (ng/mL)	Calculated Result (µg/mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
AMP_20200814B_SD	2	177.66	355.33	339.85	20.53	-3.06	3.06	6.04
	2	173.83	347.65			-5.16	5.16	
	2	158.28	316.56			-13.64	13.64	
	5	70.66	353.32	333.79	20.50	-3.61	3.61	6.14
	5	67.12	335.60			-8.44	8.44	
	5	62.49	312.44			-14.76	14.76	
	10	36.29	362.86	338.24	33.04	-1.01	1.01	9.77
	10	35.12	351.17			-4.20	4.20	
	10	30.07	300.69			-17.97	17.97	
AMP_20200828B_PK	2	184.93	369.86	394.31	22.39	0.90	0.90	5.68
	2	199.62	399.24			8.92	8.92	
	2	206.91	413.82			12.90	12.90	
	5	70.90	354.50	381.13	23.14	-3.29	3.29	6.07
	5	79.26	396.30			8.12	8.12	
	5	78.52	392.60			7.11	7.11	
	10	34.16	341.60	378.03	31.61	-6.81	6.81	8.36
	10	39.81	398.10			8.61	8.61	
	10	39.44	394.40			7.60	7.60	
AMP_20200828B_SD	2	180.84	361.68	359.26	6.74	-1.33	1.33	1.88
	2	182.23	364.45			-0.57	0.57	
	2	175.82	351.65			-4.06	4.06	
	5	72.00	360.01	353.79	14.05	-1.78	1.78	3.97
	5	72.73	363.66			-0.79	0.79	
	5	67.54	337.69			-7.87	7.87	
	10	31.56	315.63	329.74	15.95	-13.89	13.89	4.84
	10	34.70	347.04			-5.32	5.32	
	10	32.65	326.54			-10.91	10.91	
AMP_20200901B_PK	2	198.36	396.71	407.00	9.03	8.23	8.23	2.22
	2	206.81	413.63			12.84	12.84	
	2	205.33	410.65			12.03	12.03	
	5	72.83	364.14	390.95	23.43	-0.66	0.66	5.99
	5	80.25	401.23			9.46	9.46	
	5	81.50	407.49			11.17	11.17	
	10	39.46	394.56	379.86	22.23	7.64	7.64	5.85
	10	35.43	354.29			-3.34	3.34	
	10	39.07	390.74			6.60	6.60	
AMP_20200901B_CLR	2	153.53	307.06	344.17	38.72	-16.23	16.23	11.25
	2	192.16	384.31			4.85	4.85	
	2	170.58	341.15			-6.93	6.93	
	5	71.43	357.17	379.95	19.85	-2.56	2.56	5.22
	5	77.83	389.15			6.16	6.16	
	5	78.71	393.53			7.36	7.36	
	10	36.12	361.23	355.84	4.99	-1.45	1.45	1.40
	10	35.14	351.39			-4.14	4.14	
	10	35.49	354.89			-3.18	3.18	

Dilution Factor	2
Avg %Bias	7.44
Max Within-Run Precision (%CV)	11.25

Dilution Factor	5
Avg %Bias	6.21
Max Within-Run Precision (%CV)	6.14

Dilution Factor	10
Avg %Bias	6.84
Max Within-Run Precision (%CV)	9.77

Results: It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.
Comments: N/A

Acceptance Criteria: Avg %Bias must be less than 20%

Validation Study 8

PROCESSED SAMPLE STABILITY

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Sample	Time Zero Response	24H Response	48H Response	72H Response	Acceptable Range		Pass/Unstable
						Low	High	
	LQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			
	HQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			

Results:

Comments: Study not performed. Sample preparation will be completed once started without prolonged interruptions.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 8
Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

AUTOSAMPLER STABILITY
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Stability of Punctured Calibrators

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	Calibrator 1	10.17	0.26	10.25	10.18	0.26	1%
	Calibrator 2	19.12	0.48	19.12	18.75	0.48	0%
	Calibrator 3	51.40	1.28	51.95	50.49	1.30	1%
	Calibrator 4	98.78	2.45	100.57	97.48	2.50	2%
	Calibrator 5	249.03	6.18	248.65	240.60	6.17	0%
	Calibrator 6	507.28	12.57	510.65	493.84	12.65	1%

Stability of Punctured Calibrators

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)	Concentration (Time Zero Curve)	Concentration (48 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20201019B_SD & AMP_20201021B_SD	Calibrator 1	10.29	0.28	N/A	N/A	N/A	N/A	10.26	10.28	0.28	0%
	Calibrator 2	19.48	0.51	N/A	N/A	N/A	N/A	19.49	19.09	0.51	0%
	Calibrator 3	45.50	1.16	N/A	N/A	N/A	N/A	45.49	43.93	1.16	0%
	Calibrator 4	99.53	2.52	N/A	N/A	N/A	N/A	99.49	95.50	2.51	0%
	Calibrator 5	258.94	6.51	N/A	N/A	N/A	N/A	259.15	248.00	6.52	0%
	Calibrator 6	528.08	13.27	N/A	N/A	N/A	N/A	523.10	500.11	13.14	-1%

Stability of Punctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	LQC	24.38	0.61	24.53	23.99	0.62	1%
		24.29	0.61	24.64	24.09	0.62	1%
		24.27	0.61	24.44	23.89	0.61	1%
	HQC	393.02	9.74	396.93	383.92	9.84	1%
		394.78	9.79	396.35	383.36	9.82	0%
		395.82	9.81	395.06	382.11	9.79	0%

Stability of Punctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)	Concentration (Time Zero Curve)	Concentration (48 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20201019B_SD & AMP_20201021B_SD	LQC	24.67	0.64	N/A	N/A	N/A	N/A	24.58	23.95	0.64	0%
		24.69	0.64	N/A	N/A	N/A	N/A	24.75	24.11	0.64	0%
		24.66	0.64	N/A	N/A	N/A	N/A	24.77	24.13	0.64	0%
	HQC	401.55	10.09	N/A	N/A	N/A	N/A	394.43	377.20	9.91	-2%
		400.16	10.06	N/A	N/A	N/A	N/A	395.42	378.16	9.94	-1%
		398.60	10.02	N/A	N/A	N/A	N/A	397.47	380.11	9.99	0%

Stability of Unpunctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	LQC	24.38	0.61	24.38	23.84	0.61	0%
		24.29	0.61	24.56	24.01	0.62	1%
		24.27	0.61	24.62	24.07	0.62	1%
	HQC	393.02	9.74	395.69	382.73	9.81	1%
		394.78	9.79	397.53	384.50	9.85	1%
		395.82	9.81	398.57	385.51	9.88	1%

Stability of Unpunctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)	Concentration (Time Zero Curve)	Concentration (48 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20201019B_SD & AMP_20201021B_SD	LQC	24.67	0.64	N/A	N/A	N/A	N/A	24.63	24.00	0.64	0%
		24.69	0.64	N/A	N/A	N/A	N/A	24.67	24.04	0.64	0%
		24.66	0.64	N/A	N/A	N/A	N/A	24.76	24.13	0.64	0%
	HQC	401.55	10.09	N/A	N/A	N/A	N/A	396.30	378.99	9.96	-1%
		400.16	10.06	N/A	N/A	N/A	N/A	397.63	380.27	9.99	-1%
		398.60	10.02	N/A	N/A	N/A	N/A	394.17	376.96	9.91	-1%

Results: Punctured and unpunctured samples were shown to be stable for 48 hours.

Comments: AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for MDA were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 9 **MATRIX EFFECTS**

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Neat Response at Analyte RT

	LQC	HQC
1	362346	3562600
2	341261	3601120
3	354361	3637204
4	358215	3591569
5	338333	3441000
6	337385	3643637
Mean	**348650**	**3579522**
SD	10951	74195
%CV	3%	2%

Neat Response at IS RT

LQC	HQC
568223	370576
531651	368744
609232	372199
592467	360812
543609	390241
555689	346856
566812	**368238**
29523	14268
5%	4%

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at Analyte RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	325007	-7%	3285537	-8%
5	310820	-11%	3553173	-1%
7	313815	-10%	3559901	-1%
8	336070	-4%	3446874	-4%
9	281484	-19%	3341211	-7%
10	317463	-9%	3254067	-9%
11	328510	-6%	3391030	-5%
12	337111	-3%	3486792	-3%
13	312584	-10%	3499839	-2%
14	332928	-5%	3609470	1%
Mean	319579		3442790	
SD	16604		121212	
%CV	5%		4%	
Avgerage % suppression/enhancement	-8%		-4%	

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at IS RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	591599	4%	355658	-3%
5	577217	2%	382886	4%
7	583710	3%	387330	5%
8	612124	8%	374005	2%
9	515791	-9%	361226	-2%
10	577038	2%	358241	-3%
11	594049	5%	368026	0%
12	616578	9%	378096	3%
13	564120	0%	377426	2%
14	614439	8%	384105	4%
Mean	584666		372700	
SD	29954		11324	
%CV	5%		3%	
Avgerage % suppression/enhancement	3%		1%	

Results: Average percent suppression values for LQC and HQC were -8% and -4%, respectively.

Comments: N/A

Acceptance Criteria: **Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%**

Validation Study 10

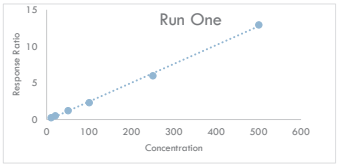
Additional Experiment

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

LINEARITY

Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
AMP_202000910B_SD	10	9.98	-0.23	0.242
	20	20.26	1.29	0.493
	50	50.20	0.41	1.224
	100	94.74	-5.26	2.311
	250	244.44	-2.22	5.964
	500	530.11	6.02	12.936



Slope	0.02576
Intercept	-0.13176
R^2	0.99916

LOQ

Run Date	Run Order	LOQ
	Target Concentration (ng/mL):	*10*
AMP_202000910B_SD	1-1	10.15
	1-2	9.79
	1-3	10.22
	1-4	10.24
	1-5	10.34
	1-6	10.70
	1-7	10.44
	1-8	10.18
	1-9	10.62
	1-10	10.48
Within Run	**Mean**	**10.32**
	SD	**0.26**
	%CV	**2.55%**
	% Bias	**3.15%**

BIAS & PRECISION

Run Date	Run Order	LQC	MQC	HQC
	Target Concentration (ng/mL)	*25*	*100*	*400*
AMP_202000910B_SD	1-1	24.79	107.81	404.17
	1-2	24.82	104.38	396.80
	1-3	24.98	107.37	416.18
Within Run	**Mean**	**24.86**	**106.52**	**405.72**
	SD	**0.10**	**1.87**	**9.78**
	%CV	**0.40%**	**1.76%**	**2.41%**
	% Bias	**-0.55%**	**6.52%**	**1.43%**

Comments: The purpose of this experiment was to verify the change in qualifier ions from 105.1 m/z and 77.1 m/z to 133.1 m/z and 79.1 m/z. Blank blood sources 4 (lot 310355), 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study.

Acceptance Criteria:

%RE Calibrators ±20% of target
Bias: ≤20%
Within-Run Precision: CV ≤20%

SUMMARY OF VALIDATION PERFORMANCE

Analyte: MDA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

Date	Reason
N/A	N/A

Deviations from SOP: On 9/10/2020 the qualifier ions for MDA were changed from 105.1 m/z and 77.1 m/z to the new ions of 133.1 m/z and 79.1 m/z due to interferences with the original ions. An additional experiment was performed on 9/10/2020 that included linearity, bias & precision, and LOQ. All parameters met acceptance criteria; therefore, the new ions were deemed acceptable for use.

Other Observations: Working Standards Verified in Validation:
Calibrators: 200805C-C-10, 200805C-C-2.5, 200805C-C-0.2, 200923C-C-10, 200923C-C-2.5, 200923C-C-0.2
Controls:200807L-Q-Mix, 200813L-Q-0.5, 200813L-St-5
Internal Standard: 200805C-IS-1, 200903C-IS-1, 200915C-IS-1

Sample Preparation Steps: Refer to Toxicology Analytical Manual v3.5, "Amphetamines Confirmation by Liquid Chromatography-Tandem Mass Spectrometry" section titled "Extraction Procedure".

Location of Raw Data: Toxicology section shared electronic storage.

Recommended Maximum Run Length (Unknown Samples): 30

Conclusion: **This method is fit for use on casework for MDA confirmation analysis in blood.**

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: MDMA
Units: ng/mL
Method: AMP.M
Instrument: LCMS-1
SOP Reference: Toxicology Analytical Manual v3.5

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes Σ|%RE|	Unweighted: = 629.22 1/x Weighting: = 385.11 1/x2 Weighting: = 377.46	Data were processed using 1/x2 weighting.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9526 - 0.9932 95% CI of Intercept = -4.4564 - 13.2224	The linearity of the method is acceptable because the individual calibration curves on all five days met the acceptance criteria. Also, other analytical data including the ion ratios and quantification values of the controls and calibrators met acceptance criteria.
1	Validation Calibration	%RE Calibrators ±20% of target	Max %RE = 11.95	N/A
1	Case Work Calibration	%RE Calibrators ±20% of target	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
1	Comparison of Validation Calibration to Casework Calibration	95% CI of slope includes 1 95% CI of intercept includes 0	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
2	Limit of Detection (LOD)	Signal to Noise ≥3.3 Acceptable detection and identification criteria	LOD = 10 ng/mL S:N= 3270	The lowest non-zero calibrator was defined as the LOD.
3	Limit of Quantitation (LOQ)	Bias: ≤20% Within-Run Precision: CV ≤20% Between-Run Precision: CV ≤20%	Bias = 2.11% Within-Run Precision= 10.43% Between-Run Precision = 5.74%	Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Number in red was > 20% target value. Numbers with * were > 20% target value due to insufficient IS.
4	Bias & Precision	%Bias ≤20% Within-Run %CV ≤20% Between-Run %CV ≤20%	Max Bias = -9.02% Max Within-Run Precision = 18.79% Max Between-Run Precision = 14.78%	Numbers in red were not within 20% of the target value.
5	Carryover	No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.	No significant carryover observed following samples containing analyte at up to 2000 ng/mL.	N/A
6	Matrix Interference	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.
6	Interference from stable isotope internal standard	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	
6	Exogenous Substances Interferences	Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies	No significant interference observed.	N/A
7	Dilution Integrity	Average %Bias must be less than 20%	It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.	N/A
8	Processed Sample Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	N/A	Study not performed. Sample preparation will be completed once started without interruptions.
8	Autosampler Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	Punctured and unpunctured samples were shown to be stable for 48 hours.	AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for MDMA were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.
9	Matrix Effects	Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%	Average percent suppression values for LQC and HQC were -16% and -8%, respectively.	N/A

Validation Study 1 **STANDARD CURVE WEIGHT VERIFICATION**

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Σ|%RE|
Unweighted:	629.22
1/x Weighting:	385.11
1/x² Weighting:	377.46

	C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
AMP_20200814B_SD	10	0.25225	1	2.522546	10	0.25225461	100	0.063632	5.7247	-42.753	42.753
	20	0.48722	1	9.744497	20	0.48722485	400	0.237388	16.78629	-16.0686	16.06857
	50	1.24941	1	62.47074	50	1.2494147	2500	1.561037	52.66755	5.3351	5.3351
	100	2.56309	1	256.3095	100	2.56309467	10000	6.569454	114.5111	14.51106	14.51106
	250	5.55288	1	1388.219	250	5.55287569	62500	30.83443	255.2596	2.103846	2.103846
	500	11.11483	1	5557.417	500	11.1148334	250000	123.5395	517.0974	3.419471	3.419471
	1000	20.70035	1	20700.35	1000	20.7003519	1000000	428.5046	968.3504	-3.16496	3.164957
AMP_20200814B_PK	10	0.22491	1	2.24908	10	0.224908	100	0.050584	4.437316	-55.6268	55.62684
	20	0.41306	1	8.26112	20	0.413056	400	0.170615	13.29467	-33.5266	33.52663
	50	0.83125	1	41.56255	50	0.831251	2500	0.690978	32.98185	-34.0363	34.0363
	100	1.73628	1	173.6282	100	1.736282	10000	3.014675	75.58758	-24.4124	24.41242
	250	4.88321	1	1220.803	250	4.883212	62500	23.84576	223.7342	-10.5063	10.50633
	500	9.36278	1	4681.388	500	9.362776	250000	87.66157	434.6166	-13.0767	13.07669
	1000	18.02597	1	18025.97	1000	18.02597	1000000	324.9356	842.4498	-15.755	15.75502
AMP_20200828B_SD	10	0.26652	1	2.665204	10	0.26652035	100	0.071033	6.396282	-36.0372	36.03718
	20	0.49992	1	9.998468	20	0.49992341	400	0.249923	17.38409	-13.0795	13.07954
	50	1.27470	1	63.73509	50	1.27470179	2500	1.624865	53.85798	7.715957	7.715957
	100	2.34730	1	234.7298	100	2.34729814	10000	5.509809	104.3521	4.352107	4.352107
	250	5.83258	1	1458.146	250	5.83258294	62500	34.01902	268.4273	7.370906	7.370906
	500	12.29250	1	6146.248	500	12.292497	250000	151.1055	572.5377	14.50754	14.50754
	1000	22.07351	1	22073.51	1000	22.0735145	1000000	487.24	1032.994	3.299418	3.299418
AMP_20200828B_PK	10	0.22748	1	2.274758	10	0.2274758	100	0.051745	4.558199	-54.418	54.41801
	20	0.43194	1	8.638729	20	0.43193646	400	0.186569	14.1835	-29.0825	29.0825
	50	0.97897	1	48.9485	50	0.97896995	2500	0.958382	39.93595	-20.1281	20.12811
	100	1.91075	1	191.0751	100	1.91075082	10000	3.650969	83.80097	-16.199	16.19903
	250	5.11929	1	1279.822	250	5.11928852	62500	26.20711	234.8478	-6.06087	6.060868
	500	9.91325	1	4956.625	500	9.91325098	250000	98.27255	460.531	-7.8938	7.893798
	1000	20.18783	1	20187.83	1000	20.1878343	1000000	407.5487	944.2229	-5.57771	5.577713
AMP_20200901B_CLR	10	0.25469	1	2.546862	10	0.2546862	100	0.064865	5.839171	-41.6083	41.60829
	20	0.52399	1	10.47983	20	0.52399136	400	0.274567	18.51713	-7.41436	7.414365
	50	1.23633	1	61.81673	50	1.23633452	2500	1.528523	52.05178	4.10356	4.10356
	100	2.74417	1	274.417	100	2.74417048	10000	7.530472	123.0355	23.03548	23.03548
	250	6.37900	1	1594.75	250	6.37900008	62500	40.69164	294.1507	17.66028	17.66028
	500	12.72574	1	6362.872	500	12.7257434	250000	161.9445	592.9334	18.58669	18.58669
	1000	24.94011	1	24940.11	1000	24.940108	1000000	622.009	1167.943	16.79435	16.79435
Sum	9650.0	209.5581	5	142042.1	9650	209.558084	6627500	3082.42			629.2219
Slope	0.02124200										
Intercept	0.13065052										
R^2	0.97932871										

Comments: Data were processed using $1/x^2$ weighting.

Acceptance Criteria: **The least complex weighting scheme that minimizes Σ|%RE|**

Validation Study 1

STANDARD CURVE WEIGHT VERIFICATION

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
10	0.25225	0.1	0.252255	1	0.02522546	10	0.006363	9.825702	-1.74298	1.742976
20	0.48722	0.05	0.487225	1	0.02436124	20	0.011869	20.71927	3.596345	3.596345
50	1.24941	0.02	1.249415	1	0.02498829	50	0.031221	56.05551	12.11103	12.11103
100	2.56309	0.01	2.563095	1	0.02563095	100	0.065695	116.9597	16.95966	16.95966
250	5.55288	0.004	5.552876	1	0.0222115	250	0.123338	255.5703	2.228125	2.228125
500	11.11483	0.002	11.11483	1	0.02222967	500	0.247079	513.4309	2.686175	2.686175
1000	20.70035	0.001	20.70035	1	0.02070035	1000	0.428505	957.8296	-4.21704	4.217036
10	0.22491	0.1	0.224908	1	0.0224908	10	0.005058	8.557873	-14.4213	14.42127
20	0.41306	0.05	0.413056	1	0.0206528	20	0.008531	17.28069	-13.5965	13.59654
50	0.83125	0.02	0.831251	1	0.01662502	50	0.01382	36.66883	-26.6623	26.66234
100	1.73628	0.01	1.736282	1	0.01736282	100	0.030147	78.6274	-21.3726	21.3726
250	4.88321	0.004	4.883212	1	0.01953285	250	0.095383	224.5237	-10.1905	10.19051
500	9.36278	0.002	9.362776	1	0.01872555	500	0.175323	432.2029	-13.5594	13.55942
1000	18.02597	0.001	18.02597	1	0.01802597	1000	0.324936	833.8414	-16.6159	16.61586
10	0.26652	0.1	0.26652	1	0.02665204	10	0.007103	10.48708	4.870832	4.870832
20	0.49992	0.05	0.499923	1	0.02499617	20	0.012496	21.30799	6.539967	6.539967
50	1.27470	0.02	1.274702	1	0.02549404	50	0.032497	57.22786	14.45572	14.45572
100	2.34730	0.01	2.347298	1	0.02347298	100	0.055098	106.955	6.95501	6.95501
250	5.83258	0.004	5.832583	1	0.02333033	250	0.136076	268.538	7.415181	7.415181
500	12.29250	0.002	12.2925	1	0.02458499	500	0.302211	568.0291	13.60582	13.60582
1000	22.07351	0.001	22.07351	1	0.02207351	1000	0.48724	1021.491	2.149149	2.149149
10	0.22748	0.1	0.227476	1	0.02274758	10	0.005175	8.67692	-13.2308	13.2308
20	0.43194	0.05	0.431936	1	0.02159682	20	0.009328	18.15602	-9.21991	9.21991
50	0.97897	0.02	0.97897	1	0.0195794	50	0.019168	43.5173	-12.9654	12.96541
100	1.91075	0.01	1.910751	1	0.01910751	100	0.03651	86.71603	-13.284	13.28397
250	5.11929	0.004	5.119289	1	0.02047715	250	0.104828	235.4686	-5.81257	5.812572
500	9.91325	0.002	9.913251	1	0.0198265	500	0.196545	457.7237	-8.45525	8.455252
1000	20.18783	0.001	20.18783	1	0.02018783	1000	0.407549	934.0686	-6.59314	6.593143
10	0.25469	0.1	0.254686	1	0.02546862	10	0.006487	9.938435	-0.61565	0.615655
20	0.52399	0.05	0.523991	1	0.02619957	20	0.013728	22.42382	12.11909	12.11909
50	1.23633	0.02	1.236335	1	0.02472669	50	0.03057	55.4491	10.8982	10.8982
100	2.74417	0.01	2.74417	1	0.0274417	100	0.075305	125.3546	25.3546	25.3546
250	6.37900	0.004	6.379	1	0.025516	250	0.162767	293.8707	17.54826	17.54826
500	12.72574	0.002	12.72574	1	0.02545149	500	0.323889	588.115	17.62301	17.62301
1000	24.94011	0.001	24.94011	1	0.02494011	1000	0.622009	1154.391	15.4391	15.4391
Sum	9650		209.55808	0.935	209.5581	35	0.79263432	9650	4.613846			385.1105
Slope	0.02156963											
Intercept	0.04031781											
R^2	0.99213051											

C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
10	0.25225	0.01	0.02522546	0.1	0.002523	1	0.000636	10.25639	2.5639	2.563899643
20	0.48722	0.0025	0.02436124	0.05	0.001218	1	0.000593	20.98001	4.900063	4.900062682
50	1.24941	0.0004	0.02498829	0.02	0.0005	1	0.000624	55.765	11.53	11.52999604
100	2.56309	0.0001	0.02563095	0.01	0.000256	1	0.000657	115.719	15.71901	15.71900914
250	5.55288	0.000016	0.0222115	0.004	8.88E-05	1	0.000493	252.1673	0.866913	0.866912949
500	11.11483	0.000004	0.02222967	0.002	4.45E-05	1	0.000494	506.0051	1.201023	1.20102272
1000	20.70035	0.000001	0.02070035	0.001	2.07E-05	1	0.000429	943.4711	-5.65289	5.652891801
10	0.22491	0.01	0.0224908	0.1	0.002249	1	0.000506	9.008339	-9.91661	9.916606052
20	0.41306	0.0025	0.0206528	0.05	0.001033	1	0.000427	17.59508	-12.0246	12.02460721
50	0.83125	0.0004	0.01662502	0.02	0.000333	1	0.000276	36.68075	-26.6385	26.63849496
100	1.73628	0.0001	0.01736282	0.01	0.000174	1	0.000301	77.98475	-22.0152	22.01524674
250	4.88321	0.000016	0.01953285	0.004	7.81E-05	1	0.000382	221.605	-11.358	11.3579896
500	9.36278	0.000004	0.01872555	0.002	3.75E-05	1	0.000351	426.0443	-14.7911	14.79113244
1000	18.02597	0.000001	0.01802597	0.001	1.8E-05	1	0.000325	821.4171	-17.8583	17.85829386
10	0.26652	0.01	0.02665204	0.1	0.002665	1	0.00071	10.90745	9.074529	9.074529249
20	0.49992	0.0025	0.02499617	0.05	0.00125	1	0.000625	21.55955	7.797762	7.797762244
50	1.27470	0.0004	0.02549404	0.02	0.00051	1	0.00065	56.91906	13.83811	13.83811102
100	2.34730	0.0001	0.02347298	0.01	0.000235	1	0.000551	105.8704	5.87044	5.870440192
250	5.83258	0.000016	0.02333033	0.004	9.33E-05	1	0.000544	264.9326	5.973049	5.97304907
500	12.29250	0.000004	0.02458499	0.002	4.92E-05	1	0.000604	559.7516	11.95032	11.95031539
1000	22.07351	0.000001	0.02207351	0.001	2.21E-05	1	0.000487	1006.14	0.613978	0.613977597
10	0.22748	0.01	0.02274758	0.1	0.002275	1	0.000517	9.125529	-8.74471	8.744707207
20	0.43194	0.0025	0.02159682	0.05	0.00108	1	0.000466	18.45675	-7.71625	7.71625354
50	0.97897	0.0004	0.0195794	0.02	0.000392	1	0.000383	43.42238	-13.1552	13.15523581
100	1.91075	0.0001	0.01910751	0.01	0.000191	1	0.000365	85.9472	-14.0528	14.05280108
250	5.11929	0.000016	0.02047715	0.004	8.19E-05	1	0.000419	232.3791	-7.04835	7.048345094
500	9.91325	0.000004	0.0198265	0.002	3.97E-05	1	0.000393	451.167	-9.76659	9.766593166
1000	20.18783	0.000001	0.02018783	0.001	2.02E-05	1	0.000408	920.0807	-7.99193	7.99193071
10	0.25469	0.01	0.02546862	0.1	0.002547	1	0.000649	10.36736	3.673634	3.673634093
20	0.52399	0.0025	0.02619957	0.05	0.00131	1	0.000686	22.65797	13.28985	13.28985363
50	1.23633	0.0004	0.02472669	0.02	0.000495	1	0.000611	55.16804	10.33608	10.33608352
100	2.74417	0.0001	0.0274417	0.01	0.000274	1	0.000753	123.983	23.98299	23.98298599
250	6.37900	0.000016	0.025516	0.004	0.000102	1	0.000651	289.8701	15.94805	15.94805049
500	12.72574	0.000004	0.02545149	0.002	5.09E-05	1	0.000648	579.5242	15.90483	15.90483464
1000	24.94011	0.000001	0.02494011	0.001	2.49E-05	1	0.000622	1136.966	13.6966	13.69659898
9650	209.55808	0.065105	0.79263432	0.935	0.022279	35	0.018239			377.4622586
Slope	0.02191146									
Intercept	0.02752212									
R^2	0.98776054									

Validation Study 1

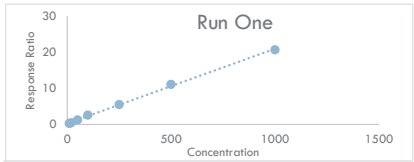
VALIDATION CURVE CALIBRATION

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

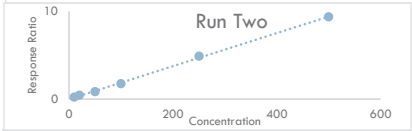
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
AMP_20200814B_SD	10	9.77	2.31	0.252
	20	20.05	0.27	0.487
	50	53.42	6.84	1.249
	100	110.93	10.93	2.563
	250	241.81	3.28	5.553
	500	485.29	2.94	11.115
	1000	904.90	9.51	20.700
AMP_20200814B_PK	10	10.09	0.94	0.225
	20	20.62	3.11	0.413
	50	44.03	11.95	0.831
	100	94.67	5.33	1.736
	250	270.77	8.31	4.883
	500	521.45	4.29	9.363
	1000	1006.24	0.62	18.026
AMP_20200828B_SD	10	9.93	0.66	0.267
	20	19.87	0.64	0.500
	50	52.86	5.73	1.275
	100	98.54	1.46	2.347
	250	246.94	1.22	5.833
	500	522.02	4.40	12.292
	1000	938.50	6.15	22.074
AMP_20200828B_PK	10	10.00	0.00	0.227
	20	20.39	1.95	0.432
	50	48.17	3.66	0.979
	100	95.50	4.50	1.911
	250	258.47	3.39	5.119
	500	501.97	0.39	9.913
	1000	1023.84	2.38	20.188
AMP_20200901B_CLR	10	9.91	0.88	0.255
	20	20.40	2.01	0.524
	50	48.15	3.70	1.236
	100	106.89	6.89	2.744
	250	248.48	0.61	6.379
	500	495.71	0.86	12.726
	1000	971.51	2.85	24.940

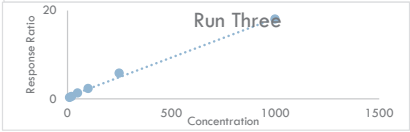
Max %RE = 11.95



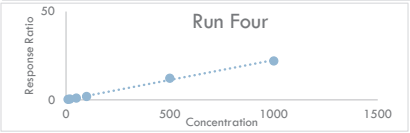
Slope	0.02070
Intercept	0.28258
R^2	0.99931



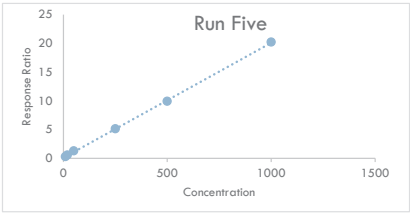
Slope	0.01887
Intercept	-0.01698
R^2	0.99947



Slope	0.01778
Intercept	0.47014
R^2	0.99751



Slope	0.02253
Intercept	0.01179
R^2	0.99826



Slope	0.01999
Intercept	0.10778
R^2	0.99989

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

LINEARITY

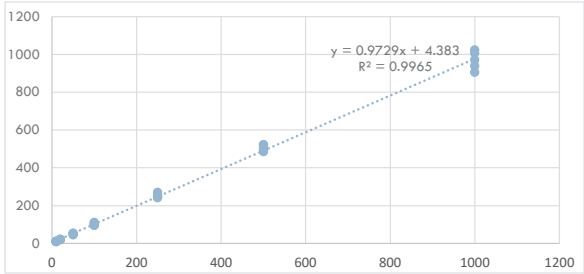
Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

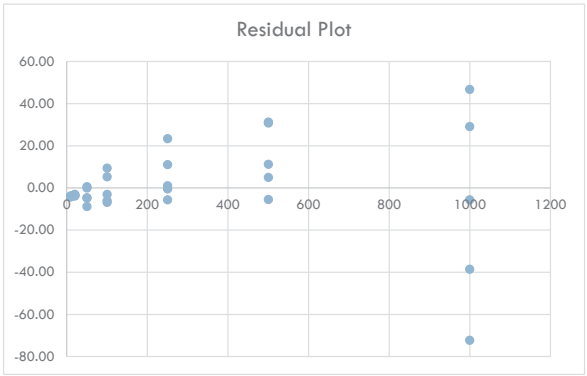
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Study Date	Target (x)	Calculated (y)	Predicted	Residual
AMP_20200814B_SD	10	9.77	14.11	-4.34
	20	20.05	23.84	-3.79
	50	53.42	53.03	0.39
	100	110.93	101.67	9.25
	250	241.81	247.61	-5.80
	500	485.29	490.83	-5.55
	1000	904.90	977.28	-72.38
AMP_20200814B_PK	10	10.09	14.11	-4.02
	20	20.62	23.84	-3.22
	50	44.03	53.03	-9.00
	100	94.67	101.67	-7.00
	250	270.77	247.61	23.17
	500	521.45	490.83	30.62
	1000	1006.24	977.28	28.96
AMP_20200828B_PK	10	10.00	14.11	-4.11
	20	20.39	23.84	-3.45
	50	48.17	53.03	-4.86
	100	95.50	101.67	-6.17
	250	258.47	247.61	10.86
	500	501.97	490.83	11.14
	1000	1023.84	977.28	46.56
AMP_20200828B_SD	10	9.93	14.11	-4.18
	20	19.87	23.84	-3.97
	50	52.86	53.03	-0.16
	100	98.54	101.67	-3.14
	250	246.94	247.61	-0.66
	500	522.02	490.83	31.18
	1000	938.50	977.28	-38.78
AMP_20200901B_CLR	10	9.91	14.11	-4.20
	20	20.40	23.84	-3.44
	50	48.15	53.03	-4.88
	100	106.89	101.67	5.21
	250	248.48	247.61	0.87
	500	495.71	490.83	4.88
	1000	971.51	977.28	-5.77

Slope	0.9729
Std err in slope, S_b	0.0100
Degrees freedom	33
Confidence level	95%
Student t	2.0345
Confidence interval	0.020
Slope	0.9729 ± 0.0203
Range	0.9526 - 0.9932
	NO

Intercept	4.3830
Std err in Intercept	4.3447
Degrees freedom	33
Confidence Level	95%
Student t	2.0345
Confidence interval	8.839
Intercept	4.383 ± 8.8394
Lower	-4.4564 - 13.2224
	YES





Comments: The linearity of the method is acceptable because the individual calibration curves on all five days met the acceptance criteria. Also, other analytical data including the ion ratios and quantification values of the controls and calibrators met acceptance criteria.

Acceptance Criteria:

95% CI of slope includes 1
95% CI of intercept includes 0

Validation Study 2

Sensitivity (LOD)

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Signal to Noise Ratio			
Concentration (ng/mL)	*10*			
AMP_20200814B_SD	2929			
	4659			
	4762			
	4789			
	1388			
	7028			
	7771			
	6318			
	3705			
AMP_20200828B_PK	2512			
	1968			
	2860			
	3959			
	1702			
	3215			
	1398			
	2219			
	401			
AMP_20200901B_CLR	3511			
	544			
	2252			
	5585			
	1373			
	1935			
	3270			
	2079			
	4154			
AMP_20200901B_PK	1282			
	484			
	1761			
	827			
	2775			
	3512			
	523			
	2043			
	2258			
Average Signal to Noise:	**3270**			

Established LOD: 10 ng/mL
Signal to Noise: 3270

Comments: The lowest non-zero calibrator was defined as the LOD.

Acceptance Criteria: Signal to Noise ≥3.3
Acceptable detection and identification criteria

Validation Study 3

SENSITIVITY (LOQ)

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LOQ
	Target Concentration (ng/mL):	*10*
AMP_20200814B_SD	1-1	10.16
	1-2	10.33
	1-3	9.71
	1-4	9.89
	1-5	10.05
	1-6	10.94
	1-7	10.17
	1-8	9.95
	1-9	9.78
Within Run	**Mean**	**10.11**
	SD	**0.37**
	%CV	**3.64%**
	% Bias	**1.10%**
AMP_20200828B_PK	2-1	10.28
	2-2	10.41
	2-3	9.98
	2-4	10.20
	2-5	4.54*
	2-6	10.25
	2-7	10.69
	2-8	10.07
	2-9	10.57
Within Run	**Mean**	**10.31**
	SD	**0.24**
	%CV	**2.34%**
	% Bias	**3.07%**
AMP_20200901B_CLR	3-1	52.40*
	3-2	10.35
	3-3	9.67
	3-4	9.97
	3-5	9.21
	3-6	10.65
	3-7	12.84
	3-8	10.12
	3-9	10.28
Within Run	**Mean**	**10.39**
	SD	**1.08**
	%CV	**10.43%**
	% Bias	**3.87%**
AMP_20200901B_PK	4-1	10.16
	4-2	9.80
	4-3	9.73
	4-4	9.48
	4-5	9.96
	4-6	10.61
	4-7	10.29
	4-8	10.24
	4-9	10.07
Within Run	**Mean**	**10.04**
	SD	**0.34**
	%CV	**3.39%**
	% Bias	**0.38%**

Mean		**10.20**
SD		**0.59**
Precision (%CV)	***Max Within-Run***	**10.43%**
	Between-Run	**5.74%**
% Bias		**2.11%**

Comments: Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Number in red was > 20% target value. Numbers with * were > 20% target value due to insufficient IS.

Acceptance Criteria:
Bias: ≤20%
Within-Run Precision: CV ≤20%
Between-Run Precision: CV ≤20%

Validation Study 4

BIAS AND PRECISION

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LQC	MQC	UTAK	HQC
	Target Concentration (ng/mL):	*25*	*100*	*79.64*	*800*
AMP_202008014B_SD	1-1	26.31	98.72	69.97	730.22
	1-2	24.26	95.22	74.11	722.29
	1-3	25.79	97.51	71.59	720.42
	1-4			75.15	
Within Run	Mean	25.46	97.15	72.71	724.31
	SD	1.06	1.78	2.36	5.20
	%CV	4.18%	1.83%	3.24%	0.72%
	% Bias	1.82%	-2.85%	-8.71%	-9.46%
AMP_202008014B_PK	2-1	23.22	77.69	94.45	854.80
	2-2	27.42	79.95	93.43	890.21
	2-3	26.24	87.58	101.32	841.35
	2-4			104.21	
Within Run	Mean	25.63	81.74	98.35	862.12
	SD	2.16	5.18	5.25	25.24
	%CV	8.44%	6.34%	5.34%	2.93%
	% Bias	2.51%	-18.26%	23.50%	7.77%
AMP_20200828B_PK	3-1	25.51	NA	73.91	770.63
	3-2	27.65	NA	71.58	758.67
	3-3	24.66	NA	71.17	768.48
	3-4			69.80	
Within Run	Mean	25.94		71.61	765.93
	SD	1.54		1.71	6.37
	%CV	5.94%		2.39%	0.83%
	% Bias	3.76%		-10.08%	-4.26%
AMP_20200828B_SD	4-1	23.38	99.15	77.71	762.49
	4-2	23.90	94.28	81.35	767.76
	4-3	24.29	96.93	71.01	746.61
	4-4			73.47	
Within Run	Mean	23.85	96.79	75.88	758.95
	SD	0.46	2.44	4.58	11.01
	%CV	1.91%	2.52%	6.03%	1.45%
	% Bias	-4.58%	-3.21%	-4.71%	-5.13%
AMP_20200901B_PK	5-1	24.07	80.07		770.54
	5-2	24.72	88.74		763.70
	5-3	24.37	84.70		744.42
Within Run	Mean	24.39	84.50		759.55
	SD	0.32	4.34		13.54
	%CV	1.32%	5.13%		1.78%
	% Bias	-2.45%	-15.50%		-5.06%
AMP_20200901B_CLR	6-1	28.12	112.89		729.58
	6-2	29.17	77.32		781.06
	6-3	25.48	93.93		772.69
Within Run	Mean	27.59	94.71		761.11
	SD	1.90	17.80		27.62
	%CV	6.89%	18.79%		3.63%
	% Bias	10.37%	-5.29%		-4.86%

Mean		25.48	90.98	79.64	772.00
SD		1.71	9.94	11.77	46.13
Precision (%CV)	*Max Within-Run*	8.44%	18.79%	6.03%	3.63%
	Between-Run	6.72%	10.92%	14.78%	5.98%
% Bias		1.91%	-9.02%	0.00%	-3.50%

Comments: Numbers in red were not within 20% of the target value.

Acceptance Criteria:

%Bias ≤20%
Within-Run %CV ≤20%
Between-Run %CV ≤20%

Validation Study 5

CARRYOVER

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Average LOQ Response*: 206683

Study Date:	Response		
	AMP_202008014B_SD	AMP_202008014B_PK	AMP_202008028B_PK
Concentrated Sample (2000 ng/mL)	15224166	14850080	20045769
Blank	4726	4480	5462
%LOD Response	**2.29%**	**2.17%**	**2.64%**
		Maximum Response in Blank:	**2.6%**

Results: No significant carryover observed following samples containing analyte at up to 2000 ng/mL.
Comments: N/A

Acceptance Criteria: **No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.**

Validation Study 6

MATRIX & IS INTERFERENCE

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

LOQ Response

	Analyte	IS
Run 1	249596	989459
Run 2	151193	672245
Run 3	202304	759057
Run 4	216388	951259
Run 5	213932	839983
Average	**206683**	**842401**

Matrix Interference — Study Date: AMP_20200814B_PK

Matrix Source	Peak at Analyte RT	
	Peak Response	Percent of LOQ Response
4	287	0.14%
5	394	0.19%
7	341	0.16%
8	1728	0.84%
9	1145	0.55%
10	861	0.42%
11	696	0.34%
12	474	0.23%
13	565	0.27%
14	1315	0.64%

Interference from Stable-Isotope Internal Standards — Study Date: AMP_20200828B_SD

Experiment	Peak at Analyte RT		Peak at IS RT	
	Peak Response	Percent of LOQ Response	Peak Response	Percent of LOQ Response
Matrix with IS but no D0 (IS = 50 ng/mL)	5966	2.89%	N/A	N/A
Matrix with D0 but no IS (D0 = 2000 ng/mL)	N/A	N/A	312	0.04%

Matrix Interference: No significant interference observed.

IS Interference: No significant interference observed.

Comments: LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.

Acceptance Criteria: **Response of blank matrix is less than 20% the average response of LOQ**

Validation Study 6 | **EXOGENOUS SUBSTANCE INTERFERENCE**

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target LQC Concentration (µg/mL): 25.48
Control Acceptance: 20%
Run Date: AMP_20200828B_SD

Group	Compound	Compound Concentration (µg/mL)	Calculated LQC Concentration (ng/mL)	% Difference from Target	Comment
Benzodiazepines	Alprazolam	1	24.52	-4%	No significant interference
	α-Hydroxyalprazolam				
	Clonazepam				
	7-Aminoclonazepam				
	Diazepam				
	Nordiazepam				
	Temazepam				
	Lorazepam				
	Oxazepam				
	Zolpidem				
Phencyclidine	Phencyclidine	1	25.88	2%	No significant interference
Opioids	Morphine	2	24.34	-4%	No significant interference
	Hydrocodone	1			
	Buprenorphine	0.5			
	Norbuprenorphine				
	Codeine				
	Fentanyl				
	Norfentanyl oxalate				
	Hydromorphone				
	Methadone				
	EDDP				
	Oxycodone				
	Oxymorphone				
	Tramadol				
	o-Desmethyltramadol				
Cocaine and Metabolites	Benzoylecgonine	2	24.46	-4%	No significant interference
	Cocaine	0.5			
	Cocaethylene				
Carisoprodol/Meprobamate	Carisoprodol	1	24.48	-4%	No significant interference
	Meprobamate				
Basic and Neutral Mix	Amitriptyline	5	24.48	-4%	No significant interference
	Benzylpiperizine				
	Chlorpheniramine				
	Cyclobenzaprine				
	Dextromethorphan				
	Diphenhydramine				
	Doxylamine				
	Fluoxetine				
	Imipramine				
	Ketamine				
	Norketamine				
	Meperidine				
	Nortriptyline				
	Propoxyphene				
	Sertraline				
	Trazodone				
	Venlafaxine				
	Zopiclone				
Over-the-Counter Drugs	Acetaminophen	10	23.11	-9%	No significant interference
	Caffeine				
	Ibuprofen				
	Naproxen				
	Pseudoephedrine				
	Theobromine	1			
Cannabinoids	Δ9-THC	0.5	24.91	-2%	No significant interference
	11-Hydroxy-Δ9-THC				
	Δ9-THC-COOH				
	Δ8-THC				
	Cannabinol				
	Cannabinolic acid				

Conclusions: No significant interference observed.

Comments: N/A

Acceptance Criteria: Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies

Validation Study 7

DILUTION INTEGRITY

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target Concentration (ng/mL): 359.98

Run Date	Dilution Factor	Diluted Result (ng/mL)	Calculated Result (µg/mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
AMP_202008014B_SD	2	183.03	366.05	353.65	18.38	1.69	1.69	5.20
	2	181.18	362.36			0.66	0.66	
	2	166.26	332.53			-7.63	7.63	
	5	75.39	376.93	359.71	20.91	4.71	4.71	5.81
	5	73.15	365.75			1.60	1.60	
	5	67.29	336.44			-6.54	6.54	
	10	38.88	388.76	362.71	34.32	8.00	8.00	9.46
	10	37.55	375.54			4.32	4.32	
	10	32.38	323.83			-10.04	10.04	
AMP_20200828B_PK	2	185.35	370.70	398.09	26.40	2.98	2.98	6.63
	2	200.09	400.18			11.17	11.17	
	2	211.69	423.38			17.61	17.61	
	5	73.05	365.25	391.78	22.98	1.46	1.46	5.87
	5	81.07	405.35			12.60	12.60	
	5	80.95	404.75			12.44	12.44	
	10	34.87	348.70	388.30	34.48	-3.13	3.13	8.88
	10	41.17	411.70			14.37	14.37	
	10	40.45	404.50			12.37	12.37	
AMP_20200828B_SD	2	185.82	371.63	367.55	8.37	3.24	3.24	2.28
	2	186.55	373.10			3.64	3.64	
	2	178.97	357.93			-0.57	0.57	
	5	73.97	369.86	364.23	14.98	2.74	2.74	4.11
	5	75.12	375.58			4.33	4.33	
	5	69.45	347.26			-3.53	3.53	
	10	32.53	325.28	340.71	17.68	-9.64	9.64	5.19
	10	36.00	360.01			0.01	0.01	
	10	33.68	336.84			-6.43	6.43	
AMP_20200901B_PK	2	200.46	400.92	407.67	6.41	11.37	11.37	1.57
	2	206.84	413.68			14.92	14.92	
	2	204.21	408.42			13.46	13.46	
	5	74.10	370.50	397.30	23.48	2.92	2.92	5.91
	5	81.42	407.12			13.10	13.10	
	5	82.85	414.27			15.08	15.08	
	10	40.10	401.02	389.11	23.90	11.40	11.40	6.14
	10	36.16	361.59			0.45	0.45	
	10	40.47	404.72			12.43	12.43	
AMP_20200901B_CLR	2	154.68	309.37	348.02	38.49	-14.06	14.06	11.06
	2	193.17	386.34			7.32	7.32	
	2	174.18	348.36			-3.23	3.23	
	5	73.72	368.59	388.54	18.30	2.39	2.39	4.71
	5	78.49	392.47			9.03	9.03	
	5	80.91	404.55			12.38	12.38	
	10	36.87	368.75	361.68	6.97	2.44	2.44	1.93
	10	35.48	354.81			-1.44	1.44	
	10	36.15	361.49			0.42	0.42	

Dilution Factor	2
Avg %Bias	7.57
Max Within-Run Precision (%CV)	11.06

Dilution Factor	5
Avg %Bias	6.99
Max Within-Run Precision (%CV)	5.91

Dilution Factor	10
Avg %Bias	6.46
Max Within-Run Precision (%CV)	9.46

Results: It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.
Comments: N/A

Acceptance Criteria: Avg %Bias must be less than 20%

Validation Study 8

PROCESSED SAMPLE STABILITY

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Sample	Time Zero Response	24H Response	48H Response	72H Response	Acceptable Range		Pass/Unstable
						Low	High	
	LQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			
	HQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			

Results:

Comments: Study not performed. Sample preparation will be completed once started without interruptions.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 8 — **AUTOSAMPLER STABILITY**

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Stability of Punctured Calibrators

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	Calibrator 1	9.92	0.26	10.04	9.86	0.26	1%
	Calibrator 2	19.66	0.48	19.83	19.36	0.48	1%
	Calibrator 3	53.76	1.26	53.68	52.20	1.26	0%
	Calibrator 4	102.48	2.37	102.10	99.17	2.37	0%
	Calibrator 5	245.29	5.64	248.62	241.33	5.72	1%
	Calibrator 6	502.16	11.52	501.53	486.70	11.50	0%
	Calibrator 7	939.29	21.51	938.11	910.27	21.49	0%

Stability of Punctured Calibrators

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	Calibrator 1	10.06	0.27	N/A	N/A	N/A	N/A	9.94	9.92	0.27	-1%
	Calibrator 2	20.07	0.50	N/A	N/A	N/A	N/A	20.17	19.72	0.50	0%
	Calibrator 3	47.16	1.12	N/A	N/A	N/A	N/A	47.13	45.55	1.12	0%
	Calibrator 4	102.15	2.38	N/A	N/A	N/A	N/A	103.84	99.89	2.42	2%
	Calibrator 5	254.66	5.88	N/A	N/A	N/A	N/A	254.36	244.11	5.87	0%
	Calibrator 6	519.93	11.97	N/A	N/A	N/A	N/A	519.39	498.04	11.95	0%
	Calibrator 7	966.98	22.22	N/A	N/A	N/A	N/A	964.94	924.94	22.17	0%

Stability of Punctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	Response Ratio
AMP_20200923B_SD & AMP_20200924B_SD	LQC	25.19	0.61	25.33	24.70	0.61	1%
		25.31	0.61	25.26	24.63	0.61	0%
		24.95	0.60	25.16	24.53	0.61	1%
	HQC	734.98	16.84	744.83	722.75	17.07	1%
		737.45	16.90	739.70	717.76	16.95	0%
		742.44	17.01	745.10	723.01	17.07	0%

Stability of Punctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	LQC	25.09	0.61	N/A	N/A	N/A	N/A	25.38	24.71	0.62	1%
		24.96	0.61	N/A	N/A	N/A	N/A	25.31	24.64	0.62	1%
		25.03	0.61	N/A	N/A	N/A	N/A	25.22	24.56	0.62	1%
	HQC	749.51	17.23	N/A	N/A	N/A	N/A	762.67	731.14	17.53	2%
		756.89	17.40	N/A	N/A	N/A	N/A	758.84	727.48	17.45	0%
		751.99	17.29	N/A	N/A	N/A	N/A	761.19	729.73	17.50	1%

Stability of Unpunctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	Response Ratio
AMP_20200923B_SD & AMP_20200924B_SD	LQC	25.19	0.61	25.20	24.57	0.61	0%
		25.31	0.61	25.37	24.73	0.61	0%
		24.95	0.60	25.32	24.69	0.61	1%
	HQC	734.98	16.84	743.87	721.82	17.04	1%
		737.45	16.90	745.37	723.27	17.08	1%
		742.44	17.01	742.68	720.66	17.02	0%

Stability of Unpunctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	LQC	25.09	0.61	N/A	N/A	N/A	N/A	25.14	24.48	0.62	0%
		24.96	0.61	N/A	N/A	N/A	N/A	25.15	24.49	0.62	1%
		25.03	0.61	N/A	N/A	N/A	N/A	25.36	24.69	0.62	1%
	HQC	749.51	17.23	N/A	N/A	N/A	N/A	760.46	729.03	17.48	1%
		756.89	17.40	N/A	N/A	N/A	N/A	756.52	725.25	17.39	0%
		751.99	17.29	N/A	N/A	N/A	N/A	756.14	724.89	17.38	1%

Results: Punctured and unpunctured samples were shown to be stable for 48 hours.

Comments: AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for MDMA were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 9 **MATRIX EFFECTS**

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Neat Response at Analyte RT

	LQC	HQC
1	393349	6488376
2	371460	6444722
3	382566	6491170
4	386798	6509970
5	369330	6214191
6	368034	6503590
Mean	**378589**	**6442003**
SD	10479	113918
%CV	3%	2%

Neat Response at IS RT

	LQC	HQC
	631729	413530
	595093	411600
	689097	420179
	660047	405451
	605456	442964
	621645	383501
Mean	**633845**	**412871**
SD	35214	19384
%CV	6%	5%

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at Analyte RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	339410	-10%	5850994	-9%
5	291989	-23%	6097799	-5%
7	297308	-21%	6018770	-7%
8	331180	-13%	5716567	-11%
9	293660	-22%	5807287	-10%
10	327260	-14%	5284226	-18%
11	332241	-12%	5955490	-8%
12	353836	-7%	6082211	-6%
13	291461	-23%	6057075	-6%
14	333293	-12%	6227281	-3%
Mean	319164		5909770	
SD	23158		267533	
%CV	7%		5%	
Avgerage % suppression/enhancement	-16%		-8%	

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at IS RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	623545	-2%	389332	-6%
5	546517	-14%	405616	-2%
7	561610	-11%	403889	-2%
8	613070	-3%	386885	-6%
9	548885	-13%	390660	-5%
10	613306	-3%	351112	-15%
11	604344	-5%	392959	-5%
12	657766	4%	403492	-2%
13	539597	-15%	396946	-4%
14	624249	-2%	414299	0%
Mean	593289		393519	
SD	40813		17209	
%CV	7%		4%	
Avgerage % suppression/enhancement	-6%		-5%	

Results: Average percent suppression values for LQC and HQC were -16% and -8%, respectively.

Comments: N/A

Acceptance Criteria: **Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%**

SUMMARY OF VALIDATION PERFORMANCE

Analyte: MDMA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

Date	Reason
N/A	N/A

Deviations from SOP: N/A

Other Observations:
Working Standards Verified in Validation:
Calibrators: 200805C-C-10, 200805C-C-2.5, 200805C-C-0.2, 200923C-C-10, 200923C-C-2.5, 200923C-C-0.2
Controls:200807L-Q-Mix, 200813L-Q-0.5, 200813L-St-5
Internal Standard: 200805C-IS-1, 200903C-IS-1, 200915C-IS-1

Sample Preparation Steps: Refer to Toxicology Analytical Manual v3.5, "Amphetamines Confirmation by Liquid Chromatography-Tandem Mass Spectrometry" section titled "Extraction Procedure".

Location of Raw Data: Toxicology section shared electronic storage.

Recommended Maximum Run Length (Unknown Samples): 30

Conclusion: **This method is fit for use on casework for MDMA confirmation analysis in blood.**

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: MDEA
Units: ng/mL
Method: AMP.M
Instrument: LCMS-1
SOP Reference: Toxicology Analytical Manual v3.5

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes Σ|%RE|	Unweighted: = 535.96 1/x Weighting: = 363.82 1/x2 Weighting: = 349.31	Data were processed using 1/x2 weighting.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9349 - 0.9812 95% CI of Intercept = -1.6720 - 9.1182	The linearity of the method is acceptable because the individual calibration curves on all five days met the acceptance criteria. Also, other analytical data including the ion ratios and quantification values of the controls and calibrators met acceptance criteria.
1	Validation Calibration	%RE Calibrators ±20% of target	Max %RE = 9.87	N/A
1	Case Work Calibration	%RE Calibrators ±20% of target	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
1	Comparison of Validation Calibration to Casework Calibration	95% CI of slope includes 1 95% CI of intercept includes 0	N/A	Not performed. All 6 calibrators used in validation will be used for casework.
2	Limit of Detection (LOD)	Signal to Noise ≥3.3 Acceptable detection and identification criteria	LOD = 10 ng/mL S:N= 4157	The lowest non-zero calibrator was defined as the LOD.
3	Limit of Quantitation (LOQ)	Bias: ≤20% Within-Run Precision: CV ≤20% Between-Run Precision: CV ≤20%	Bias = 1.75% Within-Run Precision= 10.57% Between-Run Precision = 5.64%	Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Number in red was > 20% target value. Numbers with * were > 20% target value due to insufficient IS.
4	Bias & Precision	%Bias ≤20% Within-Run %CV ≤20% Between-Run %CV ≤20%	Max Bias = -7.93% Max Within-Run Precision = 19.33% Max Between-Run Precision = 17.19%	Number in red was not within 20% of the target value.
5	Carryover	No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.	No significant carryover observed following samples containing analyte at up to 2000 ng/mL.	N/A
6	Matrix Interference	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.
6	Interference from stable isotope internal standard	Response of blank matrix is less than 20% the average response of LOQ	No significant interference observed.	
6	Exogenous Substances Interferences	Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies	No significant interference observed.	N/A
7	Dilution Integrity	Average %Bias must be less than 20%	It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.	N/A
8	Processed Sample Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	N/A	Study not performed. Sample preparation will be completed once started without prolonged interruptions.
8	Autosampler Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%	Punctured and unpunctured samples were shown to be stable for 48 hours.	AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for MDEA were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.
9	Matrix Effects	Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%	Average percent suppression values for LQC and HQC were -20% and -10%, respectively.	N/A

Validation Study 1 **STANDARD CURVE WEIGHT VERIFICATION**

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Σ|%RE|
Unweighted:	535.96
1/x Weighting:	363.82
1/x² Weighting:	349.31

	C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
AMP_20200814B_SD	10	0.28553	1	2.855284	10	0.28552844	100	0.081526	6.506682	-34.9332	34.93318
	20	0.55939	1	11.18784	20	0.55939209	400	0.31292	18.35313	-8.23434	8.234341
	50	1.42739	1	71.36973	50	1.42739461	2500	2.037455	55.9001	11.80019	11.80019
	100	2.86432	1	286.4322	100	2.86432161	10000	8.204338	118.0569	18.05688	18.05688
	250	6.28665	1	1571.663	250	6.2866534	62500	39.52201	266.0958	6.438326	6.438326
	500	11.80453	1	5902.263	500	11.8045262	250000	139.3468	504.781	0.956207	0.956207
AMP_20200814B_PK	10	0.25475	1	2.54753	10	0.254753	100	0.064899	5.175437	-48.2456	48.24563
	20	0.46347	1	9.26934	20	0.463467	400	0.214802	14.20372	-28.9814	28.98138
	50	0.94908	1	47.45375	50	0.949075	2500	0.900743	35.20955	-29.5809	29.58091
	100	1.95758	1	195.7582	100	1.957582	10000	3.832127	78.83428	-21.1657	21.16572
	250	5.44247	1	1360.617	250	5.442468	62500	29.62046	229.5791	-8.16836	8.168359
	500	9.78285	1	4891.423	500	9.782845	250000	95.70406	417.3297	-16.5341	16.53406
AMP_20200828B_SD	10	0.29981	1	2.998103	10	0.29981026	100	0.089886	7.124467	-28.7553	28.75533
	20	0.55932	1	11.18642	20	0.55932075	400	0.31284	18.35005	-8.24977	8.249769
	50	1.40843	1	70.42129	50	1.40842579	2500	1.983663	55.07957	10.15913	10.15913
	100	2.58069	1	258.0687	100	2.5806867	10000	6.659944	105.7878	5.787758	5.787758
	250	6.36041	1	1590.102	250	6.36040886	62500	40.4548	269.2862	7.714495	7.714495
	500	12.69487	1	6347.435	500	12.6948691	250000	161.1597	543.2944	8.658874	8.658874
AMP_20200828B_PK	10	0.25360	1	2.535996	10	0.25359964	100	0.064313	5.125546	-48.7445	48.74454
	20	0.48865	1	9.77291	20	0.48864551	400	0.238774	15.29287	-23.5357	23.53567
	50	1.08332	1	54.16606	50	1.08332118	2500	1.173585	41.0166	-17.9668	17.9668
	100	2.09596	1	209.5959	100	2.09595938	10000	4.393046	84.82003	-15.18	15.17997
	250	5.53419	1	1383.548	250	5.5341931	62500	30.62729	233.5468	-6.58127	6.581267
	500	10.23135	1	5115.677	500	10.2313536	250000	104.6806	436.7307	-12.6539	12.65386
AMP_20200901B_CLR	10	0.29213	1	2.921285	10	0.29212855	100	0.085339	6.792181	-32.0782	32.07819
	20	0.60799	1	12.15979	20	0.60798958	400	0.369651	20.4553	2.276507	2.276507
	50	1.41181	1	70.59072	50	1.41181448	2500	1.99322	55.22615	10.4523	10.4523
	100	3.11917	1	311.9174	100	3.11917403	10000	9.729247	129.081	29.08097	29.08097
	250	7.11643	1	1779.107	250	7.1164296	62500	50.64357	301.9892	20.79569	20.79569
	500	13.33480	1	6667.4	500	13.3348005	250000	177.8169	570.9757	14.19514	14.19514
Sum	4650.0	111.5509	5	38252.44	4650	111.550937	1627500	912.3186			535.9615
Slope	0.02311778										
Intercept	0.13510839										
R^2	0.97400078										

Comments: Data were processed using $1/x^2$ weighting.

Acceptance Criteria: **The least complex weighting scheme that minimizes Σ|%RE|**

Validation Study 1

STANDARD CURVE WEIGHT VERIFICATION

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
	10	0.28553	0.1	0.285528	1	0.02855284	10	0.008153	9.604585	-3.95415	3.954148
	20	0.55939	0.05	0.559392	1	0.0279696	20	0.015646	21.20389	6.019458	6.019458
	50	1.42739	0.02	1.427395	1	0.02854789	50	0.040749	57.96754	15.93508	15.93508
	100	2.86432	0.01	2.864322	1	0.02864322	100	0.082043	118.8276	18.8276	18.8276
	250	6.28665	0.004	6.286653	1	0.02514661	250	0.158088	263.7781	5.511243	5.511243
	500	11.80453	0.002	11.80453	1	0.02360905	500	0.278694	497.4838	-0.50324	0.503236
	10	0.25475	0.1	0.254753	1	0.0254753	10	0.00649	8.301112	-16.9889	16.98888
	20	0.46347	0.05	0.463467	1	0.02317335	20	0.01074	17.14105	-14.2947	14.29475
	50	0.94908	0.02	0.949075	1	0.0189815	50	0.018015	37.70864	-24.5827	24.58271
	100	1.95758	0.01	1.957582	1	0.01957582	100	0.038321	80.42326	-19.5767	19.57674
	250	5.44247	0.004	5.442468	1	0.02176987	250	0.118482	228.0232	-8.79071	8.790714
	500	9.78285	0.002	9.782845	1	0.01956569	500	0.191408	411.8569	-17.6286	17.62862
	10	0.29981	0.1	0.29981	1	0.02998103	10	0.008989	10.20948	2.094815	2.094815
	20	0.55932	0.05	0.559321	1	0.02796604	20	0.015642	21.20087	6.004351	6.004351
	50	1.40843	0.02	1.408426	1	0.02816852	50	0.039673	57.16413	14.32826	14.32826
	100	2.58069	0.01	2.580687	1	0.02580687	100	0.066599	106.8144	6.814436	6.814436
	250	6.36041	0.004	6.360409	1	0.02544164	250	0.161819	266.902	6.760788	6.760788
	500	12.69487	0.002	12.69487	1	0.02538974	500	0.322319	535.1937	7.038736	7.038736
	10	0.25360	0.1	0.2536	1	0.02535996	10	0.006431	8.252263	-17.4774	17.47737
	20	0.48865	0.05	0.488646	1	0.02443228	20	0.011939	18.20747	-8.96265	8.962655
	50	1.08332	0.02	1.083321	1	0.02166642	50	0.023472	43.39455	-13.2109	13.2109
	100	2.09596	0.01	2.095959	1	0.02095959	100	0.04393	86.28414	-13.7159	13.71586
	250	5.53419	0.004	5.534193	1	0.02213677	250	0.122509	231.9082	-7.23673	7.236732
	500	10.23135	0.002	10.23135	1	0.02046271	500	0.209361	430.8532	-13.8294	13.82936
	10	0.29213	0.1	0.292129	1	0.02921285	10	0.008534	9.884128	-1.15872	1.15872
	20	0.60799	0.05	0.60799	1	0.03039948	20	0.018483	23.26221	16.31103	16.31103
	50	1.41181	0.02	1.411814	1	0.02823629	50	0.039864	57.30766	14.61531	14.61531
	100	3.11917	0.01	3.119174	1	0.03119174	100	0.097292	129.6217	29.6217	29.6217
	250	7.11643	0.004	7.11643	1	0.02846572	250	0.202574	298.9227	19.56908	19.56908
	500	13.33480	0.002	13.3348	1	0.0266696	500	0.355634	562.2975	12.45951	12.45951
Sum	4650	111.55094	0.93	111.5509	30	0.76295799	4650	2.721895			363.8228
Slope	0.02361035										
Intercept	0.05876086										
R^2	0.98961522										

	C_{nom}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	%RE	|%RE|
	10	0.28553	0.01	0.02855284	0.1	0.002855	1	0.000815	10.18836	1.883605	1.883604624
	20	0.55939	0.0025	0.0279696	0.05	0.001398	1	0.000782	21.48876	7.443776	7.443775915
	50	1.42739	0.0004	0.02854789	0.02	0.000571	1	0.000815	57.30501	14.61003	14.61002833
	100	2.86432	0.0001	0.02864322	0.01	0.000286	1	0.00082	116.5967	16.59672	16.59671929
	250	6.28665	0.000016	0.02514661	0.004	0.000101	1	0.000632	257.8119	3.124755	3.1247547
	500	11.80453	0.000004	0.02360905	0.002	4.72E-05	1	0.000557	485.4951	-2.90099	2.900989913
	10	0.25475	0.01	0.0254753	0.1	0.002548	1	0.000649	8.918478	-10.8152	10.81522064
	20	0.46347	0.0025	0.02317335	0.05	0.001159	1	0.000537	17.53061	-12.3469	12.34693669
	50	0.94908	0.0004	0.0189815	0.02	0.00038	1	0.00036	37.56818	-24.8636	24.86363338
	100	1.95758	0.0001	0.01957582	0.01	0.000196	1	0.000383	79.18206	-20.8179	20.81794241
	250	5.44247	0.000016	0.02176987	0.004	8.71E-05	1	0.000474	222.9784	-10.8086	10.80864397
	500	9.78285	0.000004	0.01956569	0.002	3.91E-05	1	0.000383	402.0747	-19.5851	19.58505592
	10	0.29981	0.01	0.02998103	0.1	0.002998	1	0.000899	10.77767	7.776688	7.776687519
	20	0.55932	0.0025	0.02796604	0.05	0.001398	1	0.000782	21.48581	7.429059	7.429059009
	50	1.40843	0.0004	0.02816852	0.02	0.000563	1	0.000793	56.52231	13.04461	13.044613
	100	2.58069	0.0001	0.02580687	0.01	0.000258	1	0.000666	104.8931	4.893134	4.893134199
	250	6.36041	0.000016	0.02544164	0.004	0.000102	1	0.000647	260.8552	4.342099	4.342098914
	500	12.69487	0.000004	0.02538974	0.002	5.08E-05	1	0.000645	522.2331	4.446627	4.44662744
	10	0.25360	0.01	0.02535996	0.1	0.002536	1	0.000643	8.870887	-11.2911	11.29112902
	20	0.48865	0.0025	0.02443228	0.05	0.001222	1	0.000597	18.56955	-7.15225	7.152250686
	50	1.08332	0.0004	0.02166642	0.02	0.000433	1	0.000469	43.10756	-13.7849	13.78487294
	100	2.09596	0.0001	0.02095959	0.01	0.00021	1	0.000439	84.8919	-15.1081	15.10809711
	250	5.53419	0.000016	0.02213677	0.004	8.85E-05	1	0.00049	226.7632	-9.29471	9.294708337
	500	10.23135	0.000004	0.02046271	0.002	4.09E-05	1	0.000419	420.5815	-15.8837	15.88370698
	10	0.29213	0.01	0.02921285	0.1	0.002921	1	0.000853	10.4607	4.606995	4.606995451
	20	0.60799	0.0025	0.03039948	0.05	0.00152	1	0.000924	23.49403	17.47013	17.47013218
	50	1.41181	0.0004	0.02823629	0.02	0.000565	1	0.000797	56.66213	13.32427	13.32426686
	100	3.11917	0.0001	0.03119174	0.01	0.000312	1	0.000973	127.1127	27.11266	27.11265652
	250	7.11643	0.000016	0.02846572	0.004	0.000114	1	0.00081	292.0508	16.82033	16.82032744
	500	13.33480	0.000004	0.0266696	0.002	5.33E-05	1	0.000711	548.6385	9.727707	9.727706622
	4650	111.55094	0.0651	0.76295799	0.93	0.025052	30	0.019768			349.306376
Slope	0.02423487										
Intercept	0.03861481										
R^2	0.98454674										

Validation Study 1

LINEARITY

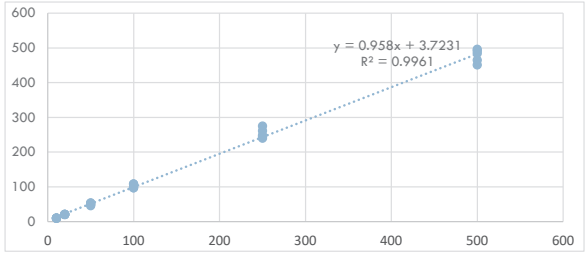
Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

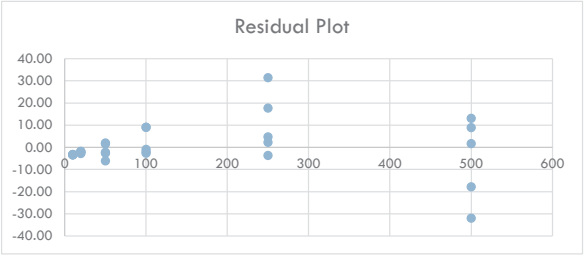
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Study Date	Target (x)	Calculated (y)	Predicted	Residual
AMP_20200814B_SD	10	9.75	13.30	-3.55
	20	20.24	22.88	-2.65
	50	53.46	51.62	1.83
	100	108.45	99.52	8.93
	250	239.44	243.22	-3.78
	500	450.63	482.72	-32.10
AMP_20200814B_PK	10	10.01	13.30	-3.29
	20	20.65	22.88	-2.23
	50	45.41	51.62	-6.21
	100	96.82	99.52	-2.70
	250	274.48	243.22	31.26
	500	495.75	482.72	13.03
AMP_20200828B_PK	10	9.80	13.30	-3.50
	20	20.98	22.88	-1.91
	50	49.25	51.62	-2.37
	100	97.40	99.52	-2.12
	250	260.89	243.22	17.66
	500	484.23	482.72	1.51
AMP_20200828B_SD	10	9.91	13.30	-3.39
	20	19.99	22.88	-2.89
	50	52.98	51.62	1.36
	100	98.53	99.52	-0.99
	250	245.39	243.22	2.17
	500	491.52	482.72	8.80
AMP_20200901B_CLR	10	9.78	13.30	-3.52
	20	20.80	22.88	-2.08
	50	48.84	51.62	-2.78
	100	108.41	99.52	8.89
	250	247.87	243.22	4.64
	500	464.81	482.72	-17.91

Slope	0.9580
Std err in slope, S_b	0.0113
Degrees freedom	28
Confidence level	95%
Student t	2.0484
Confidence interval	0.023
Slope	0.958 ± 0.0232
Range	0.9349 - 0.9812
	NO

Intercept	3.7231
Std err in Intercept	2.6338
Degrees freedom	28
Confidence Level	95%
Student t	2.0484
Confidence interval	5.395
Intercept	3.7231 ± 5.3951
Lower	-1.6720 - 9.1182
	YES





Comments: The linearity of the method is acceptable because the individual calibration curves on all five days met the acceptance criteria. Also, other analytical data including the ion ratios and quantification values of the controls and calibrators met acceptance criteria.

Acceptance Criteria:

95% CI of slope includes 1
95% CI of intercept includes 0

Validation Study 1

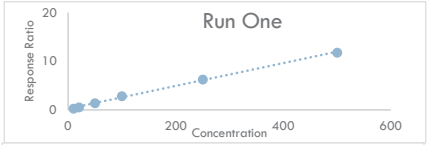
VALIDATION CURVE CALIBRATION

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

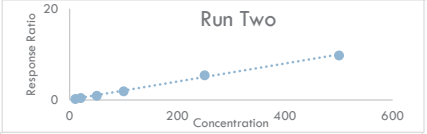
Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
AMP_20200814B_SD	10	9.75	2.45	0.286
	20	20.24	1.18	0.559
	50	53.46	6.92	1.427
	100	108.45	8.45	2.864
	250	239.44	4.22	6.287
	500	450.63	9.87	11.805
AMP_20200814B_PK	10	10.01	0.14	0.255
	20	20.65	3.27	0.463
	50	45.41	9.18	0.949
	100	96.82	3.18	1.958
	250	274.48	9.79	5.442
	500	495.75	0.85	9.783
AMP_20200828B_PK	10	9.80	1.99	0.254
	20	20.98	4.88	0.489
	50	49.25	1.49	1.083
	100	97.40	2.60	2.096
	250	260.89	4.36	5.534
	500	484.23	3.15	10.231
AMP_20200828B_SD	10	9.91	0.92	0.300
	20	19.99	0.04	0.559
	50	52.98	5.97	1.408
	100	98.53	1.47	2.581
	250	245.39	1.84	6.360
	500	491.52	1.70	12.695
AMP_20200901B_CLR	10	9.78	2.20	0.2921
	20	20.80	4.00	0.6080
	50	48.84	2.31	1.4118
	100	108.41	8.41	3.1192
	250	247.87	0.85	7.1164
	500	464.81	7.04	13.3348

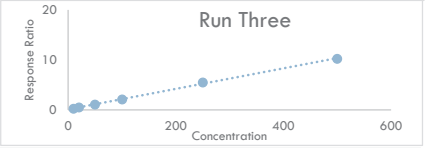
Max %RE = 9.87



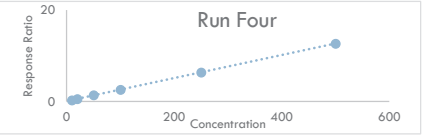
Slope	0.02341
Intercept	0.24270
R^2	0.99902



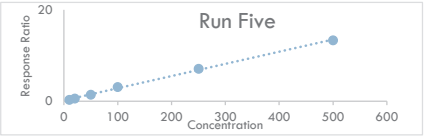
Slope	0.01977
Intercept	0.07735
R^2	0.99835



Slope	0.02053
Intercept	0.09844
R^2	0.99923



Slope	0.02523
Intercept	0.07352
R^2	0.99997



Slope	0.02665
Intercept	0.18352
R^2	0.99913

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 2

Sensitivity (LOD)

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

	Signal to Noise Ratio			
Concentration (ng/mL)	*10*			
AMP_20200814B_SD	8441			
	7976			
	2980			
	3684			
	10782			
	9267			
	9171			
	6879			
	2863			
AMP_20200828B_PK	2017			
	1609			
	2447			
	2554			
	4242			
	6556			
	6512			
	1328			
	2342			
AMP_20200901B_PK	2215			
	3339			
	2370			
	7272			
	4066			
	2492			
	4689			
	3738			
	4437			
AMP_20200901B_CLR	1264			
	4223			
	1790			
	4398			
	981			
	845			
	4450			
	2314			
	3130			
Average Signal to Noise:	**4157**			

Established LOD: 10 ng/mL
Signal to Noise: 4157

Comments: The lowest non-zero calibrator was defined as the LOD.

Acceptance Criteria:
Signal to Noise ≥3.3
Acceptable detection and identification criteria

Validation Study 3

SENSITIVITY (LOQ)

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LOQ
	Target Concentration (ng/mL):	*10*
AMP_20200814B_SD	1-1	10.30
	1-2	10.37
	1-3	9.76
	1-4	9.94
	1-5	10.14
	1-6	11.14
	1-7	10.27
	1-8	10.09
	1-9	9.86
Within Run	**Mean**	**10.21**
	SD	**0.41**
	%CV	**3.98%**
	% Bias	**2.08%**
AMP_20200828B_PK	2-1	10.32
	2-2	9.91
	2-3	10.15
	2-4	9.87
	2-5	9.89
	2-6	3.95*
	2-7	10.10
	2-8	10.25
	2-9	9.85
Within Run	**Mean**	**10.04**
	SD	**0.19**
	%CV	**1.86%**
	% Bias	**0.42%**
AMP_20200901B_CLR	3-1	53.71*
	3-2	10.17
	3-3	9.78
	3-4	9.98
	3-5	9.00
	3-6	10.44
	3-7	12.77
	3-8	10.08
	3-9	10.15
Within Run	**Mean**	**10.30**
	SD	**1.09**
	%CV	**10.57%**
	% Bias	**2.97%**
AMP_20200901B_PK	4-1	9.97
	4-2	9.78
	4-3	9.70
	4-4	10.19
	4-5	10.64
	4-6	10.37
	4-7	10.44
	4-8	10.09
	4-9	10.20
Within Run	**Mean**	**10.15**
	SD	**0.31**
	%CV	**3.01%**
	% Bias	**1.53%**

Mean		**10.18**
SD		**0.57**
Precision	***Max Within-Run***	**10.57%**
(%CV)	***Between-Run***	**5.64%**
% Bias		**1.75%**

Comments: Blank blood sources 5 (lot 264184),7 (lot 228467), 8 (lot 367648), 9 (lot 331307), 10 (lot 335183), 11 (lot 233762), 12 (lot 235659), 13 (lot 223450), and 14 (lot 279720) were used for this study. Number in red was > 20% target value. Numbers with * were > 20% target value due to insufficient IS.

Acceptance Criteria:
Bias: ≤20%
Within-Run Precision: CV ≤20%
Between-Run Precision: CV ≤20%

Validation Study 4

BIAS AND PRECISION

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Run Order	LQC	MQC	UTAK	HQC
	Target Concentration (ng/mL):	*25*	*100*	*82.34*	*400*
AMP_20200814B_SD	1-1	25.66	96.60	69.69	358.47
	1-2	24.43	93.24	74.01	350.50
	1-3	25.30	95.17	70.39	344.17
	1-4			74.35	
Within Run	Mean	25.13	95.00	72.11	351.04
	SD	0.63	1.68	2.41	7.17
	%CV	2.52%	1.77%	3.34%	2.04%
	% Bias	0.53%	-5.00%	-12.42%	-12.24%
AMP_20200814B_PK	2-1	24.26	80.62	102.78	426.73
	2-2	28.26	82.98	101.94	439.33
	2-3	27.20	90.29	107.27	415.71
	2-4			109.20	
Within Run	Mean	26.58	84.63	105.30	427.26
	SD	2.07	5.04	3.50	11.82
	%CV	7.79%	5.96%	3.32%	2.77%
	% Bias	6.30%	-15.37%	27.89%	6.81%
AMP_20200828B_PK	3-1	25.63	91.83	77.54	381.13
	3-2	28.33	100.48	75.89	368.66
	3-3	24.85	88.94	73.10	375.05
	3-4			75.10	
Within Run	Mean	26.27	93.75	75.41	374.95
	SD	1.82	6.00	1.84	6.24
	%CV	6.95%	6.41%	2.45%	1.66%
	% Bias	5.07%	-6.25%	-8.42%	-6.26%
AMP_20200828B_SD	4-1	23.38	100.15	78.93	365.75
	4-2	24.06	96.29	82.66	362.48
	4-3	24.40	96.33	70.66	360.60
	4-4			73.85	
Within Run	Mean	23.95	97.59	76.53	362.95
	SD	0.52	2.21	5.32	2.61
	%CV	2.18%	2.27%	6.96%	0.72%
	% Bias	-4.22%	-2.41%	-7.05%	-9.26%
AMP_20200901B_CLR	5-1	27.90	115.45		353.38
	5-2	29.76	78.96		370.27
	5-3	25.96	92.23		374.32
Within Run	Mean	27.87	95.54		365.99
	SD	1.90	18.47		11.10
	%CV	6.82%	19.33%		3.03%
	% Bias	11.49%	-4.46%		-8.50%
AMP_20200901B_PK	6-1	24.54	81.84		374.15
	6-2	24.87	91.08		368.35
	6-3	24.82	84.84		363.33
Within Run	Mean	24.74	85.92		368.61
	SD	0.17	4.71		5.41
	%CV	0.71%	5.48%		1.47%
	% Bias	-1.03%	-14.08%		-7.85%

Mean		25.76	92.07	82.34	375.13
SD		1.79	8.77	14.15	26.00
Precision (%CV)	*Max Within-Run*	7.79%	19.33%	6.96%	3.03%
	Between-Run	6.93%	9.52%	17.19%	6.93%
% Bias		3.02%	-7.93%	0.00%	-6.22%

Comments: Number in red was not within 20% of the target value.

Acceptance Criteria:

%Bias ≤20%
Within-Run %CV ≤20%
Between-Run %CV ≤20%

Validation Study 5

CARRYOVER

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Average LOQ Response*: 203508

Study Date:	Response		
	AMP_20200814B_SD	AMP_20200814B_PK	AMP_20200828B_PK
Concentrated Sample (2000 ng/mL)	13331962	12785352	17480700
Blank	4214	5571	7809
%LOD Response	**2.07%**	**2.74%**	**3.84%**
		Maximum Response in Blank:	**3.8%**

Results: No significant carryover observed following samples containing analyte at up to 2000 ng/mL.
Comments: N/A

Acceptance Criteria: **No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.**

Validation Study 6

MATRIX & IS INTERFERENCE

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

LOQ Response

	Analyte	IS
Run 1	255002	893087
Run 2	145439	570902
Run 3	182272	600000
Run 4	214297	845019
Run 5	220528	754901
Average	**203508**	**732782**

Matrix Interference AMP_20200814B_PK

Matrix Source	Peak at Analyte RT	
	Peak Response	Percent of LOQ Response
4	214	0.11%
5	266	0.13%
7	229	0.11%
8	821	0.40%
9	970	0.48%
10	722	0.35%
11	608	0.30%
12	363	0.18%
13	558	0.27%
14	1222	0.60%

Interference from Stable-Isotope Internal Standards Study Date: AMP_20200828B_SD

Experiment	Peak at Analyte RT		Peak at IS RT	
	Peak Response	Percent of LOQ Response	Peak Response	Percent of LOQ Response
Matrix with IS but no D0 (IS = 50 ng/mL)	8177	4.02%	N/A	N/A
Matrix with D0 but no IS (D0 = 400 ng/mL)	N/A	N/A	246	0.03%

Matrix Interference: No significant interference observed.
IS Interference: No significant interference observed.
Comments: LOQ reponse per run was taken from the lowest calibrator in analytical runs between 8/14/2020 and 9/1/2020.

Acceptance Criteria: **Response of blank matrix is less than 20% the average response of LOQ**

Validation Study 6 | **EXOGENOUS SUBSTANCE INTERFERENCE**

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target LQC Concentration (µg/mL): 25.76
Control Acceptance: 20%
Run Date: AMP_20200828B_SD

Group	Compound	Compound Concentration (µg/mL)	Calculated LQC Concentration (ng/mL)	% Difference from Target	Comment
Benzodiazepines	Alprazolam	1	24.66	-4%	No significant interference
	α-Hydroxyalprazolam				
	Clonazepam				
	7-Aminoclonazepam				
	Diazepam				
	Nordiazepam				
	Temazepam				
	Lorazepam				
	Oxazepam				
	Zolpidem				
Phencyclidine	Phencyclidine	1	26.06	1%	No significant interference
Opioids	Morphine	2	24.38	-5%	No significant interference
	Hydrocodone	1			
	Buprenorphine	0.5			
	Norbuprenorphine				
	Codeine				
	Fentanyl				
	Norfentanyl oxalate				
	Hydromorphone				
	Methadone				
	EDDP				
	Oxycodone				
	Oxymorphone				
	Tramadol				
	o-Desmethyltramadol				
Cocaine and Metabolites	Benzoylecgonine	2	24.67	-4%	No significant interference
	Cocaine	0.5			
	Cocaethylene				
Carisoprodol/Meprobamate	Carisoprodol	1	24.60	-4%	No significant interference
	Meprobamate				
Basic and Neutral Mix	Amitriptyline	5	24.77	-4%	No significant interference
	Benzylpiperizine				
	Chlorpheniramine				
	Cyclobenzaprine				
	Dextromethorphan				
	Diphenhydramine				
	Doxylamine				
	Fluoxetine				
	Imipramine				
	Ketamine				
	Norketamine				
	Meperidine				
	Nortriptyline				
	Propoxyphene				
	Sertraline				
	Trazodone				
	Venlafaxine				
	Zopiclone				
Over-the-Counter Drugs	Acetaminophen	10	23.25	-10%	No significant interference
	Caffeine				
	Ibuprofen				
	Naproxen				
	Pseudoephedrine				
	Theobromine	1			
Cannabinoids	Δ9-THC	0.5	25.14	-2%	No significant interference
	11-Hydroxy-Δ9-THC				
	Δ9-THC-COOH				
	Δ8-THC				
	Cannabinol				
	Cannabinolic acid				

Conclusions: No significant interference observed.

Comments: N/A

Acceptance Criteria: Concentrations of analytes of interest within ±20% of the average concentration obtained in the Bias and Precision studies

Validation Study 7

DILUTION INTEGRITY

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Target Concentration (ng/mL): 342.17

Run Date	Dilution Factor	Diluted Result (ng/mL)	Calculated Result (µg/mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
AMP_20200814B_SD	2	173.02	346.04	333.83	18.06	1.13	1.13	5.41
	2	171.18	342.37			0.06	0.06	
	2	156.55	313.09			-8.50	8.50	
	5	70.76	353.79	339.35	18.31	3.40	3.40	5.40
	5	69.10	345.51			0.98	0.98	
	5	63.75	318.76			-6.84	6.84	
	10	37.03	370.33	345.31	33.58	8.23	8.23	9.72
	10	35.84	358.44			4.75	4.75	
	10	30.71	307.15			-10.24	10.24	
AMP_20200828B_PK	2	181.92	363.84	385.45	21.91	6.33	6.33	5.68
	2	192.43	384.86			12.48	12.48	
	2	203.82	407.64			19.13	19.13	
	5	71.61	358.04	382.43	21.13	4.64	4.64	5.52
	5	79.00	395.01			15.44	15.44	
	5	78.85	394.23			15.22	15.22	
	10	34.14	341.35	375.27	29.47	-0.24	0.24	7.85
	10	39.47	394.70			15.35	15.35	
	10	38.98	389.76			13.91	13.91	
AMP_20200828B_SD	2	178.64	357.29	353.29	9.61	4.42	4.42	2.72
	2	180.12	360.25			5.28	5.28	
	2	171.16	342.32			0.04	0.04	
	5	71.77	358.86	353.39	13.78	4.88	4.88	3.90
	5	72.72	363.60			6.26	6.26	
	5	67.54	337.72			-1.30	1.30	
	10	31.55	315.48	327.50	13.75	-7.80	7.80	4.20
	10	34.25	342.49			0.09	0.09	
	10	32.45	324.53			-5.15	5.15	
AMP_20200901B_CLR	2	148.20	296.40	330.84	34.40	-13.38	13.38	10.40
	2	182.60	365.19			6.73	6.73	
	2	165.46	330.92			-3.29	3.29	
	5	70.43	352.15	369.65	15.35	2.92	2.92	4.15
	5	75.19	375.97			9.88	9.88	
	5	76.17	380.83			11.30	11.30	
	10	35.70	357.01	350.04	6.16	4.34	4.34	1.76
	10	34.54	345.37			0.93	0.93	
	10	34.77	347.73			1.63	1.63	
AMP_20200901B_PK	2	190.84	381.68	393.43	10.35	11.54	11.54	2.63
	2	200.59	401.17			17.24	17.24	
	2	198.72	397.44			16.15	16.15	
	5	71.09	355.44	383.83	25.11	3.88	3.88	6.54
	5	78.57	392.87			14.82	14.82	
	5	80.63	403.17			17.83	17.83	
	10	39.58	395.79	377.29	25.93	15.67	15.67	6.87
	10	34.77	347.65			1.60	1.60	
	10	38.84	388.41			13.51	13.51	

Dilution Factor	2
Avg %Bias	8.38
Max Within-Run Precision (%CV)	10.40

Dilution Factor	5
Avg %Bias	7.97
Max Within-Run Precision (%CV)	6.54

Dilution Factor	10
Avg %Bias	6.90
Max Within-Run Precision (%CV)	9.72

Results: It is suitable to dilute blood samples 2x, 5x, or 10x prior to analysis.
Comments: N/A

Acceptance Criteria: Avg %Bias must be less than 20%

Validation Study 8

PROCESSED SAMPLE STABILITY

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Run Date	Sample	Time Zero Response	24H Response	48H Response	72H Response	Acceptable Range		Pass/Unstable
						Low	High	
	LQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			
	HQC	N/A	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A
		N/A	N/A	N/A	N/A			
		N/A	N/A	N/A	N/A			

Results:

Comments: Study not performed. Sample preparation will be completed once started without prolonged interruptions.

Acceptance Criteria: Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%

Validation Study 8

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

AUTOSAMPLER STABILITY

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Stability of Punctured Calibrators

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero (Response Ratio)
AMP_20200923B_SD & AMP_20200924B_SD	Calibrator 1	9.89	0.29	9.79	9.86	0.29	-1%
	Calibrator 2	19.85	0.55	19.75	19.46	0.55	0%
	Calibrator 3	53.88	1.42	53.90	52.37	1.42	0%
	Calibrator 4	101.92	2.64	102.29	99.01	2.65	0%
	Calibrator 5	240.68	6.18	243.40	235.00	6.25	1%
	Calibrator 6	479.71	12.27	479.52	462.54	12.27	0%

Stability of Punctured Calibrators

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	Calibrator 1	10.06	0.30	N/A	N/A	N/A	N/A	10.18	10.14	0.31	1%
	Calibrator 2	20.06	0.56	N/A	N/A	N/A	N/A	20.18	19.72	0.56	1%
	Calibrator 3	47.26	1.25	N/A	N/A	N/A	N/A	47.05	45.48	1.25	0%
	Calibrator 4	102.43	2.67	N/A	N/A	N/A	N/A	103.02	99.14	2.68	1%
	Calibrator 5	254.18	6.55	N/A	N/A	N/A	N/A	252.42	242.36	6.50	-1%
	Calibrator 6	502.21	12.90	N/A	N/A	N/A	N/A	500.99	480.65	12.86	0%

Stability of Punctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	Response Ratio
AMP_20200923B_SD & AMP_20200924B_SD	LQC	25.34	0.69	24.79	24.32	0.67	-2%
		25.03	0.68	25.38	24.89	0.69	1%
		24.76	0.67	25.22	24.74	0.69	2%
	HQC	358.65	9.19	360.16	347.51	9.23	0%
		356.12	9.12	360.40	347.75	9.23	1%
		356.62	9.14	359.21	346.60	9.20	1%

Stability of Punctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	LQC	25.45	0.70	N/A	N/A	N/A	N/A	25.56	24.88	0.70	0%
		25.36	0.69	N/A	N/A	N/A	N/A	25.45	24.78	0.70	0%
		25.63	0.70	N/A	N/A	N/A	N/A	25.20	24.53	0.69	-2%
	HQC	363.52	9.35	N/A	N/A	N/A	N/A	359.53	345.04	9.24	-1%
		360.43	9.27	N/A	N/A	N/A	N/A	360.98	346.43	9.28	0%
		363.25	9.34	N/A	N/A	N/A	N/A	362.14	347.55	9.31	0%

Stability of Unpunctured Controls

		Time Zero		24 Hours			
Run Date	Sample	Concentration (Time Zero Curve)	Response Ratio	Concentration (Time Zero Curve)	Concentration (24 h Curve)	Response Ratio	Response Ratio
AMP_20200923B_SD & AMP_20200924B_SD	LQC	25.34	0.69	25.04	24.56	0.68	-1%
		25.03	0.68	25.35	24.87	0.69	1%
		24.76	0.67	24.98	24.50	0.68	1%
	HQC	358.65	9.19	360.25	347.60	9.23	0%
		356.12	9.12	358.85	346.25	9.19	1%
		356.62	9.14	361.01	348.33	9.25	1%

Stability of Unpunctured Controls

		Time Zero		24 Hours				48 Hours			
Run Date	Sample	Concentration (Time Zero	Response Ratio	Concentration (Time Zero	Concentration (24 h Curve)	Response Ratio	% Difference from Time Zero	Concentration (Time Zero	Concentration (48 h Curve)	Response Ratio	% Difference from Time
AMP_20201019B_SD & AMP_20201021B_SD	LQC	25.45	0.70	N/A	N/A	N/A	N/A	25.34	24.66	0.69	0%
		25.36	0.69	N/A	N/A	N/A	N/A	25.42	24.74	0.70	0%
		25.63	0.70	N/A	N/A	N/A	N/A	25.27	24.60	0.69	-1%
	HQC	363.52	9.35	N/A	N/A	N/A	N/A	360.98	346.44	9.28	-1%
		360.43	9.27	N/A	N/A	N/A	N/A	361.63	347.06	9.30	0%
		363.25	9.34	N/A	N/A	N/A	N/A	363.15	348.52	9.34	0%

Results: Punctured and unpunctured samples were shown to be stable for 48 hours.

Comments: AMP_20200904B_SD showed the punctured and unpunctured samples for methamphetamine at 72 hr were not stable; therefore the 72 hr data for MDEA were not included. These data can be found in a supplemental Excel located in the Toxicology section shared electronic storage.

Acceptance Criteria: **Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 20%**

Validation Study 9

MATRIX EFFECTS

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

Neat Response at Analyte RT

	LQC	HQC
1	407305	3900146
2	382858	3874153
3	397271	3911042
4	398607	3914083
5	384454	3729381
6	382650	3935315
Mean	**392191**	**3877353**
SD	10328	75192
%CV	3%	2%

Neat Response at IS RT

LQC	HQC
586466	448869
547858	451012
629070	452954
610671	437647
570033	471021
577715	425476
586969	**447830**
29109	15354
5%	3%

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at Analyte RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	332993	-15%	3520615	-9%
5	276253	-30%	3645120	-6%
7	284383	-27%	3546662	-9%
8	321140	-18%	3358029	-13%
9	291710	-26%	3403063	-12%
10	321126	-18%	2874818	-26%
11	332387	-15%	3543726	-9%
12	353234	-10%	3613103	-7%
13	278037	-29%	3579524	-8%
14	331703	-15%	3729843	-4%
Mean	312297		3481450	
SD	27318		239091	
%CV	9%		7%	
Avgerage % suppression/enhancement	-20%		-10%	

Matrix Effect Study Date: AMP_20200904B_SD

Matrix Source	Peak at IS RT			
	Peak Response at LQC	% suppression/enhancement	Peak Response at HQC	% suppression/enhancement
4	546608	-7%	418968	-6%
5	457871	-22%	427257	-5%
7	472156	-20%	426408	-5%
8	525896	-10%	401327	-10%
9	480321	-18%	406874	-9%
10	535505	-9%	336457	-25%
11	536677	-9%	417987	-7%
12	582285	-1%	433751	-3%
13	448073	-24%	420820	-6%
14	547079	-7%	444020	-1%
Mean	513247		413387	
SD	45111		29681	
%CV	9%		7%	
Avgerage % suppression/enhancement	-13%		-8%	

Results: Average percent suppression values for LQC and HQC were -20% and -10%, respectively.

Comments: N/A

Acceptance Criteria: **Average suppression/enhancement ≤25% or the ≤%CV of the suppression/enhancement 20%**

SUMMARY OF VALIDATION PERFORMANCE

Analyte: MDEA
Units: ng/mL
Instrument: LCMS-1

Analyst: SD, PK, and CLR
Study Dates: 8/14/2020 to 9/24/2020
Matrix: Blood

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

Date	Reason
N/A	N/A

Deviations from SOP: N/A

Other Observations:
Working Standards Verified in Validation:
Calibrators: 200805C-C-10, 200805C-C-2.5, 200805C-C-0.2, 200923C-C-10, 200923C-C-2.5, 200923C-C-0.2
Controls:200807L-Q-Mix, 200813L-Q-0.5, 200813L-St-5
Internal Standard: 200805C-IS-1, 200903C-IS-1, 200915C-IS-1

Sample Preparation Steps: Refer to Toxicology Analytical Manual v3.5, "Amphetamines Confirmation by Liquid Chromatography-Tandem Mass Spectrometry" section titled "Extraction Procedure".

Location of Raw Data: Toxicology section shared electronic storage.

Recommended Maximum Run Length (Unknown Samples): 30

Conclusion: **This method is fit for use on casework for MDEA confirmation analysis in blood.**