



Validation of Quantitative Methods

Purpose	To validate the method using a linear regression model for morphine, codeine, o-desmethyltramadol, 6-acetylmorphine, oxycodone, and tramadol by adjusting various parameters for these analytes including ULOQs, internal standard concentrations, and ion transitions.
Analyte	Opioids (morphine, oxymorphone, hydromorphone, codeine, o-desmethyltramadol, 6-acetylmorphine, oxycodone, hydrocodone, tramadol, norbuprenorphine, and buprenorphine)
Unit of Measure	ng/mL
Analyst Performing Validation Studies	Jami Reber, Kalya M. Yang, Cassandra Duvall, Corissa L. Rodgers
Responsible Supervisor	Dayong Lee
Start Date	April 14, 2022
Completion Date	April 27, 2022
Primary Matrix	Blood
Secondary Matrix	N/A
Lowest Calibrator Concentration	5 ng/mL for morphine, oxymorphone, hydromorphone, codeine, o-desmethyltramadol, 6-acetylmorphine, oxycodone, hydrocodone, and tramadol; 0.5 ng/mL for norbuprenorphine, and buprenorphine
Highest Calibrator Concentration	125 ng/mL for morphine, codeine, o-desmethyltramadol, 6-acetylmorphine, oxycodone, and tramadol; 500 ng/mL for oxymorphone, hydromorphone, and hydrocodone; 100 ng/mL for norbuprenorphine and buprenorphine
Equipment/Instrument	LCMS-1
Instrument Serial Number	SG1939G104
Method	OPI1.M

Validation Approval

Analyst: _____ Date

Analyst: _____ Date

Analyst: _____ Date

Analyst: _____ Date

Responsible Supervisor: _____ Date

Quality Division: _____ Date

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Morphine
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 339.78 1/x Weighting: = 238.37 1/x2 Weighting: = 231.71	1/x weighting will be used. While 1/x2 weighting gave a lower %RE, data processed using 1/x2 weighting failed to meet the SOP criteria for linearity.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9623 - 1.0039 95% CI of Intercept = -0.5101 - 2.1468	N/A
1	Validation Calibration	%RE Calibrators $\pm 20\%$ of target	Max %RE of Calibrators = 13.75	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ 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	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = -9.02% Max Within-Run Precision = 6.70% Max Between-Run Precision = 5.18%	N/A

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	$\Sigma \%RE $	C_{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
Unweighted:	339.78	5.0	0.048	1	0.237878	5	0.04757568	25	0.002263	3.830101	-23.398	23.39798
1/x Weighting:	238.37	10	0.095	1	0.950582	10	0.09505823	100	0.009036	9.342706	-6.57294	6.572938
1/x ² Weighting:	231.71	25	0.220	1	5.501025	25	0.22004102	625	0.048418	23.8529	-4.58842	4.588416
		50	0.441	1	22.04836	50	0.44096728	2500	0.194452	49.50188	-0.99623	0.996232
		75	0.671	1	50.31824	75	0.67090987	5625	0.45012	76.19764	1.596859	1.596859
		125	1.051	1	131.3185	125	1.05054839	15625	1.103652	120.2727	-3.78182	3.781817
		5.0	0.048	1	0.237721	5	0.04754413	25	0.00226	3.826437	-23.4713	23.47125
		10	0.097	1	0.970764	10	0.09707644	100	0.009424	9.577015	-4.22985	4.229853
		25	0.227	1	5.662579	25	0.22650315	625	0.051304	24.60313	-1.58746	1.587465
		50	0.435	1	21.75764	50	0.43515283	2500	0.189358	48.82694	-2.34632	2.346318
		75	0.670	1	50.27735	75	0.67036469	5625	0.449399	76.13435	1.512467	1.512467
		125	1.052	1	131.5072	125	1.05205748	15625	1.106825	120.4479	-3.64166	3.641655
		5.0	0.052	1	0.258314	5	0.05186281	25	0.00269	4.327828	-13.4435	13.44349
		10	0.114	1	1.139664	10	0.11396637	100	0.012988	11.53789	15.37894	15.37894
		25	0.291	1	7.27002	25	0.29080079	625	0.084565	32.06793	28.27172	28.27172
		50	0.481	1	24.05131	50	0.48102613	2500	0.231386	54.15262	8.305232	8.305232
		75	0.840	1	63.0352	75	0.84046934	5625	0.706389	95.88308	27.8441	27.8441
		125	1.222	1	152.722	125	1.22177583	15625	1.492736	140.1518	12.12145	12.12145
		5.0	0.050	1	0.247549	5	0.0495099	25	0.002451	4.054658	-18.9068	18.90684
		10	0.088	1	0.881925	10	0.08819255	100	0.007778	8.545618	-14.5438	14.54382
		25	0.210	1	5.249533	25	0.2099813	625	0.044092	22.68499	-9.26005	9.260047
		50	0.416	1	20.79225	50	0.41584492	2500	0.172927	46.58524	-6.82952	6.82952
		75	0.647	1	48.50134	75	0.64668458	5625	0.418201	73.38515	-2.15313	2.153135
		125	1.074	1	134.3006	125	1.07440499	15625	1.154346	123.0424	-1.56606	1.566063
		5.0	0.044	1	0.220618	5	0.04412367	25	0.001947	3.42933	-31.4134	31.41339
		10	0.090	1	0.901866	10	0.09018655	100	0.008134	8.777117	-12.2288	12.22883
		25	0.217	1	5.420453	25	0.21681813	625	0.04701	23.47873	-6.08509	6.085093
		50	0.434	1	21.71449	50	0.43428973	2500	0.188608	48.72864	-2.54673	2.546727
		75	0.690	1	51.78022	75	0.69040294	5625	0.476856	78.46074	4.614321	4.614321
		125	1.002	1	125.2953	125	1.00236277	15625	1.004731	114.6785	-8.2572	8.257201
		5.0	0.048	1	0.239106	5	0.04782126	25	0.002287	3.858612	-22.8278	22.82776
		10	0.103	1	1.03359	10	0.10335896	100	0.010683	10.3064	3.063996	3.063996
		25	0.229	1	5.723931	25	0.22895726	625	0.052421	24.88805	-0.4478	0.447805
		50	0.450	1	22.50275	50	0.45005508	2500	0.20255	50.55695	1.113909	1.113909
		75	0.615	1	46.10656	75	0.6147541	5625	0.377923	69.6781	-7.09587	7.095867
		125	1.051	1	131.378	125	1.05102397	15625	1.104651	120.3279	-3.73765	3.737645
Sum		1740.0	15.5125		32	1291.556	1740	15.5124731	147000	11.42465		339.7802
Slope			0.00861345									
Intercept			0.01458530									
R ²			0.98446320									

Comments: 1/x weighting will be used. While 1/x² weighting gave a lower %RE, data processed using 1/x² weighting failed to meet the SOP criteria for linearity.

Acceptance Criteria: The least complex weighting scheme that minimizes $\Sigma | \%RE |$

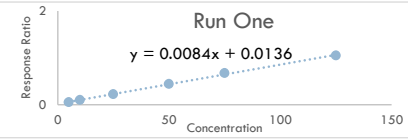
Validation Study 1

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

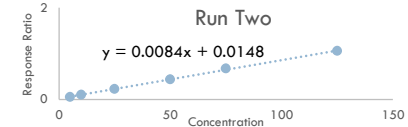
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

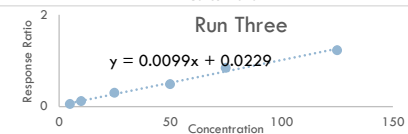
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	4.76	4.80	0.048
	10	10.30	3.00	0.095
	25	24.89	0.44	0.220
	50	50.68	1.36	0.441
	75	77.52	3.36	0.671
	125	121.84	2.53	1.051
OPI1_20220418B_JR	5	4.64	7.21	0.048
	10	10.43	4.28	0.097
	25	25.55	2.21	0.227
	50	49.93	0.13	0.435
	75	77.42	3.23	0.670
	125	122.02	2.38	1.052
OPI1_20220418B_KMY	5	4.35	13.00	0.052
	10	10.45	4.50	0.114
	25	27.80	11.20	0.291
	50	46.47	7.06	0.481
	75	81.75	9.00	0.840
	125	119.18	4.66	1.222
OPI1_20220421B_CD	5	5.27	5.47	0.050
	10	9.84	1.57	0.088
	25	24.23	3.10	0.210
	50	48.54	2.93	0.416
	75	75.80	1.07	0.647
	125	126.32	1.05	1.074
OPI1_20220421B_CLR	5	4.69	6.22	0.044
	10	10.14	1.40	0.090
	25	25.12	0.48	0.217
	50	50.85	1.70	0.434
	75	81.15	8.20	0.690
	125	118.05	5.56	1.002
OPI1_20220427B_CD	5	4.31	13.75	0.048
	10	10.95	9.52	0.103
	25	25.97	3.88	0.229
	50	52.41	4.81	0.450
	75	72.10	3.87	0.615
	125	124.26	0.59	1.051



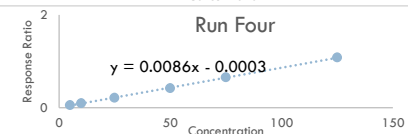
Slope 0.00843
 Intercept 0.01361
 R² 0.99861



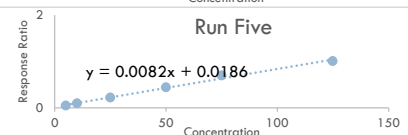
Slope 0.00841
 Intercept 0.01484
 R² 0.99878



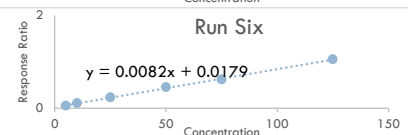
Slope 0.00987
 Intercept 0.02288
 R² 0.99091



Slope 0.00857
 Intercept -0.00030
 R² 0.99967



Slope 0.00816
 Intercept 0.01858
 R² 0.99244



Slope 0.00824
 Intercept 0.01790
 R² 0.99856

Max %RE = 13.75

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

LINEARITY

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

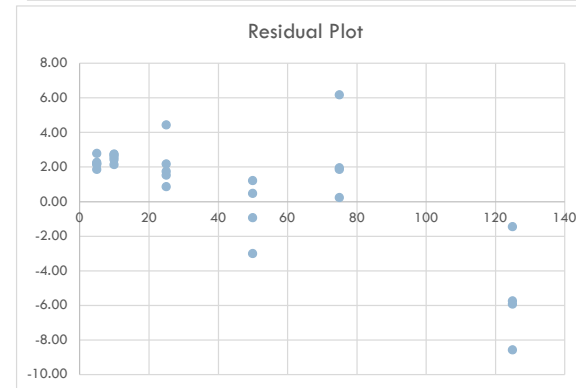
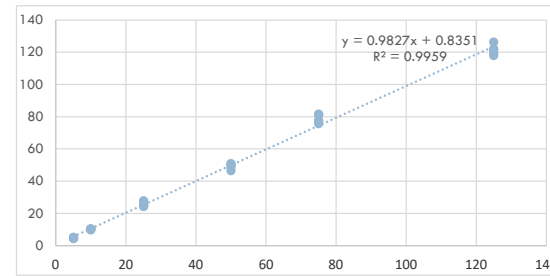
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	4.76	2.50	2.26
	10	10.30	7.72	2.58
	25	24.89	23.38	1.51
	50	50.68	49.48	1.20
	125	121.84	127.78	-5.94
OPI1_20220418B_JR	5	4.64	2.50	2.14
	10	10.43	7.72	2.71
	25	25.55	23.38	2.18
	50	49.93	49.48	0.46
	125	77.42	75.58	1.84
OPI1_20220418B_KMY	5	4.35	2.50	1.85
	10	10.45	7.72	2.73
	25	27.80	23.38	4.42
	50	46.47	49.48	-3.01
	125	119.18	127.78	-8.60
OPI1_20220421B_CD	5	5.27	2.50	2.78
	10	9.84	7.72	2.13
	25	24.23	23.38	0.85
	50	48.54	49.48	-0.94
	125	75.80	75.58	0.23
OPI1_20220421B_CLR	5	4.69	2.50	2.19
	10	10.14	7.72	2.42
	25	25.12	23.38	1.74
	50	50.85	49.48	1.37
	125	81.15	75.58	5.57
OPI1_20220427B_CD	5	4.31	2.50	1.82
	10	10.95	7.72	3.24
	25	25.97	23.38	2.59
	50	52.41	49.48	2.93
	125	72.10	75.58	-3.48

Slope	0.9831
Std err in slope, S_b	0.0102
Degrees freedom	34
Confidence level	95%
Student t	2.0322
Confidence interval	0.021
Slope	0.983 ± 0.021
Range	$0.9623 - 1.0039$

Intercept	0.8183
Std err in Intercept	0.6537
Degrees freedom	34
Confidence Level	95%
Student t	2.0322
Confidence interval	1.328
Intercept	0.818 ± 1.328
Lower	$-0.5101 - 2.1468$

YES

YES



Comments: N/A

Acceptance Criteria: 95% CI of slope includes 1
 95% CI of intercept includes 0

Validation Study 2

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	30	100	
OPI1_20220414B_JR	1-1	15.29	29.58	87.17	
	1-2	14.11	28.87	88.60	
	1-3	14.31	28.67	86.75	
	1-4	14.35	29.91	89.12	
	Within Run	Mean	14.52	29.26	87.91
		SD	0.53	0.58	1.13
		%CV	3.63%	2.00%	1.29%
	% Bias	-3.23%	-2.48%	-12.09%	
OPI1_20220418B_JR	2-1	14.55	28.85	92.72	
	2-2	14.85	28.22	91.59	
	2-3	14.84	29.80	92.54	
	Within Run	Mean	14.74	28.96	92.28
		SD	0.17	0.80	0.61
		%CV	1.17%	2.75%	0.66%
		% Bias	-1.71%	-3.47%	-7.72%
OPI1_20220414B_KMY	3-1	14.95	26.14	86.15	
	3-2	13.79	25.65	84.82	
	3-3	12.96	26.91	86.74	
	3-4	14.86	27.30	82.92	
	Within Run	Mean	14.14	26.50	85.16
		SD	0.95	0.74	1.69
		%CV	6.70%	2.81%	1.99%
	% Bias	-5.73%	-11.67%	-14.84%	
OPI1_20220421B_CD	4-1	14.62	30.75	95.99	
	4-2	15.55	29.92	103.03	
	4-3	15.92	30.99	93.37	
	Within Run	Mean	15.36	30.55	97.46
		SD	0.67	0.56	4.99
		%CV	4.34%	1.85%	5.12%
		% Bias	2.43%	1.84%	-2.54%
OPI1_20220421B_CLR	5-1	15.24	30.18	93.58	
	5-2	15.55	30.21	92.36	
	5-3	15.08	29.35	95.83	
	Within Run	Mean	15.29	29.92	93.93
		SD	0.24	0.49	1.76
		%CV	1.58%	1.64%	1.87%
		% Bias	1.92%	-0.28%	-6.07%
OPI1_20220427B_CD	6-1	15.15	28.58	90.53	
	6-2	14.96	29.34	86.43	
	6-3	15.16	28.89	90.42	
	Within Run	Mean	15.09	28.93	89.13
		SD	0.11	0.38	2.34
		%CV	0.73%	1.33%	2.62%
		% Bias	0.60%	-3.55%	-10.87%
Mean		14.80	28.91	90.53	
SD		0.67	1.45	4.69	
Precision (%CV)	Max Within-Run	6.70%	2.81%	5.12%	
	Between-Run	4.55%	5.03%	5.18%	
% Bias		-0.95%	-3.27%	-9.02%	

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Oxymorphone
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 783.98 1/x Weighting: = 295.24 1/x2 Weighting: = 265.8	1/x2 weighting will be used.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9388 - 0.9563 95% CI of Intercept = 1.5835 - 5.1846	The linearity of the method is acceptable because the individual calibration curves on all six 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 $\pm 20\%$ of target	Max %RE of Calibrators = 11.04	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ of target	N/A	Not performed. All 8 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 8 calibrators used in validation will be used for casework.
2	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = -9.03% Max Within-Run Precision = 6.97% Max Between-Run Precision = 3.47%	N/A

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Unweighted:
 1/x Weighting:
 1/x² Weighting:

Σ |%RE|
 783.98
 295.24
 265.80

C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
5.0	0.010	1	0.049399	5	0.00987979	25	9.76E-05	1.335583	-73.2883	73.28835
10	0.019	1	0.194893	10	0.01948933	100	0.00038	6.564245	-34.3576	34.35755
25	0.045	1	1.136039	25	0.04544155	625	0.002065	20.68516	-17.2594	17.25937
50	0.094	1	4.679229	50	0.09358458	2500	0.008758	46.88035	-6.23929	6.239295
75	0.143	1	10.6894	75	0.14252537	5625	0.020313	73.50962	-1.98717	1.98717
125	0.235	1	29.32678	125	0.23461427	15625	0.055044	123.6163	-1.10697	1.10697
250	0.460	1	115.0016	250	0.46000627	62500	0.211606	246.2548	-1.4981	1.498095
500	0.873	1	436.3615	500	0.87272301	250000	0.761645	470.8189	-5.83623	5.836227
5.0	0.009	1	0.044946	5	0.00898915	25	8.08E-05	0.850974	-82.9805	82.98053
10	0.020	1	0.200507	10	0.02005074	100	0.000402	6.869718	-31.3028	31.30282
25	0.045	1	1.120759	25	0.04515037	625	0.002039	20.52973	-17.8931	17.89308
50	0.090	1	4.500714	50	0.09001427	2500	0.008103	44.93771	-10.1246	10.12458
75	0.145	1	10.86339	75	0.14484523	5625	0.02098	74.77189	-0.30415	0.304152
125	0.232	1	29.0413	125	0.23233044	15625	0.053977	122.3736	-2.1011	2.101095
250	0.454	1	113.4674	250	0.45386948	62500	0.205998	242.9157	-2.83374	2.833735
500	0.887	1	443.5399	500	0.88707989	250000	0.786911	478.6306	-4.27388	4.273878
5.0	0.01116	1	0.0558	5	0.01116	25	0.000125	2.032159	-59.3568	59.35682
10	0.02294	1	0.2294	10	0.02294	100	0.000526	8.441797	-15.582	15.58203
25	0.05881	1	1.47025	25	0.05881	625	0.003459	27.95909	-11.83637	11.83637
50	0.10211	1	5.1057	50	0.102114	2500	0.010427	51.52131	3.042628	3.042628
75	0.18063	1	13.54725	75	0.18063	5625	0.032627	94.24281	25.65707	25.65707
125	0.27529	1	34.41125	125	0.27529	15625	0.075785	145.7484	16.59875	16.59875
250	0.50527	1	126.3175	250	0.50527	62500	0.255298	270.8833	8.353319	8.353319
500	0.99853	1	499.265	500	0.99853	250000	0.997062	539.2719	7.854389	7.854389
5.0	0.01019	1	0.050942	5	0.0101884	25	0.000104	1.5035	-69.93	69.93001
10	0.02065	1	0.206543	10	0.02065435	100	0.000427	7.198147	-28.0185	28.01853
25	0.04850	1	1.212523	25	0.04850094	625	0.002352	22.34981	-10.8008	10.80077
50	0.10189	1	5.094254	50	0.10188508	2500	0.010381	51.39676	2.793522	2.793522
75	0.14817	1	11.11282	75	0.14817091	5625	0.021955	76.58143	2.108572	2.108572
125	0.24704	1	30.88015	125	0.24704123	15625	0.061029	130.3779	4.302359	4.302359
250	0.47669	1	119.1726	250	0.47669022	62500	0.227234	255.3327	2.133079	2.133079
500	0.90904	1	454.518	500	0.90903595	250000	0.826346	490.5772	-1.88457	1.884567
5.0	0.011	1	0.053788	5	0.01075768	25	0.000116	1.81325	-63.735	63.73499
10	0.021	1	0.209512	10	0.02095121	100	0.000439	7.359674	-26.4033	26.40326
25	0.049	1	1.220977	25	0.04883908	625	0.002385	22.53379	-9.86482	9.864821
50	0.101	1	5.043941	50	0.10087882	2500	0.010177	50.84924	1.698471	1.698471
75	0.160	1	12.02773	75	0.16036969	5625	0.025718	83.21893	10.95857	10.95857
125	0.234	1	29.19061	125	0.23352485	15625	0.054534	123.0235	-1.58118	1.581183
250	0.469	1	117.2738	250	0.4690952	62500	0.22005	251.2002	0.480064	0.480064
500	0.922	1	461.1218	500	0.9222436	250000	0.850533	497.7636	-0.44728	0.447279
5.0	0.011	1	0.053358	5	0.01067166	25	0.000114	1.766448	-64.671	64.67104
10	0.02136	1	0.213575	10	0.0213575	100	0.000456	7.580737	-24.1926	24.19263
25	0.05043	1	1.260634	25	0.05042535	625	0.002543	23.3969	-6.41239	6.412389
50	0.09996	1	4.997786	50	0.09995572	2500	0.009991	50.34697	0.693931	0.693931
75	0.14677	1	11.00774	75	0.14676991	5625	0.021541	75.81913	1.092172	1.092172
125	0.25104	1	31.38047	125	0.25104373	15625	0.063023	132.5558	6.044602	6.044602
250	0.46230	1	115.5754	250	0.46230165	62500	0.213723	247.5037	-0.99852	0.998516
500	0.93795	1	468.9736	500	0.93794714	250000	0.879745	506.3081	1.261618	1.261618
Sum	6240.0		48	3762.48	6240	11.8246376	2022000	7.018623		783.9752

Comments: 1/x² weighting will be used.

Slope 0.00183786
 Intercept 0.00742518
 R² 0.99612220

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

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE	C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
5.0	0.010	0.2	0.00988	1	0.00197596	5	1.95E-05	4.159014	-16.8197	16.81973	5.0	0.010	0.04	0.00197596	0.2	0.000395	1	3.9044E-06	4.685887	-6.28225	6.28225177
10	0.019	0.1	0.0194893	1	0.00194893	10	3.8E-05	9.272937	-7.27063	7.270631	10	0.019	0.01	0.00194893	0.1	0.000195	1	3.7983E-06	9.636465	-3.63535	3.635349104
25	0.045	0.04	0.045442	1	0.00181766	25	8.26E-05	23.08398	-7.66408	7.664083	25	0.045	0.0016	0.00181766	0.04	7.27E-05	1	3.3039E-06	23.00636	-7.97454	7.97454095
50	0.094	0.02	0.093585	1	0.00187169	50	0.000175	48.70434	-2.59132	2.591315	50	0.094	0.0004	0.00187169	0.02	3.74E-05	1	3.5032E-06	47.80838	-4.38325	4.383245059
75	0.143	0.0133333	0.142525	1	0.00190034	75	0.000271	74.74926	-0.33433	0.334326	75	0.143	0.000178	0.00190034	0.0133333	2.53E-05	1	3.6113E-06	73.02138	-2.63816	2.638159292
125	0.235	0.008	0.234614	1	0.00187691	125	0.00044	123.7564	-0.9949	0.994902	125	0.235	0.000064	0.00187691	0.008	1.5E-05	1	3.5228E-06	120.4631	-3.62949	3.629485255
250	0.460	0.004	0.460006	1	0.00184003	250	0.000846	243.7037	-2.51854	2.51854	250	0.460	0.000016	0.00184003	0.004	7.36E-06	1	3.3857E-06	236.5791	-5.36834	5.368343866
500	0.873	0.002	0.872723	1	0.00174545	500	0.001523	463.3399	-7.33202	7.332023	500	0.873	0.000004	0.00174545	0.002	3.49E-06	1	3.0466E-06	449.1999	-10.16	10.16002168
5.0	0.009	0.2	0.008989	1	0.00179783	5	1.62E-05	3.685039	-26.2992	26.29922	5.0	0.009	0.04	0.00179783	0.2	0.00036	1	3.2322E-06	4.227052	-15.459	15.45895837
10	0.020	0.1	0.020051	1	0.00200507	10	4.02E-05	9.571707	-4.28293	4.282934	10	0.020	0.01	0.00200507	0.1	0.000201	1	4.0203E-06	9.925692	-0.74308	0.743083087
25	0.045	0.04	0.04515	1	0.00180601	25	8.15E-05	22.92902	-8.2839	8.283904	25	0.045	0.0004	0.00180601	0.04	7.22E-05	1	3.2617E-06	22.85636	-8.57457	8.574568194
50	0.090	0.02	0.090014	1	0.00180029	50	0.000162	46.80433	-6.39134	6.391344	50	0.090	0.0004	0.00180029	0.02	3.6E-05	1	3.241E-06	45.96905	-8.0619	8.061895735
75	0.145	0.0133333	0.144845	1	0.00193127	75	0.00028	75.98382	1.31176	1.31176	75	0.145	0.000178	0.00193127	0.0133333	2.58E-05	1	3.7298E-06	74.21651	-1.04465	1.044651705
125	0.232	0.008	0.23233	1	0.00185864	125	0.000432	122.541	-1.96721	1.967212	125	0.232	0.000064	0.00185864	0.008	1.49E-05	1	3.4546E-06	119.2866	-4.57074	4.570738596
250	0.454	0.004	0.453869	1	0.00181548	250	0.000824	240.4378	-3.82487	3.824871	250	0.454	0.000016	0.00181548	0.004	7.26E-06	1	3.296E-06	233.4176	-6.63295	6.6329486
500	0.887	0.002	0.88708	1	0.00177416	500	0.001574	470.9802	-5.80396	5.803958	500	0.887	0.000004	0.00177416	0.002	3.55E-06	1	3.1476E-06	456.5962	-8.68076	8.680764748
5.0	0.01116	0.2	0.01116	1	0.002232	5	2.49E-05	4.840304	-3.19392	3.193921	5.0	0.01116	0.04	0.002232	0.2	0.000446	1	4.9818E-06	5.345417	6.90833	6.908330109
10	0.02294	0.1	0.02294	1	0.002294	10	5.26E-05	11.10929	11.09288	11.09288	10	0.02294	0.01	0.002294	0.1	0.000229	1	5.2624E-06	11.41416	14.14161	14.141609
25	0.05881	0.04	0.05881	1	0.0023524	25	0.000138	30.19829	20.79317	20.79317	25	0.05881	0.0016	0.0023524	0.04	9.41E-05	1	5.5338E-06	29.99344	19.57374	19.57374425
50	0.10211	0.02	0.102114	1	0.00204228	50	0.000209	53.24346	6.486924	6.486924	50	0.10211	0.0004	0.00204228	0.02	4.08E-05	1	4.1709E-06	52.20254	14.405023	14.40502262
75	0.18063	0.0133333	0.18063	1	0.0024084	75	0.000435	95.02747	26.70329	26.70329	75	0.18063	0.000178	0.0024084	0.0133333	3.21E-05	1	5.8004E-06	92.65188	23.53584	23.53583689
125	0.27529	0.008	0.27529	1	0.00220232	125	0.000606	145.4029	16.32228	16.32228	125	0.27529	0.000064	0.00220232	0.008	1.76E-05	1	4.8502E-06	141.4182	13.13456	13.13456457
250	0.50527	0.004	0.50527	1	0.00202108	250	0.001021	267.7917	7.116694	7.116694	250	0.50527	0.000016	0.00202108	0.004	8.08E-06	1	4.0848E-06	258.8978	3.959127	3.959127303
500	0.99853	0.002	0.99853	1	0.00199706	500	0.001994	530.2908	6.058164	6.058164	500	0.99853	0.000004	0.00199706	0.002	3.99E-06	1	3.9882E-06	514.0123	2.802464	2.802464498
5.0	0.01019	0.2	0.010188	1	0.00203768	5	2.08E-05	4.323246	-13.5351	13.53509	5.0	0.01019	0.04	0.00203768	0.2	0.000408	1	4.1521E-06	4.844874	-3.10252	3.102524754
10	0.02065	0.1	0.020654	1	0.00206543	10	4.27E-05	9.892929	-1.07071	1.070709	10	0.02065	0.01	0.00206543	0.1	0.000207	1	4.266E-06	10.23665	2.366538	2.366536298
25	0.04850	0.04	0.048501	1	0.00194004	25	9.41E-05	24.7121	-1.1516	1.1516	25	0.04850	0.0016	0.00194004	0.04	7.76E-05	1	3.7637E-06	24.58248	-1.67008	1.670079571
50	0.10189	0.02	0.101885	1	0.0020377	50	0.000208	53.12164	6.243284	6.243284	50	0.10189	0.0004	0.0020377	0.02	4.08E-05	1	4.1522E-06	52.08458	4.169165	4.169165384
75	0.14817	0.0133333	0.148171	1	0.00197561	75	0.000293	77.75365	3.671538	3.671538	75	0.14817	0.000178	0.00197561	0.0133333	2.63E-05	1	3.903E-06	75.92981	1.239752	1.239751941
125	0.24704	0.008	0.247041	1	0.00197633	125	0.000488	130.3697	4.295724	4.295724	125	0.24704	0.000064	0.00197633	0.008	1.58E-05	1	3.9059E-06	128.8852	1.49215	1.492150452
250	0.47669	0.004	0.47669	1	0.00190676	250	0.000909	252.5824	1.032952	1.032952	250	0.47669	0.000016	0.00190676	0.004	7.63E-06	1	3.6357E-06	245.1743	-1.93029	1.930291703
500	0.90904	0.002	0.909036	1	0.00181807	500	0.001653	482.6646	-3.46708	3.467079	500	0.90904	0.000004	0.00181807	0.002	3.64E-06	1	3.3054E-06	467.9074	-6.41853	6.41852869
5.0	0.011	0.2	0.010758	1	0.00215154	5	2.31E-05	4.626199	-7.47602	7.476017	5.0	0.011	0.04	0.00215154	0.2	0.00043	1	4.6291E-06	5.13815	2.76301	2.763010258
10	0.021	0.1	0.020951	1	0.00209512	10	4.39E-05	10.50911	0.509116	0.509116	10	0.021	0.01	0.00209512	0.1	0.00021	1	4.3895E-06	10.38959	3.895902	3.895901724
25	0.049	0.04	0.048839	1	0.00195356	25	9.54E-05	24.89205	-0.4318	0.431804	25	0.049	0.0016	0.00195356	0.04	7.81E-05	1	3.8164E-06	24.75668	-0.97327	0.973274665
50	0.101	0.02	0.100879	1	0.00201758	50	0.000204	52.58613	5.172263	5.172263	50	0.101	0.0004	0.00201758	0.02	4.04E-05	1	4.0706E-06	51.56618	1.32354	1.32354216
75	0.160	0.0133333	0.16037	1	0.00213826	75	0.000343	84.2455	12.32733	12.32733	75	0.160	0.000178	0.00213826	0.0133333	2.85E-05	1	4.5722E-06	82.2143	9.619069	9.619068895
125	0.234	0.008	0.233525	1	0.0018682	125	0.000436	123.1766	-1.45871	1.458708	125	0.234	0.000064	0.0018682	0.008	1.49E-05	1	3.4902E-06	119.9019	-4.07848	4.078477515
250	0.469	0.004	0.469095	1	0.00187638	250	0.00088	248.5405	-0.58379	0.583789	250	0.469	0.000016	0.00187638	0.004	7.51E-06	1	3.5208E-06	241.2615	-3.49539	3.495391945
500	0.922	0.002	0.922244	1	0.00184449	500	0.001701	489.6933	-2.06133	2.061331	500	0.922	0.000004	0.00184449	0.002	3.69E-06	1	3.4021E-06	474.7116	-5.05768	5.057682124
5.0	0.011	0.2	0.010672	1	0.00213433	5	2.28E-05	4.580424	-8.39153	8.391526	5.0	0.011	0.04	0.00213433	0.2	0.000427	1	4.5554E-06	5.093837	1.876743	1.876743405
10	0.02136	0.1	0.021357	1	0.00213575	10	4.56E-05	10.26712	2.671237	2.671237	10	0.02136	0.01	0.00213575	0.1	0.000214	1	4.5614E-06	10.5989	5.988962	5.988961843
25	0.05043	0.04	0.050425	1	0.00201701	25	0.000102	25.73622	2.944867	2.944867	25	0.05043	0.0016	0.00201701	0.04	8.07E-05	1	4.0683E-06	25.57389	2.295541	2.295540576
50	0.09996	0.02	0.099956	1	0.00199911	50	0.0002	52.09488	4.189767	4.189767	50	0.09996	0.0004	0.00199911	0.02	4E-05	1	3.9956E-06	51.09062	2.18124	2.18124008
75	0.14677	0.0133333	0.14677	1	0.00195663	75	0.000287	77.0808	2.677443	2.677443	75	0.14677	0.000178	0.00195663	0.0133333	2.61E-05	1	3.8298E-06	75.20806	0.277409	0.277409374
125	0.25104	0.008	0.251044	1	0.00200835	125	0.000504	132.4997	5.999735	5.999735	125	0.25104	0.000064	0.00200835	0.008	1.61E-05	1	4.0335E-06	128.9272	3.141733	3.141732957
250	0.46230	0.004	0.462302	1	0.00184921	250	0.000855	244.9252	-2.02992	2.029924	250	0.46230	0.000016	0.00184921	0.004	7.4E-06	1	3.41			

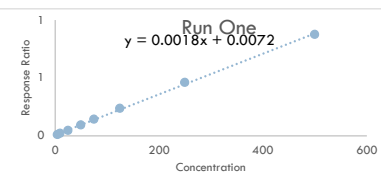
Validation Study 1

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

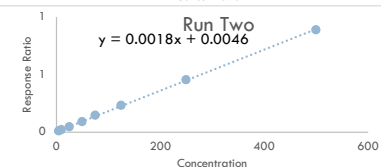
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

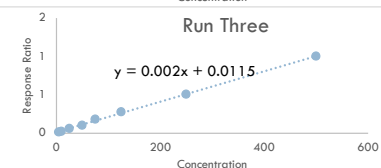
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	4.96	0.80	0.010
	10	10.20	2.00	0.019
	25	24.35	2.60	0.045
	50	50.60	1.20	0.094
	75	77.28	3.04	0.143
	125	127.49	1.99	0.235
	250	250.38	0.15	0.460
	500	475.40	4.92	0.873
OPI1_20220418B_JR	5	4.82	3.67	0.009
	10	10.82	8.18	0.020
	25	24.44	2.26	0.045
	50	48.78	2.45	0.090
	75	78.52	4.70	0.145
	125	125.99	0.79	0.232
	250	246.18	1.53	0.454
	500	481.21	3.76	0.887
OPI1_20220418B_KMY	5	4.85	3.00	0.011
	10	10.30	3.00	0.023
	25	26.90	7.60	0.059
	50	46.95	6.10	0.102
	75	83.28	11.04	0.181
	125	127.09	1.67	0.275
	250	233.53	6.59	0.505
	500	461.82	7.64	0.999
OPI1_20220421B_CD	5	4.91	1.84	0.010
	10	10.31	3.12	0.021
	25	24.69	1.24	0.049
	50	52.26	4.52	0.102
	75	76.16	1.54	0.148
	125	127.21	1.77	0.247
	250	245.80	1.68	0.477
	500	469.05	6.19	0.909
OPI1_20220421B_CLR	5	4.93	1.39	0.011
	10	10.20	2.01	0.021
	25	24.62	1.51	0.049
	50	51.53	3.06	0.101
	75	82.29	9.72	0.160
	125	120.12	3.91	0.234
	250	241.92	3.23	0.469
	500	476.23	4.75	0.922
OPI1_20220427B_CD	5	4.88	2.46	0.011
	10	10.39	3.90	0.021
	25	25.39	1.56	0.050
	50	50.95	1.89	0.100
	75	75.10	0.14	0.147
	125	128.91	3.13	0.251
	250	237.92	4.83	0.462
	500	483.35	3.33	0.938



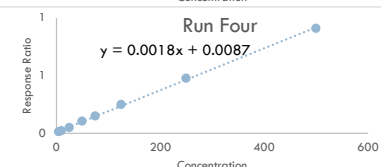
Slope 0.00175
 Intercept 0.00721
 R² 0.99917



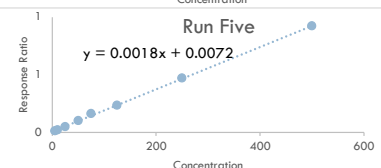
Slope 0.00177
 Intercept 0.00461
 R² 0.99970



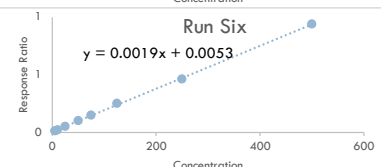
Slope 0.00198
 Intercept 0.01148
 R² 0.99881



Slope 0.00182
 Intercept 0.00874
 R² 0.99922



Slope 0.00184
 Intercept 0.00717
 R² 0.99950



Slope 0.00186
 Intercept 0.00534
 R² 0.99961

Max %RE = 11.04

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

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

LINEARITY

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

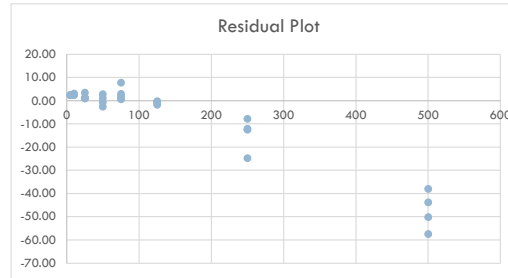
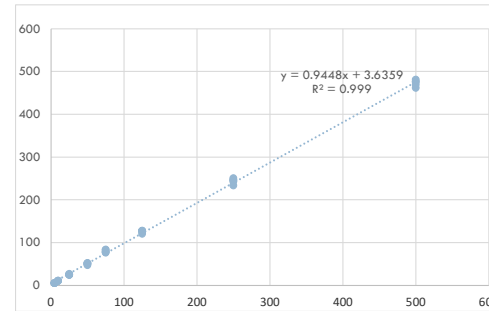
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	4.96	2.50	2.46
	10	10.20	7.72	2.48
	25	24.35	23.38	0.97
	50	50.60	49.48	1.12
	75	77.28	75.58	1.70
	125	127.49	127.78	-0.29
	250	250.38	258.28	-7.90
	500	475.40	519.28	-43.88
OPI1_20220418B_JR	5	4.82	2.50	2.32
	10	10.82	7.72	3.10
	25	24.44	23.38	1.06
	50	48.78	49.48	-0.70
	75	78.52	75.58	2.95
	125	125.99	127.78	-1.79
	250	246.18	258.28	-12.10
	500	481.21	519.28	-38.07
OPI1_20220418B_KMY	5	4.85	2.50	2.35
	10	10.30	7.72	2.58
	25	26.90	23.38	3.52
	50	46.95	49.48	-2.53
	75	83.28	75.58	7.70
	125	127.09	127.78	-0.69
	250	233.53	258.28	-24.75
	500	461.82	519.28	-57.46
OPI1_20220421B_CD	5	4.91	2.50	2.41
	10	10.31	7.72	2.60
	25	24.69	23.38	1.31
	50	52.26	49.48	2.78
	75	76.16	75.58	0.58
	125	127.21	127.78	-0.57
	250	245.80	258.28	-12.48
	500	469.05	519.28	-50.23
OPI1_20220421B_CLR	5	4.93	2.50	2.43
	10	10.20	7.72	2.48
	25	24.62	23.38	1.24
	50	51.53	49.48	2.05
	75	82.29	75.58	6.71
	125	120.12	127.78	-7.66
	250	241.92	258.28	-16.35
	500	476.23	519.28	-43.04
OPI1_20220427B_CD	5	4.88	2.50	2.38
	10	10.39	7.72	2.67
	25	25.39	23.38	2.01
	50	50.95	49.48	1.47
	75	75.10	75.58	-0.47
	125	128.91	127.78	1.13
	250	237.92	258.28	-20.36
	500	483.35	519.28	-35.93

Slope	0.9476
Std err in slope, S_b	0.0044
Degrees freedom	46
Confidence level	95%
Student t	2.0129
Confidence interval	0.009
Slope	0.948 ± 0.009
Range	0.9388 - 0.9563

Intercept	3.3841
Std err in Intercept	0.8945
Degrees freedom	46
Confidence Level	95%
Student t	2.0129
Confidence interval	1.801
Intercept	3.384 ± 1.801
Lower	1.5835 - 5.1846

NO

NO



Comments: The linearity of the method is acceptable because the individual calibration curves on all six 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

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	100	400	
OPI1_20220414B_JR	1-1	15.61	100.72	376.06	
	1-2	15.65	100.80	367.67	
	1-3	16.02	100.22	373.41	
	1-4	15.60	101.53	374.15	
	Within Run	Mean	15.72	100.82	372.82
		SD	0.20	0.54	3.61
		%CV	1.28%	0.54%	0.97%
	% Bias	4.80%	0.82%	-6.79%	
OPI1_20220418B_JR	2-1	15.71	98.25	373.70	
	2-2	16.08	98.26	376.42	
	2-3	16.40	98.87	375.16	
	Within Run	Mean	16.06	98.46	375.09
		SD	0.35	0.35	1.36
		%CV	2.16%	0.36%	0.36%
		% Bias	7.08%	-1.54%	-6.23%
OPI1_20220418B_KMY	3-1	15.31	93.14	343.52	
	3-2	15.11	89.94	348.71	
	3-3	13.85	95.30	360.30	
	3-4	16.43	97.44	337.81	
	Within Run	Mean	15.18	93.96	347.59
		SD	1.06	3.20	9.57
		%CV	6.97%	3.41%	2.75%
	% Bias	1.17%	-6.05%	-13.10%	
OPI1_20220421B_CD	4-1	15.29	101.41	371.39	
	4-2	15.43	101.31	376.99	
	4-3	15.92	102.10	363.09	
	Within Run	Mean	15.55	101.61	370.49
		SD	0.33	0.43	6.99
		%CV	2.11%	0.42%	1.89%
		% Bias	3.65%	1.61%	-7.38%
OPI1_20220421B_CLR	5-1	15.90	98.52	364.97	
	5-2	15.52	99.22	366.59	
	5-3	15.62	99.39	364.97	
	Within Run	Mean	15.68	99.04	365.51
		SD	0.20	0.46	0.93
		%CV	1.27%	0.47%	0.25%
		% Bias	4.52%	-0.96%	-8.62%
OPI1_20220427B_CD	6-1	15.50	94.79	361.27	
	6-2	15.32	99.74	338.91	
	6-3	15.49	97.35	355.06	
	Within Run	Mean	15.44	97.29	351.75
		SD	0.10	2.47	11.55
		%CV	0.68%	2.54%	3.28%
		% Bias	2.90%	-2.71%	-12.06%

Mean		15.59	98.42	363.51
SD		0.54	3.10	12.61
Precision (%CV)	Max Within-Run	6.97%	3.41%	3.28%
	Between-Run	3.47%	3.15%	3.47%
% Bias		4.02%	-1.47%	-9.03%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Hydromorphone
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 201.47 1/x Weighting: = 190.86 1/x2 Weighting: = 195.03	1/x2 weighting will be used. While 1/x weighting gave a lower %RE, data processed using 1/x weighting failed to meet the SOP criteria for linearity.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9870 - 1.0044 95% CI of Intercept = -1.6134 - 1.9544	N/A
1	Validation Calibration	%RE Calibrators $\pm 20\%$ of target	Max %RE of Calibrators = 12.44	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ of target	N/A	Not performed. All 8 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 8 calibrators used in validation will be used for casework.
2	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = -7.22% Max Within-Run Precision = 5.88% Max Between-Run Precision = 3.94%	N/A

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	$\Sigma \%RE $	C_{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
Unweighted:	201.47	5.0	0.011	1	0.053818	5	0.01076356	25	0.000116	5.033012	0.660238	0.660238
1/x Weighting:	190.86	10	0.020	1	0.200795	10	0.02007953	100	0.000403	9.585419	-4.14581	4.145813
1/x ² Weighting:	195.03	25	0.048	1	1.20928	25	0.0483712	625	0.00234	23.41062	-6.35751	6.357509
		50	0.097	1	4.839401	50	0.09678802	2500	0.009368	47.07032	-5.85935	5.859351
		75	0.152	1	11.42459	75	0.15232793	5625	0.023204	74.21084	-1.05221	1.05221
		125	0.245	1	30.67761	125	0.2454209	15625	0.060231	119.7023	-4.23816	4.238162
		250	0.491	1	122.7134	250	0.49085375	62500	0.240937	239.6372	-4.14511	4.145112
		500	0.967	1	483.7046	500	0.9674093	250000	0.935881	472.5142	-5.49717	5.497168
		5.0	0.010	1	0.049373	5	0.00987468	25	9.75E-05	4.598647	-8.02707	8.027069
		10	0.020	1	0.201356	10	0.02013562	100	0.000405	9.612831	-3.87169	3.871686
		25	0.050	1	1.250285	25	0.05001142	625	0.002501	24.21214	-3.15142	3.151421
		50	0.098	1	4.918153	50	0.09836306	2500	0.009675	47.93969	-4.32002	4.320017
		75	0.156	1	11.86364	75	0.15551523	5625	0.024185	75.78837	1.024493	1.024493
		125	0.251	1	31.43574	125	0.2514859	15625	0.063245	122.6661	-1.86715	1.867149
		250	0.498	1	124.4558	250	0.49782323	62500	0.247828	243.043	-2.78281	2.782811
		500	1.015	1	507.7132	500	1.01542638	250000	1.031091	495.9785	-0.8043	0.804296
		5.0	0.01067	1	0.053325	5	0.010665	25	0.000114	4.984851	-0.30299	0.302987
		10	0.02306	1	0.23055	10	0.023055	100	0.000532	11.03943	10.39434	10.39434
		25	0.06019	1	1.504725	25	0.060189	625	0.003623	29.18559	16.74237	16.74237
		50	0.10180	1	5.0902	50	0.101804	2500	0.010364	49.52147	-0.95707	0.957066
		75	0.19110	1	14.3325	75	0.1911	5625	0.036519	93.15747	24.20996	24.20996
		125	0.28223	1	35.279	125	0.282232	15625	0.079655	137.6907	10.15253	10.15253
		250	0.53937	1	134.8415	250	0.539366	62500	0.290916	263.3436	5.337421	5.337421
		500	1.08442	1	542.212	500	1.084424	250000	1.175975	529.6954	5.939075	5.939075
		5.0	0.01041	1	0.052066	5	0.01041323	25	0.000108	4.861818	-2.76365	2.763649
		10	0.02046	1	0.204561	10	0.02045614	100	0.000418	9.769457	-2.30543	2.305428
		25	0.04983	1	1.245738	25	0.04982951	625	0.002483	24.12325	-3.507	3.506998
		50	0.09848	1	4.923779	50	0.09847858	2500	0.009697	47.89498	-4.21004	4.210039
		75	0.15117	1	11.33745	75	0.15116599	5625	0.022851	73.64304	-1.80928	1.809281
		125	0.25451	1	31.81316	125	0.25450529	15625	0.064773	124.1415	-0.68677	0.686768
		250	0.50828	1	127.069	250	0.50827585	62500	0.258344	248.1508	-0.73967	0.739671
		500	1.02440	1	512.1982	500	1.02439647	250000	1.049388	500.3619	0.072381	0.072381
		5.0	0.009	1	0.046094	5	0.00921874	25	8.5E-05	4.27811	-14.4378	14.4378
		10	0.020	1	0.200291	10	0.02002907	100	0.000401	9.560762	-4.39238	4.392383
		25	0.050	1	1.248704	25	0.04994817	625	0.002495	24.18124	-3.27504	3.275044
		50	0.098	1	4.919842	50	0.09839683	2500	0.009682	47.8565	-4.287	4.287004
		75	0.159	1	11.93385	75	0.15911805	5625	0.025319	77.52895	3.371931	3.371931
		125	0.246	1	30.69187	125	0.245535	15625	0.060287	119.7581	-4.19356	4.193556
		250	0.500	1	124.8994	250	0.49959777	62500	0.249598	243.9101	-2.43595	2.435947
		500	1.025	1	512.4584	500	1.02491674	250000	1.050454	500.6161	0.12323	0.12323
		5.0	0.010	1	0.052121	5	0.01042411	25	0.000109	4.867138	-2.65725	2.657247
		10	0.022	1	0.216207	10	0.02162072	100	0.000467	10.33855	3.385508	3.385508
		25	0.051	1	1.265794	25	0.05063176	625	0.002564	24.51529	-1.93886	1.93886
		50	0.103	1	5.138677	50	0.10277354	2500	0.010562	49.99525	-0.0095	0.009499
		75	0.154	1	11.5855	75	0.15447333	5625	0.023862	75.25923	0.345639	0.345639
		125	0.268	1	33.4668	125	0.26773441	15625	0.071682	130.6062	4.484938	4.484938
		250	0.498	1	124.4832	250	0.49793293	62500	0.247937	243.0966	-2.76137	2.761368
		500	1.038	1	519.1778	500	1.03835554	250000	1.078182	507.1832	1.436648	1.436648
		Sum	6240.0	12.7917	48	4140.683	6240	12.7917095	2022000	8.490956		201.4711

Comments: 1/x² weighting will be used. While 1/x weighting gave a lower %RE, data processed using 1/x weighting failed to meet the SOP criteria for linearity.

Slope 0.00204638
 Intercept 0.00046408
 R² 0.99771886

Acceptance Criteria: The least complex weighting scheme that minimizes $\Sigma | \%RE |$

Validation Study 1

STANDARD CURVE WEIGHT VERIFICATION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

C _{room}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE	C _{room}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
5.0	0.011	0.2	0.010764	1	0.00215271	5	2.32E-05	5.161278	3.225559	3.225559	5.0	0.011	0.04	0.00215271	0.2	0.000431	1	4.63E-06	5.226323	4.526456	4.526456
10	0.020	0.1	0.02008	1	0.00200795	10	4.03E-05	9.709012	-2.90988	2.909879	10	0.020	0.01	0.00200795	0.1	0.000201	1	4.03E-06	9.754964	-2.45036	2.450362
25	0.048	0.04	0.048371	1	0.00193485	25	9.36E-05	23.52003	-5.9199	5.919986	25	0.048	0.0016	0.00193485	0.04	7.74E-05	1	3.74E-06	23.50799	-5.96803	5.968025
50	0.097	0.02	0.096788	1	0.00193576	50	0.000187	47.15544	-5.68911	5.689113	50	0.097	0.0004	0.00193576	0.02	3.87E-05	1	3.75E-06	47.04418	-5.91164	5.911639
75	0.152	0.0133333	0.152328	1	0.00203104	75	0.000309	74.2681	-0.97586	0.975861	75	0.152	0.000178	0.00203104	0.0133333	2.71E-05	1	4.13E-06	74.04301	-1.27599	1.275985
125	0.245	0.008	0.245421	1	0.00196337	125	0.000482	119.7129	-4.22971	4.229706	125	0.245	0.000064	0.00196337	0.008	1.57E-05	1	3.85E-06	119.297	-4.56242	4.562417
250	0.491	0.004	0.490854	1	0.00196342	250	0.000964	239.5247	-4.19012	4.190125	250	0.491	0.000016	0.00196342	0.004	7.85E-06	1	3.85E-06	238.6058	-4.55769	4.557687
500	0.967	0.002	0.967409	1	0.00193482	500	0.001872	472.1626	-5.56748	5.567479	500	0.967	0.000004	0.00193482	0.002	3.87E-06	1	3.74E-06	470.267	-5.9466	5.946601
5.0	0.010	0.2	0.009875	1	0.00197494	5	1.95E-05	4.727358	-5.45283	5.452831	5.0	0.010	0.04	0.00197494	0.2	0.000395	1	3.9E-06	4.794225	-4.1155	4.115499
10	0.020	0.1	0.020136	1	0.00201356	10	4.05E-05	9.736397	-2.63603	2.636033	10	0.020	0.01	0.00201356	0.1	0.000201	1	4.05E-06	9.782233	-2.17767	2.177668
25	0.050	0.04	0.050011	1	0.00200446	25	0.00011	24.32073	-2.7171	2.717099	25	0.050	0.0016	0.00200446	0.04	8E-05	1	4E-06	24.30533	-2.77868	2.778675
50	0.098	0.02	0.098363	1	0.00196726	50	0.000194	47.92432	-4.15136	4.151359	50	0.098	0.0004	0.00196726	0.02	3.93E-05	1	3.87E-06	47.80983	-4.38034	4.380342
75	0.156	0.0133333	0.155515	1	0.00207354	75	0.000322	75.82403	1.09871	1.098711	75	0.156	0.000178	0.00207354	0.0133333	2.78E-05	1	4.3E-06	75.59241	0.789876	0.7898761
125	0.251	0.008	0.251486	1	0.00201189	125	0.000506	122.6736	-1.86113	1.861127	125	0.251	0.000064	0.00201189	0.008	1.61E-05	1	4.05E-06	122.2453	-2.20378	2.203780
250	0.498	0.004	0.497823	1	0.00199129	250	0.000991	242.9269	-2.82922	2.829222	250	0.498	0.000016	0.00199129	0.004	7.97E-06	1	3.97E-06	241.9938	-3.2025	3.202498
500	1.015	0.002	1.015426	1	0.00203085	500	0.002062	495.6029	-0.87942	0.879424	500	1.015	0.000004	0.00203085	0.002	4.06E-06	1	4.12E-06	493.6089	-1.27823	1.278228
5.0	0.01067	0.2	0.010665	1	0.002133	5	2.27E-05	5.113166	2.263323	2.263323	5.0	0.01067	0.04	0.002133	0.2	0.000427	1	4.55E-06	5.178413	3.56826	3.568259
10	0.02306	0.1	0.023055	1	0.0023055	10	5.32E-05	11.16154	11.61535	11.61535	10	0.02306	0.01	0.0023055	0.1	0.000231	1	5.32E-06	11.20139	12.01388	12.013884
25	0.06019	0.04	0.060189	1	0.00240756	25	0.000145	29.28907	17.15627	17.15627	25	0.06019	0.0016	0.00240756	0.04	9.63E-05	1	5.8E-06	29.25281	17.01126	17.011259
50	0.10180	0.02	0.101804	1	0.00203608	50	0.000207	49.60407	-0.79186	0.79186	50	0.10180	0.0004	0.00203608	0.02	4.07E-05	1	4.15E-06	49.48251	-1.03495	1.034947
75	0.19110	0.0133333	0.1911	1	0.002548	75	0.000487	93.19529	24.26038	24.26038	75	0.19110	0.000178	0.002548	0.0133333	3.4E-05	1	6.49E-06	92.89073	23.8543	23.854304
125	0.28223	0.008	0.282232	1	0.00225786	125	0.000637	137.6828	10.14622	10.14622	125	0.28223	0.000064	0.00225786	0.008	1.81E-05	1	5.1E-06	137.1914	9.753151	9.753151
250	0.53937	0.004	0.539366	1	0.00215746	250	0.001164	263.2067	5.282675	5.282675	250	0.53937	0.000016	0.00215746	0.004	8.63E-06	1	4.65E-06	262.1884	4.875342	4.875341
500	1.08442	0.002	1.084424	1	0.00216885	500	0.002352	529.2851	5.857026	5.857026	500	1.08442	0.000004	0.00216885	0.002	4.34E-06	1	4.7E-06	527.1497	5.429939	5.429938
5.0	0.01041	0.2	0.010413	1	0.00208265	5	2.17E-05	4.990259	-0.19481	0.194814	5.0	0.01041	0.04	0.00208265	0.2	0.000417	1	4.34E-06	5.056022	1.20443	1.204429
10	0.02046	0.1	0.020456	1	0.00204561	10	4.18E-05	9.892862	-1.07138	1.071382	10	0.02046	0.01	0.00204561	0.1	0.000205	1	4.18E-06	9.938042	-0.61958	0.619584
25	0.04983	0.04	0.04983	1	0.00199318	25	9.93E-05	24.23192	-3.07231	3.07231	25	0.04983	0.0016	0.00199318	0.04	7.97E-05	1	3.97E-06	24.2169	-3.1324	3.132395
50	0.09848	0.02	0.098476	1	0.00196951	50	0.000194	47.97925	-4.04149	4.041494	50	0.09848	0.0004	0.00196951	0.02	3.96E-05	1	3.88E-06	47.86453	-4.27094	4.270937
75	0.15117	0.0133333	0.151166	1	0.00201555	75	0.000305	73.70088	-1.73215	1.732155	75	0.15117	0.000178	0.00201555	0.0133333	2.69E-05	1	4.06E-06	73.47817	-2.0291	2.029104
125	0.25451	0.008	0.254505	1	0.00203604	125	0.000518	124.1476	-0.68196	0.681957	125	0.25451	0.000064	0.00203604	0.008	1.63E-05	1	4.15E-06	123.713	-1.02956	1.029562
250	0.50828	0.004	0.508276	1	0.0020331	250	0.001033	248.0296	-0.78818	0.788179	250	0.50828	0.000016	0.0020331	0.004	8.13E-06	1	4.13E-06	247.0749	-1.17002	1.170024
500	1.02440	0.002	1.024396	1	0.00204879	500	0.002099	499.9818	-0.00365	0.003647	500	1.02440	0.000004	0.00204879	0.002	4.1E-06	1	4.2E-06	497.9694	-0.40613	0.406128
5.0	0.009	0.2	0.009219	1	0.00184375	5	1.7E-05	4.407151	-11.857	11.85698	5.0	0.009	0.04	0.00184375	0.2	0.000369	1	3.4E-06	4.475362	-10.4928	10.492763
10	0.020	0.1	0.020029	1	0.00200291	10	4.01E-05	9.684381	-3.15619	3.156195	10	0.020	0.01	0.00200291	0.1	0.0002	1	4.01E-06	9.730436	-2.69564	2.695643
25	0.050	0.04	0.049948	1	0.00199793	25	9.98E-05	24.28985	-2.84059	2.840594	25	0.050	0.0016	0.00199793	0.04	7.99E-05	1	3.99E-06	24.27459	-2.90165	2.901652
50	0.098	0.02	0.098397	1	0.00196794	50	0.000194	47.94081	-4.11838	4.11838	50	0.098	0.0004	0.00196794	0.02	3.94E-05	1	3.87E-06	47.82625	-4.3475	4.347501
75	0.159	0.0133333	0.159118	1	0.00212157	75	0.000338	77.5828	3.443739	3.443739	75	0.159	0.000178	0.00212157	0.0133333	2.83E-05	1	4.5E-06	77.34379	3.126509	3.126509
125	0.246	0.008	0.245535	1	0.00196428	125	0.000482	119.7686	-4.18515	4.185147	125	0.246	0.000064	0.00196428	0.008	1.57E-05	1	3.86E-06	119.3524	-4.51804	4.518044
250	0.500	0.004	0.499598	1	0.00199839	250	0.000998	243.7932	-2.48271	2.482714	250	0.500	0.000016	0.00199839	0.004	7.99E-06	1	3.99E-06	242.8564	-2.85744	2.857444
500	1.025	0.002	1.024917	1	0.00204983	500	0.002101	500.2357	0.04715	0.04715	500	1.025	0.000004	0.00204983	0.002	4.1E-06	1	4.2E-06	498.2223	-0.35555	0.355545
5.0	0.010	0.2	0.010424	1	0.00208482	5	2.17E-05	4.995874	-0.08852	0.088521	5.0	0.010	0.04	0.00208482	0.2	0.000417	1	4.35E-06	5.061314	1.226289	1.226289
10	0.022	0.1	0.021621	1	0.00216207	10	4.67E-05	10.46137	4.613713	4.613713	10	0.022	0.01	0.00216207	0.1	0.000216	1	4.67E-06	10.50416	5.041642	5.041641
25	0.051	0.04	0.050632	1	0.00202527	25	0.000103	24.62355	-1.50578	1.505782	25	0.051	0.0016	0.00202527	0.04	8.1E-05	1	4.1E-06	24.60689	-1.57244	1.572444
50	0.103	0.02	0.102774	1	0.00205647	50	0.000211	50.07737	0.154735	0.154735	50	0.103	0.0004	0.00205647	0.02	4.11E-05	1	4.22E-06	49.95384	-0.09233	0.092326
75	0.154	0.0133333	0.154473	1	0.00205964	75	0.000318	75.31542	0.420554	0.420554	75	0.154	0.000178	0.00205964	0.0133333	3.4E-05	1	4.24E-06	75.05593	0.114567	0.114566
125	0.268	0.008	0.267734	1	0.00214188	125	0.000573	130.6055	4.48444	4.48444	125	0.268	0.000064	0.00214188	0.008	1.71E-05	1	4.59E-06	130.1439	4.115144	4.115143
250	0.498	0.004	0.497933	1	0.00199173	250	0.000992	242.9805	-2.8078	2.8078	250	0.498	0.000016	0.00199173	0.004	7.97E-06	1	3.97E-06	242.0471	-3.18117	3.181165
500	1.038	0.002	1.038356	1	0.00207671	500	0.002156	506.7961	1.35922	1.35922	500	1.038	0.000004	0.00207671	0.002	4.15E-06	1	4.3			

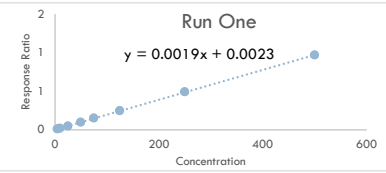
Validation Study 1

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

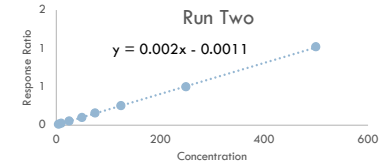
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

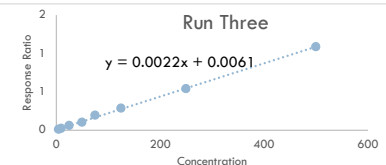
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	5.06	1.20	0.011
	10	9.84	1.60	0.020
	25	24.39	2.44	0.048
	50	49.27	1.46	0.097
	75	77.81	3.75	0.152
	125	125.66	0.53	0.245
	250	251.80	0.72	0.491
	500	496.72	0.66	0.967
OPI1_20220418B_JR	5	4.99	0.23	0.010
	10	10.08	0.76	0.020
	25	24.89	0.45	0.050
	50	48.86	2.28	0.098
	75	77.19	2.92	0.156
	125	124.77	0.18	0.251
	250	246.90	1.24	0.498
	500	503.52	0.70	1.015
OPI1_20220418B_KMY	5	4.87	2.60	0.011
	10	10.32	3.20	0.023
	25	26.68	6.72	0.060
	50	45.01	9.98	0.102
	75	84.33	12.44	0.191
	125	124.47	0.42	0.282
	250	237.72	4.91	0.539
	500	477.77	4.45	1.084
OPI1_20220421B_CD	5	5.02	0.45	0.010
	10	10.01	0.10	0.020
	25	24.60	1.61	0.050
	50	48.76	2.48	0.098
	75	74.93	0.10	0.151
	125	126.25	1.00	0.255
	250	252.28	0.91	0.508
	500	508.61	1.72	1.024
OPI1_20220421B_CLR	5	4.94	1.20	0.009
	10	10.26	2.56	0.020
	25	24.97	0.12	0.050
	50	48.80	2.40	0.098
	75	78.66	4.88	0.159
	125	121.16	3.07	0.246
	250	246.12	1.55	0.500
	500	504.48	0.90	1.025
OPI1_20220427B_CD	5	4.93	1.35	0.010
	10	10.36	3.61	0.022
	25	24.43	2.29	0.051
	50	49.71	0.59	0.103
	75	74.77	0.30	0.154
	125	129.69	3.75	0.268
	250	241.30	3.48	0.498
	500	503.31	0.66	1.038



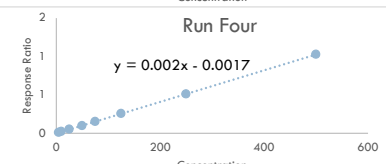
Slope 0.00194
 Intercept 0.00234
 R² 0.99991



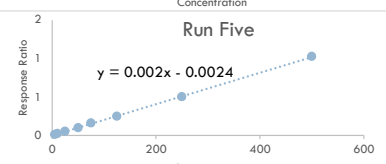
Slope 0.00203
 Intercept -0.00106
 R² 0.99988



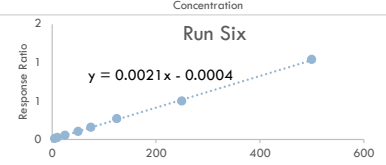
Slope 0.00216
 Intercept 0.00607
 R² 0.99912



Slope 0.00205
 Intercept -0.00173
 R² 0.99998



Slope 0.00204
 Intercept -0.00240
 R² 0.99971



Slope 0.00206
 Intercept -0.00043
 R² 0.99946

Max %RE = 12.44

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

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

LINEARITY

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

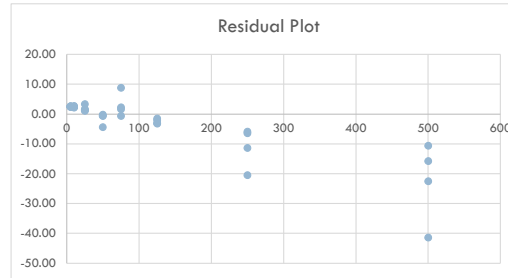
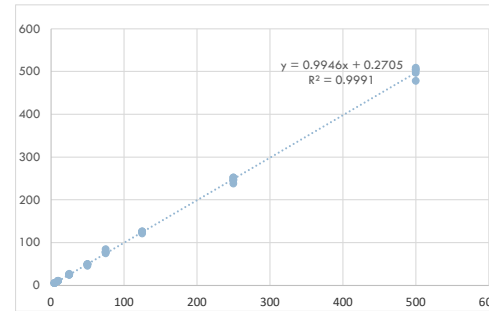
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	5.06	2.50	2.56
	10	9.84	7.72	2.12
	25	24.39	23.38	1.01
	50	49.27	49.48	-0.21
	75	77.81	75.58	2.23
	125	125.66	127.78	-2.12
	250	251.80	258.28	-6.48
OPI1_20220418B_JR	500	496.72	519.28	-22.56
	5	4.99	2.50	2.49
	10	10.08	7.72	2.36
	25	24.89	23.38	1.51
	50	48.86	49.48	-0.62
	75	77.19	75.58	1.62
	125	124.77	127.78	-3.00
OPI1_20220418B_KMY	250	246.90	258.28	-11.38
	500	503.52	519.28	-15.76
	5	4.87	2.50	2.37
	10	10.32	7.72	2.60
	25	26.68	23.38	3.30
	50	45.01	49.48	-4.47
	75	84.33	75.58	8.75
OPI1_20220421B_CD	125	124.47	127.78	-3.31
	250	237.72	258.28	-20.56
	500	477.77	519.28	-41.51
	5	5.02	2.50	2.53
	10	10.01	7.72	2.29
	25	24.60	23.38	1.22
	50	48.76	49.48	-0.72
OPI1_20220421B_CLR	75	74.93	75.58	-0.65
	125	126.25	127.78	-1.53
	250	252.28	258.28	-5.99
	500	508.61	519.28	-10.66
	5	4.94	2.50	2.44
	10	10.26	7.72	2.54
	25	24.97	23.38	1.59
OPI1_20220427B_CD	50	48.80	49.48	-0.68
	75	78.66	75.58	3.09
	125	121.16	127.78	-6.61
	250	246.12	258.28	-12.16
	500	504.48	519.28	-14.80
	5	4.93	2.50	2.44
	10	10.36	7.72	2.64
OPI1_20220427B_CD	25	24.43	23.38	1.05
	50	49.71	49.48	0.23
	75	74.77	75.58	-0.80
	125	129.69	127.78	1.91
	250	241.30	258.28	-16.98
	500	503.31	519.28	-15.96

Slope	0.9957
Std err in slope, S_b	0.0043
Degrees freedom	46
Confidence level	95%
Student t	2.0129
Confidence interval	0.009
Slope	0.996 ± 0.009
Range	0.9870 - 1.0044

Intercept	0.1705
Std err in Intercept	0.8862
Degrees freedom	46
Confidence Level	95%
Student t	2.0129
Confidence interval	1.784
Intercept	0.171 ± 1.784
Lower	-1.6134 - 1.9544

YES

YES



Comments: N/A

Acceptance Criteria: 95% CI of slope includes 1
 95% CI of intercept includes 0

Validation Study 2

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	100	400	
OPI1_20220414B_JR	1-1	15.08	99.24	374.76	
	1-2	15.56	102.49	381.83	
	1-3	15.10	102.14	377.55	
	1-4	15.51	101.35	379.97	
	Within Run	Mean	15.31	101.31	378.53
		SD	0.26	1.46	3.06
		%CV	1.68%	1.44%	0.81%
	% Bias	2.08%	1.31%	-5.37%	
OPI1_20220418B_JR	2-1	16.04	98.79	374.42	
	2-2	15.79	98.13	378.97	
	2-3	15.27	98.01	375.59	
	Within Run	Mean	15.70	98.31	376.33
		SD	0.40	0.42	2.36
		%CV	2.52%	0.43%	0.63%
		% Bias	4.69%	-1.69%	-5.92%
OPI1_20220418B_KMY	3-1	15.54	96.45	353.49	
	3-2	14.16	91.17	353.85	
	3-3	13.71	94.03	372.79	
	3-4	15.21	96.68	343.56	
	Within Run	Mean	14.66	94.58	355.92
		SD	0.86	2.57	12.21
		%CV	5.88%	2.72%	3.43%
	% Bias	-2.30%	-5.42%	-11.02%	
OPI1_20220421B_CD	4-1	15.85	101.03	383.89	
	4-2	15.27	102.71	384.27	
	4-3	15.95	102.58	359.76	
	Within Run	Mean	15.69	102.11	375.97
		SD	0.37	0.93	14.05
		%CV	2.36%	0.92%	3.74%
		% Bias	4.60%	2.11%	-6.01%
OPI1_20220421B_CLR	5-1	15.77	100.24	381.71	
	5-2	15.99	99.45	378.74	
	5-3	15.78	100.09	376.61	
	Within Run	Mean	15.85	99.93	379.02
		SD	0.13	0.42	2.56
		%CV	0.81%	0.42%	0.68%
		% Bias	5.64%	-0.07%	-5.25%
OPI1_20220427B_CD	6-1	15.99	96.39	371.86	
	6-2	15.59	99.49	345.14	
	6-3	15.94	96.32	365.56	
	Within Run	Mean	15.84	97.40	360.85
		SD	0.22	1.81	13.96
		%CV	1.38%	1.86%	3.87%
		% Bias	5.60%	-2.60%	-9.79%

Mean		15.46	98.84	370.72
SD		0.61	3.03	12.73
Precision (%CV)	Max Within-Run	5.88%	2.72%	3.87%
	Between-Run	3.94%	3.07%	3.43%
% Bias		3.38%	-1.06%	-7.22%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Codeine
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 423.44 1/x Weighting: = 300.58 1/x2 Weighting: = 290.59	1/x2 weighting will be used.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9244 - 0.9693 95% CI of Intercept = 0.2593 - 3.1325	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 $\pm 20\%$ of target	Max %RE of Calibrators = 11.49	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ 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	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = -7.48% Max Within-Run Precision = 8.54% Max Between-Run Precision = 7.31%	HQC 5-3 concentration out of acceptable range.

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Unweighted: 423.44
 1/x Weighting: 300.58
 1/x² Weighting: 290.59

Σ| %RE |
 423.44
 300.58
 290.59

C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
5.0	0.04965	1	0.248234	5	0.04964675	25	0.002465	3.611275	-27.7745	27.77449
10	0.08274	1	0.827365	10	0.08273649	100	0.006845	8.053306	-19.4669	19.46694
25	0.18782	1	4.695394	25	0.18781577	625	0.035275	22.15935	-11.3626	11.36259
50	0.40241	1	20.12066	50	0.40241327	2500	0.161936	50.96733	1.93467	1.93467
75	0.59391	1	44.54292	75	0.59390557	5625	0.352724	76.67363	2.23151	2.23151
125	0.90202	1	112.7527	125	0.90202175	15625	0.813643	118.0357	-5.5714	5.571405
5.0	0.04286	1	0.214311	5	0.04286219	25	0.001837	2.700504	-45.9899	45.98992
10	0.08189	1	0.818897	10	0.08188968	100	0.006706	7.939629	-20.8037	20.80371
25	0.18558	1	4.639442	25	0.18557767	625	0.034439	21.85891	-12.5644	12.56438
50	0.35827	1	17.91357	50	0.35827143	2500	0.128358	45.04165	-9.9167	9.916701
75	0.54964	1	41.22299	75	0.54963981	5625	0.302104	70.73131	-5.69159	5.691585
125	0.83514	1	104.3931	125	0.83514489	15625	0.697467	109.0581	-12.7535	12.75354
5.0	0.05296	1	0.264815	5	0.052963	25	0.002805	4.056455	-18.8709	18.87089
10	0.10240	1	1.02403	10	0.102403	100	0.010486	10.69338	6.933767	6.933767
25	0.27216	1	6.80395	25	0.272158	625	0.07407	33.48162	33.92646	33.92646
50	0.46253	1	23.1263	50	0.462526	2500	0.21393	59.03698	18.07397	18.07397
75	0.76496	1	57.372	75	0.76496	5625	0.585164	99.63631	32.84841	32.84841
125	1.14830	1	143.5373	125	1.148298	15625	1.318588	151.0963	20.87707	20.87707
5.0	0.04699	1	0.234929	5	0.04698588	25	0.002208	3.254075	-34.9185	34.91849
10	0.09107	1	0.910713	10	0.09107133	100	0.008294	9.172192	-8.27808	8.278083
25	0.19803	1	4.950831	25	0.19803324	625	0.039217	23.53096	-5.87614	5.876142
50	0.39286	1	19.64289	50	0.39285776	2500	0.154337	49.68459	-0.63083	0.630829
75	0.52968	1	39.72571	75	0.52967618	5625	0.280557	68.05135	-9.26486	9.26486
125	0.85175	1	106.4687	125	0.85174976	15625	0.725478	111.2871	-10.9703	10.97029
5.0	0.046	1	0.232185	5	0.04643691	25	0.002156	3.180381	-36.3924	36.39238
10	0.098	1	0.977771	10	0.09777712	100	0.00956	10.07239	0.723895	0.723895
25	0.217	1	5.41476	25	0.21659041	625	0.046911	26.02212	4.088463	4.088463
50	0.399	1	19.95022	50	0.39900448	2500	0.159205	50.50973	1.019465	1.019465
75	0.596	1	44.71048	75	0.59613968	5625	0.355383	76.97354	2.631391	2.631391
125	0.942	1	117.7753	125	0.94220279	15625	0.887746	123.4297	-1.25622	1.25622
Sum	1450.0		11.4838	25	945.5125	1450	11.4837589	122500	7.419896	423.4425
Slope	0.00744924									
Intercept	0.02274550									
R ²	0.96185692									

Comments: 1/x² weighting will be used.

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

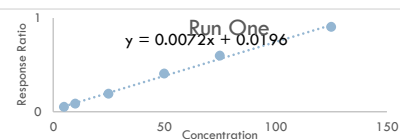
Validation Study 1

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

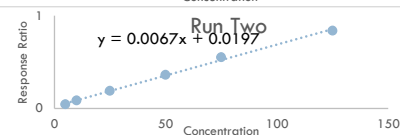
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

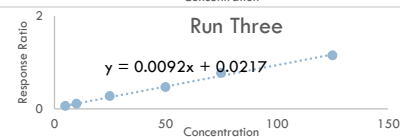
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	5.12	2.40	0.050
	10	9.59	4.10	0.083
	25	23.79	4.84	0.188
	50	52.78	5.56	0.402
	75	78.66	4.88	0.594
	125	120.30	3.76	0.902
OPI1_20220418B_JR	5	4.88	2.37	0.043
	10	10.45	4.48	0.082
	25	25.24	0.95	0.186
	50	49.87	0.26	0.358
	75	77.17	2.89	0.550
	125	117.89	5.69	0.835
OPI1_20220418B_KMY	5	4.93	1.40	0.053
	10	10.00	0.00	0.102
	25	27.42	9.68	0.272
	50	46.94	6.12	0.463
	75	77.96	3.95	0.765
	125	117.28	6.18	1.148
OPI1_20220421B_CD	5	4.76	4.77	0.047
	10	10.86	8.61	0.091
	25	25.66	2.64	0.198
	50	52.62	5.23	0.393
	75	71.55	4.61	0.530
	125	116.11	7.11	0.852
OPI1_20220427B_CD	5	4.70	6.06	0.046
	10	11.15	11.49	0.098
	25	26.08	4.33	0.217
	50	49.01	1.98	0.399
	75	73.79	1.62	0.596
	125	117.29	6.17	0.942



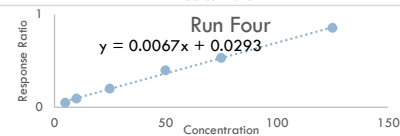
Slope 0.00725
 Intercept 0.01955
 R² 0.99600



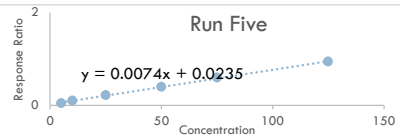
Slope 0.00667
 Intercept 0.01970
 R² 0.99708



Slope 0.00922
 Intercept 0.02168
 R² 0.99497



Slope 0.00667
 Intercept 0.02931
 R² 0.99721



Slope 0.00744
 Intercept 0.02348
 R² 0.99895

Max %RE = 11.49

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

LINEARITY

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

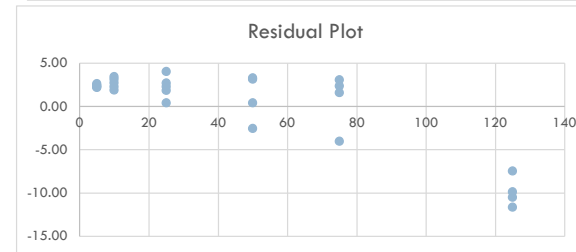
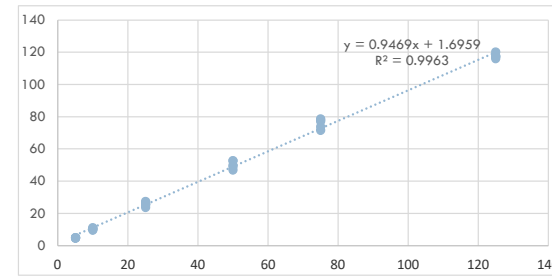
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	5.12	2.50	2.62
	10	9.59	7.72	1.87
	25	23.79	23.38	0.41
	50	52.78	49.48	3.30
	75	78.66	75.58	3.08
OPI1_20220418B_JR	125	120.30	127.78	-7.48
	5	4.88	2.50	2.38
	10	10.45	7.72	2.73
	25	25.24	23.38	1.86
	50	49.87	49.48	0.39
OPI1_20220418B_KMY	75	77.17	75.58	1.59
	125	117.89	127.78	-9.89
	5	4.93	2.50	2.43
	10	10.00	7.72	2.28
	25	27.42	23.38	4.04
OPI1_20220418B_KMY	50	46.94	49.48	-2.54
	75	77.96	75.58	2.38
	125	117.28	127.78	-10.50
	5	4.76	2.50	2.26
	10	10.86	7.72	3.14
OPI1_20220421B_CD	25	25.66	23.38	2.28
	50	52.62	49.48	3.14
	75	71.55	75.58	-4.03
	125	116.11	127.78	-11.67
	5	4.70	2.50	2.20
OPI1_20220427B_CD	10	11.15	7.72	3.43
	25	26.08	23.38	2.71
	50	49.01	49.48	-0.47
	75	73.79	75.58	-1.79
	125	117.29	127.78	-10.49

Slope	0.9469
Std err in slope, S_b	0.0110
Degrees freedom	28
Confidence level	95%
Student t	2.0484
Confidence interval	0.022
Slope	0.947 ± 0.022
Range	0.9244 - 0.9693

Intercept	1.6959
Std err in Intercept	0.7013
Degrees freedom	28
Confidence Level	95%
Student t	2.0484
Confidence interval	1.437
Intercept	1.696 ± 1.437
Lower	0.2593 - 3.1325

NO

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

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	30	100	
OPI1_20220414B_JR	1-1	16.29	31.74	90.24	
	1-2	16.43	31.05	92.63	
	1-3	16.46	30.85	89.43	
	1-4	15.88	31.56	91.02	
	Within Run	Mean	16.27	31.30	90.83
		SD	0.27	0.42	1.36
		%CV	1.64%	1.34%	1.50%
	% Bias	8.43%	4.33%	-9.17%	
OPI1_20220418B_JR	2-1	16.90	33.24	97.17	
	2-2	17.79	34.05	97.80	
	2-3	17.74	33.57	100.90	
	Within Run	Mean	17.48	33.62	98.62
		SD	0.51	0.41	2.00
		%CV	2.89%	1.21%	2.03%
		% Bias	16.52%	12.08%	-1.38%
OPI1_20220418B_KMY	3-1	15.45	30.08	86.03	
	3-2	14.65	27.06	90.40	
	3-3	13.56	28.22	98.77	
	3-4	16.61	30.58	86.13	
	Within Run	Mean	15.07	28.99	90.33
		SD	1.29	1.64	5.98
		%CV	8.54%	5.65%	6.62%
	% Bias	0.45%	-3.38%	-9.67%	
OPI1_20220421B_CD	4-1	15.71	33.72	92.67	
	4-2	15.31	34.09	102.99	
	4-3	16.46	30.89	101.29	
	Within Run	Mean	15.83	32.90	98.98
		SD	0.58	1.75	5.54
		%CV	3.69%	5.33%	5.59%
		% Bias	5.51%	9.67%	-1.02%
OPI1_20220427B_CD	5-1	15.06	28.45	89.52	
	5-2	14.47	29.02	82.38	
	5-3	15.27	28.76	79.56	
	Within Run	Mean	14.93	28.74	83.82
		SD	0.41	0.28	5.13
		%CV	2.75%	0.99%	6.12%
		% Bias	-0.44%	-4.18%	-16.18%

Mean		15.89	31.00	92.29
SD		1.13	2.22	6.75
Precision (%CV)	Max Within-Run	8.54%	5.65%	6.62%
	Between-Run	7.09%	7.15%	7.31%
% Bias		6.10%	3.70%	-7.48%

Comments: HQC 5-3 concentration out of acceptable range.

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: O-desmethyltramadol
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 291.92 1/x Weighting: = 194.05 1/x2 Weighting: = 173.79	1/x weighting will be used. While 1/x2 weighting gave a lower %RE, data processed using 1/x2 weighting failed to meet the SOP criteria for linearity.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9998 - 1.0341 95% CI of Intercept = -1.9129 - 0.2772	N/A
1	Validation Calibration	%RE Calibrators $\pm 20\%$ of target	Max %RE of Calibrators = 12.42	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ 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	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = 1.59% Max Within-Run Precision = 5.20% Max Between-Run Precision = 5.50%	N/A

Validation Study 1

Analyte: O-desmethyltramadol
 Units: ng/mL
 Instrument: LCMS-1

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Unweighted: $\Sigma | \%RE |$
 1/x Weighting: 291.92
 1/x² Weighting: 194.05
 173.79

C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
5.0	0.094	1	0.468731	5	0.09374615	25	0.008788	5.972945	19.4589	19.4589
10	0.181	1	1.805119	10	0.18051187	100	0.032585	10.28865	2.886545	2.886545
25	0.430	1	10.75215	25	0.4300859	625	0.184974	22.70242	-9.19033	9.190333
50	0.912	1	45.60241	50	0.91204829	2500	0.831832	46.67513	-6.64974	6.64974
75	1.431	1	107.33	75	1.43106641	5625	2.047951	72.49099	-3.34535	3.34535
125	2.391	1	298.8521	125	2.39081714	15625	5.716007	120.2288	-3.81696	3.816962
5.0	0.089	1	0.443625	5	0.0887249	25	0.007872	5.723189	14.46378	14.46378
10	0.181	1	1.812933	10	0.18129332	100	0.032867	10.32752	3.275236	3.275236
25	0.438	1	10.94469	25	0.43778752	625	0.191658	23.08549	-7.65802	7.658024
50	0.890	1	44.51517	50	0.89030531	2500	0.79264	45.59354	-8.81292	8.812922
75	1.463	1	109.7293	75	1.46305696	5625	2.140536	74.08219	-1.22375	1.223745
125	2.437	1	304.605	125	2.43684005	15625	5.938189	122.518	-1.98563	1.985626
5.0	0.09819	1	0.490925	5	0.098185	25	0.00964	6.193732	23.87465	23.87465
10	0.21141	1	2.1141	10	0.21141	100	0.044694	11.82552	18.25521	18.25521
25	0.56277	1	14.06918	25	0.562767	625	0.316707	29.30195	17.20779	17.20779
50	0.96020	1	48.0102	50	0.960204	2500	0.921992	49.07039	-1.85923	1.859229
75	1.79460	1	134.5948	75	1.794597	5625	3.220578	90.57293	20.7639	20.7639
125	2.74034	1	342.5423	125	2.740338	15625	7.509452	137.6139	10.09112	10.09112
5.0	0.09359	1	0.467928	5	0.09358561	25	0.008758	5.96496	19.29919	19.29919
10	0.18436	1	1.843603	10	0.18436034	100	0.033989	10.48008	4.800764	4.800764
25	0.44303	1	11.07587	25	0.44303469	625	0.19628	23.34649	-6.61405	6.614051
50	0.94624	1	47.31204	50	0.94624085	2500	0.895372	48.37586	-3.24828	3.248278
75	1.40776	1	105.5821	75	1.40776109	5625	1.981791	71.33179	-4.89095	4.890953
125	2.46637	1	308.2957	125	2.46636561	15625	6.082959	123.9866	-0.81075	0.810749
5.0	0.094	1	0.472275	5	0.09445494	25	0.008922	6.0082	20.164	20.164
10	0.184	1	1.84328	10	0.18432796	100	0.033977	10.47847	4.784659	4.784659
25	0.452	1	11.28836	25	0.45153447	625	0.203883	23.76926	-4.92294	4.922941
50	0.933	1	46.66713	50	0.93334253	2500	0.871128	47.7343	-4.5314	4.531398
75	1.499	1	112.4001	75	1.49866747	5625	2.246004	75.85345	1.137934	1.137934
125	2.400	1	300.0136	125	2.40010878	15625	5.760522	120.691	-3.44723	3.447231
5.0	0.093	1	0.463014	5	0.09260285	25	0.008575	5.916077	18.32154	18.32154
10	0.187	1	1.873909	10	0.18739088	100	0.035115	10.63082	6.308151	6.308151
25	0.458	1	11.46134	25	0.45845361	625	0.21018	24.11342	-3.54632	3.546316
50	0.940	1	47.00663	50	0.94013252	2500	0.883849	48.07203	-3.85593	3.855933
75	1.413	1	105.9977	75	1.41330304	5625	1.997425	71.60744	-4.52341	4.523412
125	2.534	1	316.8051	125	2.5344404	15625	6.423388	127.3726	1.898072	1.898072
Sum	1740.0	34.0339	30	2909.552	1740	34.0338904	147000	57.83108		291.9246

Comments: 1/x weighting will be used. While 1/x² weighting gave a lower %RE, data processed using 1/x² weighting failed to meet the SOP criteria for linearity.

Slope 0.02010462
 Intercept -0.02633766
 R² 0.99095727

Acceptance Criteria: The least complex weighting scheme that minimizes $\Sigma | \%RE |$

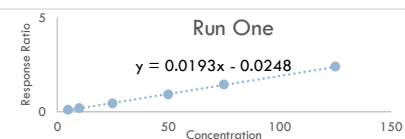
Validation Study 1

Analyte: O-desmethyltramadol
 Units: ng/mL
 Instrument: LCMS-1

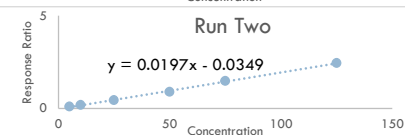
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

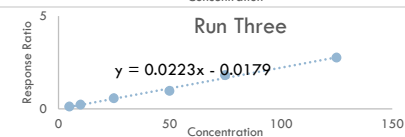
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	5.38	7.60	0.094
	10	9.97	0.30	0.181
	25	23.16	7.36	0.430
	50	48.63	2.74	0.912
	75	76.07	1.43	1.431
OPI1_20220418B_JR	5	5.33	6.58	0.089
	10	10.14	1.39	0.181
	25	23.47	6.13	0.438
	50	46.98	6.04	0.890
	75	76.74	2.32	1.463
OPI1_20220418B_KMY	5	5.01	0.20	0.098
	10	10.10	1.00	0.211
	25	25.91	3.64	0.563
	50	43.79	12.42	0.960
	75	81.32	8.43	1.795
OPI1_20220421B_CD	5	5.32	6.46	0.094
	10	10.03	0.27	0.184
	25	23.43	6.27	0.443
	50	49.51	0.98	0.946
	75	73.43	2.10	1.408
OPI1_20220421B_CLR	5	5.26	5.17	0.094
	10	9.91	0.92	0.184
	25	23.73	5.07	0.452
	50	48.66	2.68	0.933
	75	77.90	3.87	1.499
OPI1_20220427B_CD	5	5.29	5.77	0.093
	10	10.12	1.16	0.187
	25	23.92	4.31	0.458
	50	48.46	3.09	0.940
	75	72.56	3.26	1.413
	125	129.66	3.73	2.534



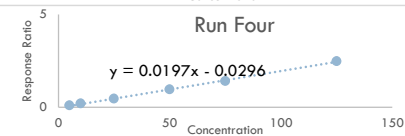
Slope: 0.01926
 Intercept: -0.02476
 R^2 : 0.99942



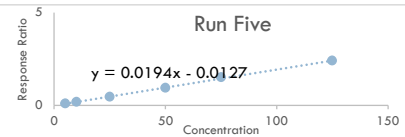
Slope: 0.01968
 Intercept: -0.03487
 R^2 : 0.99866



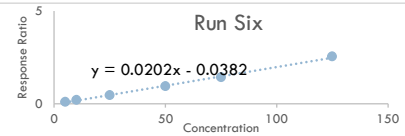
Slope: 0.02233
 Intercept: -0.01788
 R^2 : 0.99245



Slope: 0.01972
 Intercept: -0.02964
 R^2 : 0.99900



Slope: 0.01944
 Intercept: -0.01270
 R^2 : 0.99889



Slope: 0.02019
 Intercept: -0.03818
 R^2 : 0.99794

Max %RE = 12.42

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

LINEARITY

Analyte: O-desmethyltramadol
 Units: ng/mL
 Instrument: LCMS-1

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

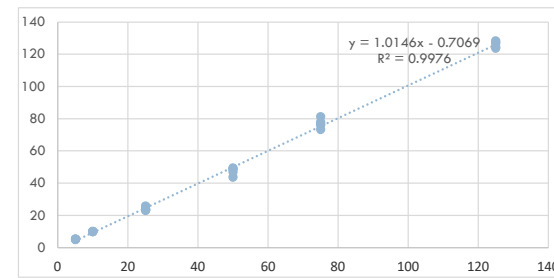
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	5.38	2.50	2.88
	10	9.97	7.72	2.25
	25	23.16	23.38	-0.22
	50	48.63	49.48	-0.85
	125	126.80	127.78	-0.98
OPI1_20220418B_JR	5	5.33	2.50	2.83
	10	10.14	7.72	2.42
	25	23.47	23.38	0.09
	50	46.98	49.48	-2.50
	125	76.74	75.58	1.17
OPI1_20220418B_KMY	5	5.01	2.50	2.51
	10	10.10	7.72	2.38
	25	25.91	23.38	2.53
	50	43.79	49.48	-5.69
	125	81.32	75.58	5.74
OPI1_20220421B_CD	5	5.32	2.50	2.83
	10	10.03	7.72	2.31
	25	23.43	23.38	0.05
	50	49.51	49.48	0.03
	125	73.43	75.58	-2.15
OPI1_20220421B_CLR	5	5.26	2.50	2.76
	10	9.91	7.72	2.19
	25	23.73	23.38	0.35
	50	48.66	49.48	-0.82
	125	77.90	75.58	2.33
OPI1_20220427B_CD	5	5.29	2.50	2.79
	10	10.12	7.72	2.40
	25	23.92	23.38	0.55
	50	48.46	49.48	-1.02
	125	72.56	75.58	-3.02

Slope	1.0169
Std err in slope, S_b	0.0084
Degrees freedom	34
Confidence level	95%
Student t	2.0322
Confidence interval	0.017
Slope	1.017 ± 0.017
Range	0.9998 - 1.0341

Intercept	-0.8179
Std err in Intercept	0.5388
Degrees freedom	34
Confidence Level	95%
Student t	2.0322
Confidence interval	1.095
Intercept	-0.818 ± 1.095
Lower	-1.9129 - 0.2772

YES

YES



Comments: N/A

Acceptance Criteria: 95% CI of slope includes 1
 95% CI of intercept includes 0

Validation Study 2

BIAS AND PRECISION

Analyte: O-desmethyltramadol
 Units: ng/mL
 Instrument: LCMS-1

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	30	100	
OPI1_20220414B_JR	1-1	15.14	30.13	101.14	
	1-2	15.13	29.78	101.09	
	1-3	14.93	29.89	101.40	
	1-4	15.24	29.92	100.47	
	Within Run	Mean	15.11	29.93	101.03
		SD	0.13	0.15	0.39
		%CV	0.86%	0.49%	0.39%
	% Bias	0.73%	-0.23%	1.03%	
OPI1_20220418B_JR	2-1	15.53	29.32	100.29	
	2-2	15.36	29.86	100.88	
	2-3	15.27	29.62	101.30	
	Within Run	Mean	15.39	29.60	100.82
		SD	0.13	0.27	0.51
		%CV	0.85%	0.92%	0.50%
		% Bias	2.58%	-1.34%	0.82%
OPI1_20220418B_KMY	3-1	14.94	28.20	93.91	
	3-2	15.21	28.07	90.38	
	3-3	14.12	29.11	84.85	
	3-4	16.02	29.28	92.88	
	Within Run	Mean	15.07	28.67	90.51
		SD	0.78	0.62	4.05
		%CV	5.20%	2.16%	4.48%
	% Bias	0.48%	-4.45%	-9.50%	
OPI1_20220421B_CD	4-1	15.16	30.55	104.92	
	4-2	14.95	30.57	107.04	
	4-3	15.42	30.04	103.90	
	Within Run	Mean	15.17	30.39	105.29
		SD	0.23	0.30	1.60
		%CV	1.53%	1.00%	1.52%
		% Bias	1.17%	1.29%	5.29%
OPI1_20220421B_CLR	5-1	15.43	29.77	102.98	
	5-2	15.51	29.72	102.97	
	5-3	15.40	29.87	102.12	
	Within Run	Mean	15.45	29.79	102.69
		SD	0.05	0.08	0.49
		%CV	0.35%	0.26%	0.48%
		% Bias	2.98%	-0.71%	2.69%
OPI1_20220427B_CD	6-1	15.13	29.03	99.48	
	6-2	15.03	30.24	92.96	
	6-3	15.58	29.02	99.09	
	Within Run	Mean	15.24	29.43	97.17
		SD	0.29	0.70	3.66
		%CV	1.93%	2.38%	3.76%
		% Bias	1.62%	-1.91%	-2.83%

Mean		15.22	29.60	99.20
SD		0.37	0.67	5.46
Precision (%CV)	Max Within-Run	5.20%	2.38%	4.48%
	Between-Run	2.43%	2.26%	5.50%
% Bias		1.59%	-1.22%	-0.42%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: 6-Acetylmorphine
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 490.82 1/x Weighting: = 238.91 1/x2 Weighting: = 209.9	1/x2 weighting will be used.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9937 - 1.0916 95% CI of Intercept = -4.3604 - 1.8913	N/A
1	Validation Calibration	%RE Calibrators $\pm 20\%$ of target	Max %RE of Calibrators = 19.17	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ 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	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = -5.02% Max Within-Run Precision = 8.03% Max Between-Run Precision = 5.94%	N/A

Validation Study 1

Analyte: 6-Acetylmorphine
 Units: ng/mL
 Instrument: LCMS-1

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Unweighted: 490.82
 1/x Weighting: 238.91
 1/x² Weighting: 209.90

Σ |%RE|
 490.82
 238.91
 209.90

C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
5.0	0.13336	1	0.66682	5	0.1333641	25	0.017786	7.387003	47.74006	47.74006
10	0.26827	1	2.682711	10	0.2682711	100	0.071969	11.65804	16.58044	16.58044
25	0.65533	1	16.38337	25	0.65533495	625	0.429464	23.91216	-4.35137	4.35137
50	1.31980	1	65.98988	50	1.31979767	2500	1.741866	44.94848	-10.103	10.10303
75	2.12953	1	159.7148	75	2.12953052	5625	4.5349	70.58394	-5.88808	5.888081
125	3.75995	1	469.9936	125	3.75994903	15625	14.13722	122.2016	-2.23871	2.238715
5.0	0.13296	1	0.664801	5	0.13296011	25	0.017678	7.374213	47.48426	47.48426
10	0.26920	1	2.691974	10	0.26919744	100	0.072467	11.68737	16.87371	16.87371
25	0.68154	1	17.03839	25	0.68153572	625	0.464491	24.74165	-1.03339	1.033394
50	1.41655	1	70.82753	50	1.41655057	2500	2.006616	48.0116	-3.9768	3.976804
75	2.40574	1	180.4304	75	2.40573846	5625	5.787578	79.32845	5.771264	5.771264
125	4.11960	1	514.9497	125	4.11969738	15625	16.97108	133.5878	6.870214	6.870214
5.0	0.12260	1	0.61302	5	0.122604	25	0.015032	7.046347	40.92695	40.92695
10	0.27659	1	2.76589	10	0.276589	100	0.076501	11.92138	19.21382	19.21382
25	0.73636	1	18.40903	25	0.736361	625	0.542228	26.47737	5.909494	5.909494
50	1.27437	1	63.7187	50	1.274374	2500	1.624029	43.51041	-12.9792	12.97918
75	2.45668	1	184.2507	75	2.456676	5625	6.035257	80.94109	7.92145	7.92145
125	3.83343	1	479.179	125	3.833432	15625	14.6952	124.528	-0.37759	0.377588
5.0	0.13655	1	0.682725	5	0.13654504	25	0.018645	7.487709	49.75417	49.75417
10	0.27034	1	2.703376	10	0.27033762	100	0.073082	11.72347	17.23468	17.23468
25	0.66431	1	16.60781	25	0.66431258	625	0.441311	24.19638	-3.21447	3.214474
50	1.39288	1	69.64401	50	1.39288023	2500	1.940115	47.26222	-5.47557	5.475569
75	2.23044	1	167.2829	75	2.2304382	5625	4.974855	73.77859	-1.62855	1.628546
125	3.97881	1	497.3507	125	3.9788057	15625	15.83089	129.1304	3.304338	3.304338
5.0	0.11767	1	0.588367	5	0.11767335	25	0.013847	6.890247	37.80494	37.80494
10	0.24197	1	2.41971	10	0.24197102	100	0.05855	10.82541	8.25406	8.25406
25	0.65944	1	16.48608	25	0.65944333	625	0.434866	24.04223	-3.8311	3.831099
50	1.19027	1	59.51356	50	1.19027129	2500	1.416746	40.84779	-18.3044	18.30442
75	2.06640	1	154.98	75	2.0663996	5625	4.270007	68.58527	-8.55298	8.552977
125	3.75966	1	469.9571	125	3.75965673	15625	14.13502	122.1924	-2.24612	2.246118
5.0	0.123	1	0.616709	5	0.1233419	25	0.015213	7.069708	41.39417	41.39417
10	0.258	1	2.583684	10	0.25836839	100	0.066754	11.34453	13.44533	13.44533
25	0.636	1	15.90378	25	0.6361513	625	0.404688	23.30482	-6.78072	6.780723
50	1.377	1	68.83983	50	1.37679652	2500	1.895569	46.75302	-6.49396	6.493962
75	2.223	1	166.7465	75	2.22328627	5625	4.943002	73.55217	-1.93044	1.930445
125	4.043	1	505.3909	125	4.04312715	15625	16.34688	131.1668	4.933428	4.933428
Sum	1740.0		51.3617	30	4469.268	1740	51.3616693	147000	136.5214	490.8233
Slope	0.03158644									
Intercept	-0.09996505									
R ²	0.99229170									

Comments: 1/x² weighting will be used.

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

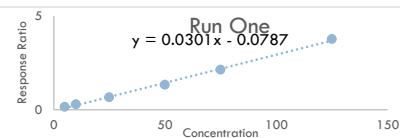
Validation Study 1

Analyte: 6-Acetylmorphine
 Units: ng/mL
 Instrument: LCMS-1

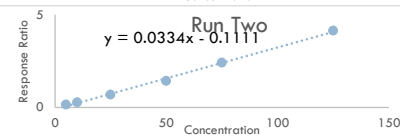
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

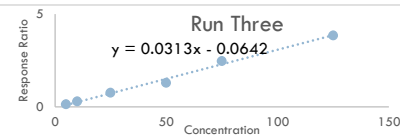
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	5.08	1.60	0.133
	10	9.90	1.00	0.268
	25	23.73	5.08	0.655
	50	47.47	5.06	1.320
	75	76.39	1.85	2.130
	125	134.64	7.71	3.760
OPI1_20220418B_JR	5	5.17	3.33	0.133
	10	9.61	3.86	0.269
	25	23.08	7.70	0.682
	50	47.07	5.86	1.417
	75	79.37	5.82	2.406
	125	135.32	8.25	4.120
OPI1_20220418B_KMY	5	5.00	0.00	0.123
	10	10.09	0.90	0.277
	25	25.30	1.20	0.736
	50	43.09	13.82	1.274
	75	82.19	9.59	2.457
	125	127.72	2.18	3.833
OPI1_20220421B_CD	5	5.14	2.79	0.137
	10	9.71	2.90	0.270
	25	23.17	7.33	0.664
	50	48.05	3.89	1.393
	75	76.66	2.22	2.230
	125	136.39	9.11	3.979
OPI1_20220421B_CLR	5	4.95	1.06	0.118
	10	10.18	1.78	0.242
	25	23.70	5.19	0.659
	50	55.97	11.93	1.190
	75	83.78	11.71	2.066
	125	101.03	19.17	3.760
OPI1_20220427B_CD	5	5.15	2.98	0.123
	10	9.75	2.49	0.258
	25	22.63	9.50	0.636
	50	47.87	4.27	1.377
	75	76.72	2.29	2.223
	125	138.74	10.99	4.043



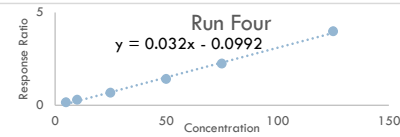
Slope 0.03013
 Intercept -0.07873
 R² 0.99730



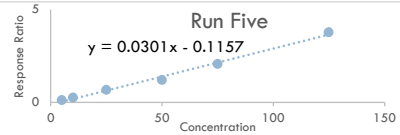
Slope 0.03342
 Intercept -0.11110
 R² 0.99716



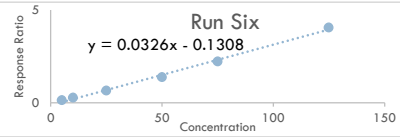
Slope 0.03133
 Intercept -0.06424
 R² 0.99195



Slope 0.03196
 Intercept -0.09924
 R² 0.99701



Slope 0.03010
 Intercept -0.11568
 R² 0.99283



Slope 0.03257
 Intercept -0.13080
 R² 0.99574

Max %RE = 19.17

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1 **LINEARITY**

Analyte: 6-Acetylmorphine
 Units: ng/mL
 Instrument: LCMS-1

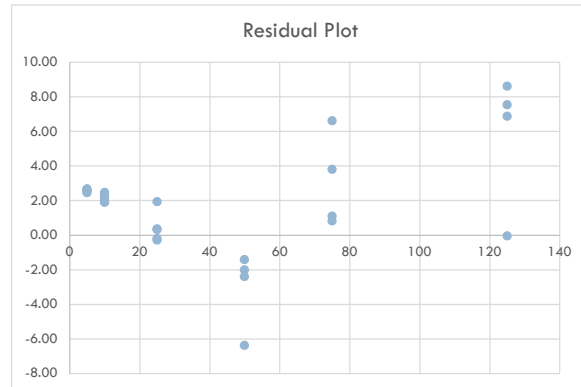
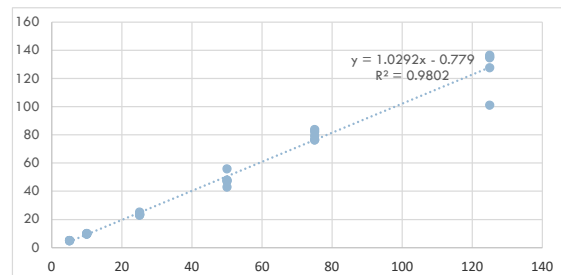
Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	5.08	2.50	2.58
	10	9.90	7.72	2.18
	25	23.73	23.38	0.35
	50	47.47	49.48	-2.01
	125	134.64	127.78	6.86
OPI1_20220418B_JR	5	5.17	2.50	2.67
	10	9.61	7.72	1.90
	25	23.08	23.38	-0.30
	50	47.07	49.48	-2.41
	125	79.37	75.58	3.79
OPI1_20220418B_KMY	5	5.00	2.50	2.50
	10	10.09	7.72	2.37
	25	25.30	23.38	1.92
	50	43.09	49.48	-6.39
	125	82.19	75.58	6.61
OPI1_20220421B_CD	5	5.14	2.50	2.64
	10	9.71	7.72	1.99
	25	23.17	23.38	-0.21
	50	48.05	49.48	-1.42
	125	76.66	75.58	1.09
OPI1_20220421B_CLR	5	4.95	2.50	2.45
	10	10.18	7.72	2.46
	25	23.70	23.38	0.32
	50	55.97	49.48	6.49
	125	83.78	75.58	8.21
OPI1_20220427B_CD	5	5.15	2.50	2.65
	10	9.75	7.72	2.03
	25	22.63	23.38	-0.75
	50	47.87	49.48	-1.61
	125	76.72	75.58	1.14
125	138.74	127.78	10.96	

Slope	1.0427
Std err in slope, S_b	0.0241
Degrees freedom	34
Confidence level	95%
Student t	2.0322
Confidence interval	0.049
Slope	1.043 ± 0.049
Range	0.9937 - 1.0916

Intercept	-1.2345
Std err in Intercept	1.5381
Degrees freedom	34
Confidence Level	95%
Student t	2.0322
Confidence interval	3.126
Intercept	-1.235 ± 3.126
Lower	-4.3604 - 1.8913

YES **YES**



Comments: N/A

Acceptance Criteria: 95% CI of slope includes 1
 95% CI of intercept includes 0

Validation Study 2

BIAS AND PRECISION

Analyte: 6-Acetylmorphine
 Units: ng/mL
 Instrument: LCMS-1

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	30	100	
OPI1_20220414B_JR	1-1	13.72	29.77	110.49	
	1-2	14.18	30.18	113.24	
	1-3	14.04	29.43	111.10	
	1-4	13.80	29.17	111.58	
	Within Run	Mean	13.94	29.64	111.60
		SD	0.21	0.44	1.18
		%CV	1.53%	1.48%	1.06%
	% Bias	-7.10%	-1.21%	11.60%	
OPI1_20220418B_JR	2-1	13.56	27.06	97.63	
	2-2	13.57	26.60	98.13	
	2-3	13.55	26.42	96.57	
	Within Run	Mean	13.56	26.69	97.45
		SD	0.01	0.33	0.80
		%CV	0.08%	1.24%	0.82%
		% Bias	-9.59%	-11.02%	-2.55%
OPI1_20220418B_KMY	3-1	14.57	27.34	96.23	
	3-2	14.19	25.64	95.30	
	3-3	12.66	27.59	104.25	
	3-4	15.38	28.48	93.70	
	Within Run	Mean	14.20	27.26	97.37
		SD	1.14	1.19	4.70
		%CV	8.03%	4.35%	4.83%
	% Bias	-5.33%	-9.12%	-2.63%	
OPI1_20220421B_CD	4-1	14.84	28.07	103.79	
	4-2	14.16	29.30	104.40	
	4-3	14.63	28.96	102.53	
	Within Run	Mean	14.55	28.78	103.57
		SD	0.35	0.63	0.95
		%CV	2.40%	2.21%	0.92%
		% Bias	-3.02%	-4.08%	3.57%
OPI1_20220421B_CLR	5-1	14.41	28.59	105.93	
	5-2	14.36	28.82	105.22	
	5-3	14.51	28.79	106.11	
	Within Run	Mean	14.43	28.73	105.76
		SD	0.08	0.13	0.47
		%CV	0.52%	0.44%	0.45%
		% Bias	-3.83%	-4.23%	5.76%
OPI1_20220427B_CD	6-1	15.06	29.01	108.62	
	6-2	14.70	31.34	101.84	
	6-3	14.67	29.41	112.87	
	Within Run	Mean	14.81	29.92	107.78
		SD	0.22	1.25	5.56
		%CV	1.47%	4.17%	5.16%
		% Bias	-1.26%	-0.27%	7.78%

Mean		14.23	28.50	103.98
SD		0.63	1.39	6.17
Precision (%CV)	Max Within-Run	8.03%	4.35%	5.16%
	Between-Run	4.42%	4.86%	5.94%
% Bias		-5.02%	-4.99%	3.92%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Oxycodone
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 254.64 1/x Weighting: = 183.03 1/x2 Weighting: = 177.02	1/x weighting will be used. While 1/x2 weighting gave a lower %RE, data processed using 1/x2 weighting failed to meet the SOP criteria for linearity.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9689 - 1.0088 95% CI of Intercept = -0.7350 - 1.8108	N/A
1	Validation Calibration	%RE Calibrators $\pm 20\%$ of target	Max %RE of Calibrators = 10.96	The calculated R2 value for run three was below 0.99; however, this calculation does not account for the weighting scheme used. When 1/x weighting is applied, the R2 value for this run is above 0.99 and therefore passes.
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ 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	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = -5.27% Max Within-Run Precision = 8.25% Max Between-Run Precision = 4.27%	N/A

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	$\Sigma \%RE $	$\Sigma \%RE $	$\Sigma \%RE $	C_{room}	y	w	wxy	wx	wy	wx^2	wy^2	C_{found}	$\%RE$	$ \%RE $
Unweighted:	254.64			5.0	0.033	1	0.162966	5	0.03259322	25	0.001062	4.316332	-13.6734	13.67336
1/x Weighting:	183.03			10	0.062	1	0.623933	10	0.06239327	100	0.003893	9.21891	-7.8109	7.810896
1/x ² Weighting:	177.02			25	0.146	1	3.638135	25	0.14552541	625	0.021178	22.89546	-8.41816	8.418155
				50	0.299	1	14.92805	50	0.29856103	2500	0.089139	48.07224	-3.85553	3.855525
				75	0.454	1	34.03096	75	0.4537461	5625	0.205886	73.60263	-1.86316	1.863158
				125	0.737	1	92.17963	125	0.73743705	15625	0.543813	120.2743	-3.78058	3.780581
				5.0	0.032	1	0.158542	5	0.03170843	25	0.001005	4.170769	-16.5846	16.58462
				10	0.063	1	0.630439	10	0.06304388	100	0.003975	9.325945	-6.74055	6.740546
				25	0.150	1	3.749229	25	0.14996915	625	0.022491	23.62653	-5.4939	5.493897
				50	0.294	1	14.69499	50	0.29389971	2500	0.086377	47.30538	-5.38925	5.389248
				75	0.454	1	34.05261	75	0.45403477	5625	0.206148	73.65012	-1.79984	1.799837
				125	0.718	1	89.7746	125	0.71819682	15625	0.515807	117.109	-6.31284	6.312839
				5.0	0.03273	1	0.163655	5	0.032731	25	0.001071	4.338999	-13.22	13.22003
				10	0.07559	1	0.75593	10	0.075593	100	0.005714	11.39047	13.90474	13.90474
				25	0.19106	1	4.776375	25	0.191055	625	0.036502	30.3858	21.54319	21.54319
				50	0.31880	1	15.9399	50	0.318798	2500	0.101632	51.40154	2.803076	2.803076
				75	0.59371	1	44.5284	75	0.593712	5625	0.352494	96.62923	28.83898	28.83898
				125	0.86454	1	108.068	125	0.864544	15625	0.747436	141.1854	12.9483	12.9483
				5.0	0.03292	1	0.164586	5	0.03291726	25	0.001084	4.369641	-12.6072	12.60718
				10	0.06479	1	0.647924	10	0.0647924	100	0.004198	9.613605	-3.86395	3.86395
				25	0.15625	1	3.906167	25	0.15624669	625	0.024413	24.65928	-1.36288	1.362876
				50	0.30210	1	15.10504	50	0.30210085	2500	0.091265	48.65459	-2.69081	2.690814
				75	0.45323	1	33.99192	75	0.45322563	5625	0.205413	73.51701	-1.97732	1.977324
				125	0.74947	1	93.68428	125	0.7494742	15625	0.561712	122.2546	-2.19634	2.196339
				5.0	0.033	1	0.163624	5	0.03272485	25	0.001071	4.337987	-13.2403	13.24026
				10	0.065	1	0.645024	10	0.06450244	100	0.004161	9.565902	-4.34098	4.340979
				25	0.153	1	3.815717	25	0.15262869	625	0.023296	24.06406	-3.74375	3.743753
				50	0.310	1	15.50348	50	0.31006967	2500	0.096143	49.96559	-0.06882	0.068819
				75	0.480	1	35.98887	75	0.47985166	5625	0.230258	77.89741	3.863211	3.863211
				125	0.701	1	87.5892	125	0.7007136	15625	0.491	114.2327	-8.61385	8.613851
				5.0	0.033	1	0.163455	5	0.03269101	25	0.001069	4.332419	-13.3516	13.35163
				10	0.067	1	0.66598	10	0.06659796	100	0.004435	9.910649	-0.89351	0.893515
				25	0.151	1	3.785043	25	0.15140171	625	0.022922	23.8622	-4.55118	4.551183
				50	0.313	1	15.64782	50	0.31295633	2500	0.097942	50.44049	0.880983	0.880983
				75	0.457	1	34.26674	75	0.45688984	5625	0.208748	74.11983	-1.17356	1.173564
				125	0.768	1	96.00057	125	0.76800459	15625	0.589831	125.3031	0.242494	0.242494
				Sum	1740.0	10.8053	30	904.5918	1740	10.8053312	147000	5.604582		254.6437

Comments: 1/x weighting will be used. While 1/x² weighting gave a lower %RE, data processed using 1/x² weighting failed to meet the SOP criteria for linearity.

Slope 0.00607844
 Intercept 0.00635664
 R² 0.98416770

Acceptance Criteria: The least complex weighting scheme that minimizes $\Sigma | \%RE |$

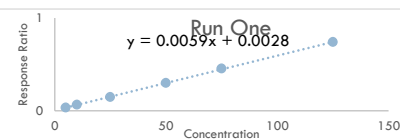
Validation Study 1

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

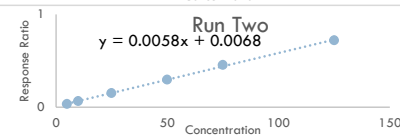
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

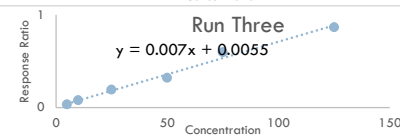
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	5.05	1.00	0.033
	10	10.10	1.00	0.062
	25	24.16	3.36	0.146
	50	50.06	0.12	0.299
	75	76.31	1.75	0.454
	125	124.32	0.54	0.737
OPI1_20220418B_JR	5	4.83	3.48	0.032
	10	10.21	2.05	0.063
	25	25.13	0.51	0.150
	50	49.84	0.33	0.294
	75	77.33	3.10	0.454
	125	122.68	1.86	0.718
OPI1_20220418B_KMY	5	4.57	8.60	0.033
	10	10.56	5.60	0.076
	25	26.68	6.72	0.191
	50	44.52	10.96	0.319
	75	82.92	10.56	0.594
	125	120.74	3.41	0.865
OPI1_20220421B_CD	5	4.85	3.04	0.033
	10	10.17	1.75	0.065
	25	25.46	1.83	0.156
	50	49.83	0.33	0.302
	75	75.09	0.12	0.453
	125	124.60	0.32	0.749
OPI1_20220421B_CLR	5	4.70	6.10	0.033
	10	10.08	0.83	0.065
	25	25.03	0.11	0.153
	50	51.72	3.45	0.310
	75	80.51	7.35	0.480
	125	117.96	5.63	0.701
OPI1_20220427B_CD	5	4.88	2.43	0.033
	10	10.43	4.30	0.067
	25	24.32	2.74	0.151
	50	50.77	1.53	0.313
	75	74.33	0.89	0.457
	125	125.27	0.22	0.768



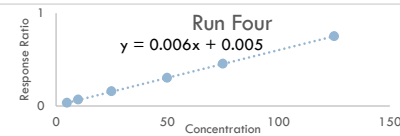
Slope 0.00591
 Intercept 0.00283
 R² 0.99972



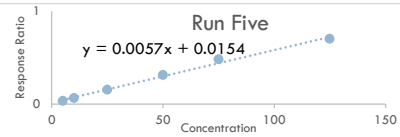
Slope 0.00576
 Intercept 0.00681
 R² 0.99907



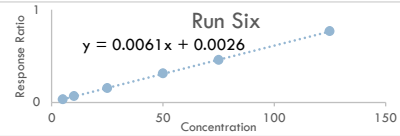
Slope 0.00705
 Intercept 0.00549
 R² 0.98914



Slope 0.00596
 Intercept 0.00500
 R² 0.99997



Slope 0.00568
 Intercept 0.01538
 R² 0.99294



Slope 0.00611
 Intercept 0.00263
 R² 0.99983

Max %RE = 10.96

Comments: The calculated R2 value for run three was below 0.99; however, this calculation does not account for the weighting scheme used. When 1/x weighting is applied, the R2 value for this run is above 0.99 and therefore passes.

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

LINEARITY

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

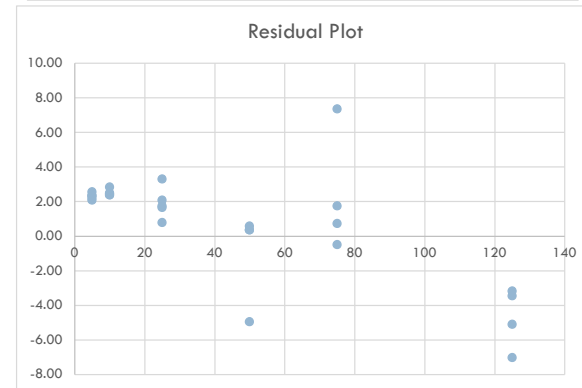
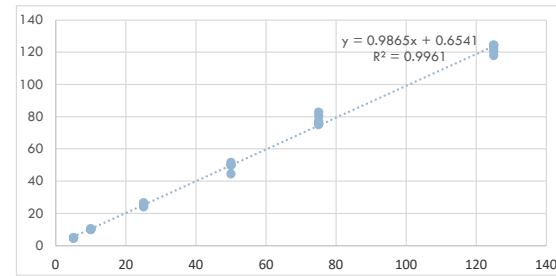
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	5.05	2.50	2.55
	10	10.10	7.72	2.38
	25	24.16	23.38	0.78
	50	50.06	49.48	0.58
	125	124.32	127.78	-3.46
OPI1_20220418B_JR	5	4.83	2.50	2.33
	10	10.21	7.72	2.49
	25	25.13	23.38	1.75
	50	49.84	49.48	0.36
	125	77.33	75.58	1.75
OPI1_20220418B_KMY	5	4.57	2.50	2.07
	10	10.56	7.72	2.84
	25	26.68	23.38	3.30
	50	44.52	49.48	-4.96
	125	82.92	75.58	7.34
OPI1_20220421B_CD	5	4.85	2.50	2.35
	10	10.17	7.72	2.46
	25	25.46	23.38	2.08
	50	49.83	49.48	0.36
	125	75.09	75.58	-0.49
OPI1_20220421B_CLR	5	4.70	2.50	2.20
	10	10.08	7.72	2.37
	25	25.03	23.38	1.65
	50	51.72	49.48	2.25
	125	80.51	75.58	4.93
OPI1_20220427B_CD	5	4.88	2.50	2.38
	10	10.43	7.72	2.71
	25	24.32	23.38	0.94
	50	50.77	49.48	1.29
	125	74.33	75.58	-1.24
125	125.27	127.78	-2.50	

Slope	0.9889
Std err in slope, S_b	0.0098
Degrees freedom	34
Confidence level	95%
Student t	2.0322
Confidence interval	0.020
Slope	0.989 ± 0.02
Range	0.9689 - 1.0088

Intercept	0.5379
Std err in Intercept	0.6264
Degrees freedom	34
Confidence Level	95%
Student t	2.0322
Confidence interval	1.273
Intercept	0.538 ± 1.273
Lower	-0.7350 - 1.8108

YES

YES



Comments: N/A

Acceptance Criteria: 95% CI of slope includes 1
 95% CI of intercept includes 0

Validation Study 2

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	30	100	
OPI1_20220414B_JR	1-1	15.62	30.76	94.62	
	1-2	15.20	30.96	93.24	
	1-3	15.42	31.08	94.92	
	1-4	15.90	30.96	95.82	
	Within Run	Mean	15.54	30.94	94.65
		SD	0.30	0.13	1.07
		%CV	1.92%	0.43%	1.13%
	% Bias	3.57%	3.13%	-5.35%	
OPI1_20220418B_JR	2-1	16.16	30.63	96.94	
	2-2	15.86	31.06	98.30	
	2-3	16.17	31.33	99.61	
	Within Run	Mean	16.07	31.01	98.28
		SD	0.18	0.36	1.33
		%CV	1.09%	1.15%	1.36%
		% Bias	7.10%	3.36%	-1.72%
OPI1_20220418B_KMY	3-1	15.17	28.16	89.12	
	3-2	14.42	27.33	91.07	
	3-3	13.56	28.73	95.19	
	3-4	16.46	29.66	88.82	
	Within Run	Mean	14.90	28.47	91.05
		SD	1.23	0.98	2.93
		%CV	8.25%	3.44%	3.22%
	% Bias	-0.65%	-5.10%	-8.95%	
OPI1_20220421B_CD	4-1	15.53	31.03	96.06	
	4-2	15.12	30.78	95.20	
	4-3	15.73	30.87	93.56	
	Within Run	Mean	15.46	30.90	94.94
		SD	0.31	0.13	1.27
		%CV	2.01%	0.42%	1.34%
		% Bias	3.06%	2.98%	-5.06%
OPI1_20220421B_CLR	5-1	15.76	31.01	97.93	
	5-2	16.25	30.42	98.47	
	5-3	15.64	30.26	97.66	
	Within Run	Mean	15.88	30.56	98.02
		SD	0.32	0.39	0.41
		%CV	2.02%	1.29%	0.42%
		% Bias	5.88%	1.88%	-1.98%
OPI1_20220427B_CD	6-1	15.89	29.61	95.36	
	6-2	15.26	31.13	87.46	
	6-3	15.81	30.21	91.54	
	Within Run	Mean	15.65	30.32	91.45
		SD	0.34	0.76	3.95
		%CV	2.20%	2.52%	4.32%
		% Bias	4.34%	1.06%	-8.55%

Mean		15.55	30.30	94.54
SD		0.66	1.09	3.43
Precision (%CV)	Max Within-Run	8.25%	3.44%	4.32%
	Between-Run	4.27%	3.59%	3.63%
% Bias		3.88%	1.22%	-5.27%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Hydrocodone
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 632.83 1/x Weighting: = 268.85 1/x2 Weighting: = 224.25	1/x2 weighting will be used.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 1.0302 - 1.0566 95% CI of Intercept = -5.7433 - -0.3184	The linearity of the method is acceptable because the individual calibration curves on all six 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 $\pm 20\%$ of target	Max %RE of Calibrators = 10.48	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ of target	N/A	Not performed. All 8 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 8 calibrators used in validation will be used for casework.
2	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = 5.65% Max Within-Run Precision = 7.24% Max Between-Run Precision = 3.96%	N/A

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	$\Sigma \%RE $	C_{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
Unweighted:	632.83	5.0	0.010	1	0.05141	5	0.01028202	25	0.000106	7.737742	54.75483	54.75483
1/x Weighting:	268.85	10	0.020	1	0.20137	10	0.02013701	100	0.000405	12.28748	22.87483	22.87483
1/x ² Weighting:	224.25	25	0.047	1	1.172895	25	0.04691579	625	0.002201	24.65041	-1.39834	1.398342
		50	0.098	1	4.916882	50	0.09833764	2500	0.00967	48.39029	-3.21943	3.219429
		75	0.151	1	11.33019	75	0.1510692	5625	0.022822	72.73481	-3.02025	3.020249
		125	0.248	1	30.96237	125	0.24769892	15625	0.061355	117.3458	-6.1234	6.123397
		250	0.507	1	126.7863	250	0.50714511	62500	0.257196	237.124	-5.1504	5.150398
		500	1.021	1	510.3209	500	1.02064179	250000	1.04171	474.1895	-5.1621	5.162104
		5.0	0.010	1	0.048294	5	0.00965882	25	9.33E-05	7.450029	49.00057	49.00057
		10	0.019	1	0.193176	10	0.1931756	100	0.000373	11.90917	19.09168	19.09168
		25	0.049	1	1.202164	25	0.04830656	625	0.002312	25.19092	0.76392	0.76392
		50	0.097	1	4.87455	50	0.09749101	2500	0.009504	47.99942	-4.00115	4.001151
		75	0.154	1	11.5524	75	0.15403203	5625	0.023726	74.10286	-1.19645	1.196454
		125	0.255	1	31.84893	125	0.25479146	15625	0.064919	120.6202	-3.50387	3.503874
		250	0.507	1	126.8254	250	0.50730173	62500	0.257355	237.1963	-5.12148	5.121475
		500	1.079	1	539.7301	500	1.07946014	250000	1.165234	501.3441	0.268818	0.268818
		5.0	0.01028	1	0.05141	5	0.010282	25	0.000106	7.737732	54.75464	54.75464
		10	0.02291	1	0.2291	10	0.02291	100	0.000525	13.56769	35.67688	35.67688
		25	0.06026	1	1.506375	25	0.060255	625	0.003631	30.80871	23.23486	23.23486
		50	0.10320	1	5.1601	50	0.103202	2500	0.010651	50.63601	1.272023	1.272023
		75	0.19158	1	14.3682	75	0.191576	5625	0.036701	91.43554	21.91406	21.91406
		125	0.28642	1	35.802	125	0.286416	15625	0.082034	135.2202	8.176182	8.176182
		250	0.56635	1	139.0863	250	0.556345	62500	0.30952	259.8381	3.935227	3.935227
		500	1.15896	1	579.4805	500	1.158961	250000	1.343191	538.0472	7.609433	7.609433
		5.0	0.01028	1	0.051408	5	0.01028158	25	0.000106	7.737538	54.75075	54.75075
		10	0.02024	1	0.202423	10	0.02024234	100	0.00041	12.33611	23.36112	23.36112
		25	0.04774	1	1.193616	25	0.04774463	625	0.00228	25.03307	0.132262	0.132262
		50	0.09732	1	4.865805	50	0.0973161	2500	0.00947	47.91867	-4.16266	4.162655
		75	0.14285	1	10.71388	75	0.14285177	5625	0.020407	68.94108	-8.07856	8.078563
		125	0.25638	1	32.04781	125	0.25638251	15625	0.065732	121.3547	-2.91624	2.916243
		250	0.51777	1	129.4425	250	0.51777004	62500	0.288086	242.0292	-3.18832	3.188318
		500	1.06247	1	531.2357	500	1.06247144	250000	1.128846	493.5009	-1.29981	1.299813
		5.0	0.010	1	0.051598	5	0.01031957	25	0.000106	7.755077	55.10154	55.10154
		10	0.020	1	0.198844	10	0.01988442	100	0.000395	12.17087	21.7087	21.7087
		25	0.050	1	1.24636	25	0.04985441	625	0.002485	26.00708	4.028332	4.028332
		50	0.098	1	4.918291	50	0.09836582	2500	0.009676	48.4033	-3.19341	3.193406
		75	0.161	1	12.04432	75	0.16059095	5625	0.025789	77.13071	2.840943	2.840943
		125	0.248	1	30.97165	125	0.24777321	15625	0.061392	117.38	-6.09596	6.095961
		250	0.502	1	125.4458	250	0.50178337	62500	0.251787	234.6487	-6.14054	6.140537
		500	1.087	1	543.3651	500	1.08673025	250000	1.180983	504.7005	0.940094	0.940094
		5.0	0.010	1	0.050412	5	0.01008239	25	0.000102	7.645579	52.91157	52.91157
		10	0.020	1	0.204169	10	0.02041692	100	0.000417	12.41671	24.16711	24.16711
		25	0.049	1	1.231389	25	0.04925557	625	0.002426	25.73062	2.922483	2.922483
		50	0.101	1	5.065272	50	0.10130545	2500	0.010263	49.76043	-0.47914	0.479138
		75	0.151	1	11.30445	75	0.15072596	5625	0.022718	72.67635	-3.23154	3.231536
		125	0.265	1	33.0953	125	0.26476238	15625	0.070099	125.2234	0.178739	0.178739
		250	0.501	1	125.3071	250	0.50122852	62500	0.25123	234.3925	-6.243	6.243001
		500	1.115	1	557.3822	500	1.11476445	250000	1.2427	517.643	3.528598	3.528598
Sum		6240.0			13.2052	48	4339.337	6240	13.2052178	2022000	9.333243	632.826
Slope		0.00216605										
Intercept		-0.00647835										
R ²		0.99656997										

Comments: 1/x² weighting will be used.

Acceptance Criteria: The least complex weighting scheme that minimizes $\Sigma | \%RE |$

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

C _{room}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	[%RE]	C _{room}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	[%RE]
5.0	0.010	0.2	0.010282	1	0.0020564	5	2.11E-05	5.448977	8.979545	8.979545	5.0	0.010	0.04	0.0020564	0.2	0.000411	1	4.23E-06	5.098606	1.972127	1.972126809
10	0.020	0.1	0.020137	1	0.0020137	10	4.05E-05	10.08389	0.838899	0.838899	10	0.020	0.01	0.0020137	0.1	0.000201	1	4.05E-06	9.840321	-1.58679	1.586785202
25	0.047	0.04	0.046916	1	0.00187663	25	8.8E-05	22.67826	-9.28697	9.28697	25	0.047	0.0016	0.00187663	0.04	7.51E-05	1	3.52E-06	22.7249	-9.10039	9.100394905
50	0.098	0.02	0.098338	1	0.00196675	50	0.000193	46.86254	-6.27492	6.274916	50	0.098	0.0004	0.00196675	0.02	3.93E-05	1	3.87E-06	47.46647	-5.06707	5.067069565
75	0.151	0.013333	0.151069	1	0.00201426	75	0.000304	71.6628	-4.4496	4.449597	75	0.151	0.000178	0.00201426	0.013333	2.69E-05	1	4.06E-06	72.8382	-2.8824	2.882401653
125	0.248	0.008	0.247699	1	0.00198159	125	0.000491	117.1089	-6.31291	6.312908	125	0.248	0.000064	0.00198159	0.008	1.59E-05	1	3.93E-06	119.3315	-4.53482	4.534818241
250	0.507	0.004	0.507145	1	0.00202858	250	0.001029	239.1294	-4.34825	4.348248	250	0.507	0.000016	0.00202858	0.004	8.11E-06	1	4.12E-06	244.1637	-2.33451	2.334513803
500	1.021	0.002	1.020642	1	0.00204128	500	0.002083	480.6327	-3.87345	3.87345	500	1.021	0.000004	0.00204128	0.002	4.08E-06	1	4.17E-06	491.2321	-1.75359	1.753588968
5.0	0.010	0.2	0.009659	1	0.00193176	5	1.87E-05	5.155878	3.117565	3.117565	5.0	0.010	0.04	0.00193176	0.2	0.000386	1	3.73E-06	4.976753	-4.02493	4.02493141
10	0.019	0.1	0.019318	1	0.00193176	10	3.73E-05	9.698493	-3.01507	3.015067	10	0.019	0.01	0.00193176	0.1	0.000193	1	3.73E-06	9.446044	-5.53956	5.539558582
25	0.048	0.04	0.048087	1	0.00192346	25	9.25E-05	23.22838	-7.08446	7.084463	25	0.048	0.0016	0.00192346	0.04	7.68E-06	1	3.7E-06	23.28822	-6.84713	6.847134701
50	0.097	0.02	0.097491	1	0.00194982	50	0.00019	46.46436	-7.07127	7.071273	50	0.097	0.0004	0.00194982	0.02	3.9E-05	1	3.8E-06	47.05911	-5.88178	5.881776245
75	0.154	0.013333	0.154032	1	0.00205376	75	0.000316	73.05625	-2.59166	2.591661	75	0.154	0.000178	0.00205376	0.013333	2.74E-05	1	4.22E-06	74.26376	-0.98165	0.981652464
125	0.255	0.008	0.254791	1	0.00203833	125	0.000519	120.4446	-3.64435	3.644347	125	0.255	0.000064	0.00203833	0.008	1.63E-05	1	4.15E-06	122.744	-1.80477	1.804765865
250	0.507	0.004	0.507302	1	0.00202921	250	0.001029	239.203	-4.31878	4.318784	250	0.507	0.000016	0.00202921	0.004	8.12E-06	1	4.12E-06	244.2391	-2.30437	2.304371193
500	1.079	0.002	1.07946	1	0.00215892	500	0.00233	508.2957	-1.659139	1.659139	500	1.079	0.000004	0.00215892	0.002	4.32E-06	1	4.66E-06	519.5324	-3.906488	3.906487949
5.0	0.01028	0.2	0.010282	1	0.0020564	5	2.11E-05	5.448977	8.979349	8.979349	5.0	0.01028	0.04	0.0020564	0.2	0.000411	1	4.23E-06	5.098596	1.971926	1.971926435
10	0.02291	0.1	0.02291	1	0.002291	10	5.25E-05	11.38806	13.88061	13.88061	10	0.02291	0.01	0.002291	0.1	0.0002291	1	5.25E-06	11.17454	11.74544	11.74544106
25	0.06026	0.04	0.060255	1	0.0024102	25	0.000145	28.95184	15.80737	15.80737	25	0.06026	0.0016	0.0024102	0.04	9.64E-05	1	5.81E-06	29.15404	16.57219	16.5721929
50	0.10320	0.02	0.103202	1	0.00206404	50	0.000213	49.15031	-1.69938	1.699384	50	0.10320	0.0004	0.00206404	0.02	4.13E-05	1	4.26E-06	49.80695	-0.3861	0.3861035
75	0.19158	0.013333	0.191576	1	0.00255435	75	0.000489	90.71361	20.95148	20.95148	75	0.19158	0.000178	0.00255435	0.013333	3.41E-05	1	6.52E-06	92.328	23.104	23.10399662
125	0.28642	0.008	0.286416	1	0.00229133	125	0.000656	135.318	8.254361	8.254361	125	0.28642	0.000064	0.00229133	0.008	1.83E-05	1	5.25E-06	137.9602	10.36812	10.36812416
250	0.55635	0.004	0.556345	1	0.00222538	250	0.001238	262.2687	4.90746	4.90746	250	0.55635	0.000016	0.00222538	0.004	8.9E-06	1	4.95E-06	267.8362	7.134474	7.134474168
500	1.15896	0.002	1.158961	1	0.00231792	500	0.002686	545.6859	9.137172	9.137172	500	1.15896	0.000004	0.00231792	0.002	4.64E-06	1	5.37E-06	557.7842	11.55684	11.55683718
5.0	0.01028	0.2	0.010282	1	0.00205632	5	2.11E-05	5.448769	8.975387	8.975387	5.0	0.01028	0.04	0.00205632	0.2	0.000411	1	4.23E-06	5.098394	1.967873	1.967873006
10	0.02024	0.1	0.020242	1	0.00202423	10	4.1E-05	10.13343	1.334301	1.334301	10	0.02024	0.01	0.00202423	0.1	0.000202	1	4.1E-06	9.891003	-1.08997	1.089967905
25	0.04774	0.04	0.047745	1	0.00190979	25	9.12E-05	23.06807	-7.72771	7.727713	25	0.04774	0.0016	0.00190979	0.04	7.72E-06	1	3.65E-06	23.1237	-7.50521	7.505206641
50	0.09732	0.02	0.097316	1	0.00194632	50	0.000189	46.3821	-7.2358	7.2358	50	0.09732	0.0004	0.00194632	0.02	3.89E-05	1	3.79E-06	46.97495	-6.0501	6.050095274
75	0.14285	0.013333	0.142852	1	0.00190469	75	0.000272	67.79805	-9.6026	9.602604	75	0.14285	0.000178	0.00190469	0.013333	2.54E-05	1	3.63E-06	68.88439	-8.15415	8.154149136
125	0.25638	0.008	0.256383	1	0.00205106	125	0.000526	121.1929	-3.04572	3.045716	125	0.25638	0.000064	0.00205106	0.008	1.64E-05	1	4.21E-06	123.5096	-1.19234	1.19234033
250	0.51777	0.004	0.51777	1	0.00207108	250	0.001072	244.1264	-2.34944	2.349438	250	0.51777	0.000016	0.00207108	0.004	8.28E-06	1	4.29E-06	249.2759	-0.28965	0.289645354
500	1.06247	0.002	1.062471	1	0.00212494	500	0.002258	500.3057	0.061143	0.061143	500	1.06247	0.000004	0.00212494	0.002	4.25E-06	1	4.52E-06	511.3583	2.271669	2.271669149
5.0	0.010	0.2	0.01032	1	0.00206391	5	2.13E-05	5.466637	9.33274	9.33274	5.0	0.010	0.04	0.00206391	0.2	0.000413	1	4.26E-06	5.116673	2.33361	2.33360538
10	0.020	0.1	0.019884	1	0.00198844	10	3.95E-05	9.965094	-0.34906	0.349059	10	0.020	0.01	0.00198844	0.1	0.000199	1	3.95E-06	9.718788	-2.81212	2.812117665
25	0.050	0.04	0.049854	1	0.00199418	25	9.94E-05	24.06032	-3.75871	3.758708	25	0.050	0.0016	0.00199418	0.04	7.98E-05	1	3.98E-06	24.18801	-3.44474	3.444744024
50	0.098	0.02	0.098366	1	0.00196732	50	0.000194	46.8758	-6.24841	6.248406	50	0.098	0.0004	0.00196732	0.02	3.93E-05	1	3.87E-06	47.40003	-5.03995	5.039948756
75	0.161	0.013333	0.160591	1	0.00214121	75	0.000344	76.14099	1.521317	1.521317	75	0.161	0.000178	0.00214121	0.013333	2.85E-05	1	4.58E-06	77.41958	3.2261	3.226100699
125	0.248	0.008	0.247773	1	0.00198219	125	0.000491	117.1438	-6.28496	6.284959	125	0.248	0.000064	0.00198219	0.008	1.59E-05	1	3.93E-06	119.3672	-4.50623	4.506225149
250	0.502	0.004	0.501783	1	0.00200713	250	0.001007	236.6077	-5.35692	5.356923	250	0.502	0.000016	0.00200713	0.004	8.03E-06	1	4.03E-06	241.5839	-3.36643	3.36643168
500	1.087	0.002	1.08673	1	0.00217346	500	0.002362	511.7149	2.342982	2.342982	500	1.087	0.000004	0.00217346	0.002	4.35E-06	1	4.72E-06	523.0304	4.606088	4.606088343
5.0	0.010	0.2	0.010082	1	0.00201648	5	2.03E-05	5.355089	7.101781	7.101781	5.0	0.010	0.04	0.00201648	0.2	0.000403	1	4.07E-06	5.002555	0.051094	0.051093585
10	0.020	0.1	0.020417	1	0.00204169	10	4.17E-05	10.21554	2.155378	2.155378	10	0.020	0.01	0.00204169	0.1	0.000204	1	4.17E-06	9.975003	-0.24997	0.249970462
25	0.049	0.04	0.049256	1	0.00197022	25	9.7E-05	23.77869	-4.88526	4.885259	25	0.049	0.0016	0.00197022	0.04	7.88E-05	1	3.88E-06	23.85069	-4.59725	4.597254077
50	0.101	0.02	0.101305	1	0.00202611	50	0.000205	48.25834	-3.48333	3.483326	50	0.101	0.0004	0.00202611	0.02	4.05E-05	1	4.11E-06	48.88442	-2.21115	2.211153084
75	0.151	0.013333	0.150726	1	0.00200968	75	0.000303	71.50137	-4.86484	4.86484	75	0.151	0.000178	0.00200968	0.013333	2.69E-05	1	4.04E-06	72.67305	-3.1028	3.10280417
125	0.265	0.008	0.264762	1	0.0021181	125	0.000561	125.134	0.107205	0.107205	125	0.265	0.000064	0.0021181	0.008	1.69E-05	1	4.49E-06	127.5415	0.032333	0.032333297
250	0.501	0.004	0.501229	1	0.00200491	250	0.001005	236.3467	-5.4613	5.461305	250	0.501	0.000016	0.00200491	0.004	8.02E-06	1	4.02E-06	241.317	-3.47322	3.473219161
500	1.115	0.002	1.114764	1	0.00222953	500	0.														

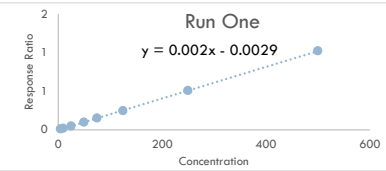
Validation Study 1

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

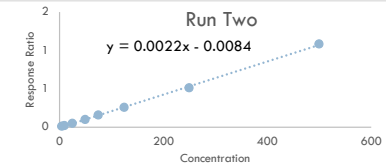
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

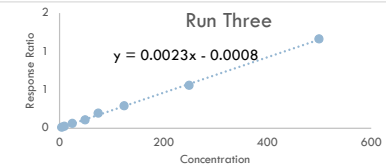
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	5.05	1.00	0.010
	10	10.02	0.20	0.020
	25	23.51	5.96	0.047
	50	49.41	1.18	0.098
	75	75.96	1.28	0.151
	125	124.63	0.30	0.248
	250	255.31	2.12	0.507
	500	513.95	2.79	1.021
OPI1_20220418B_JR	5	5.09	1.85	0.010
	10	9.84	1.65	0.019
	25	23.96	4.16	0.048
	50	48.22	3.56	0.097
	75	75.98	1.31	0.154
	125	125.45	0.36	0.255
	250	249.43	0.23	0.507
	500	530.36	6.07	1.079
OPI1_20220418B_KMY	5	4.89	2.20	0.010
	10	10.31	3.10	0.023
	25	26.33	5.32	0.060
	50	44.76	10.48	0.103
	75	82.68	10.24	0.192
	125	123.37	1.30	0.286
	250	239.17	4.33	0.556
	500	497.72	0.46	1.159
OPI1_20220421B_CD	5	5.06	1.27	0.010
	10	10.03	0.32	0.020
	25	23.75	5.00	0.048
	50	48.48	3.04	0.097
	75	71.19	5.08	0.143
	125	127.82	2.26	0.256
	250	258.21	3.28	0.518
	500	529.92	5.98	1.062
OPI1_20220421B_CLR	5	5.09	1.74	0.010
	10	9.77	2.32	0.020
	25	24.44	2.26	0.050
	50	48.18	3.65	0.098
	75	78.63	4.84	0.161
	125	121.29	2.97	0.248
	250	245.60	1.76	0.502
	500	531.86	6.37	1.087
OPI1_20220427B_CD	5	5.04	0.86	0.010
	10	10.04	0.38	0.020
	25	23.98	4.10	0.049
	50	49.13	1.74	0.101
	75	73.01	2.65	0.151
	125	128.13	2.50	0.265
	250	242.41	3.04	0.501
	500	538.92	7.78	1.115



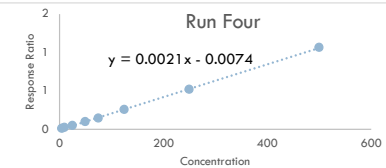
Slope 0.00204
 Intercept -0.00292
 R² 0.99995



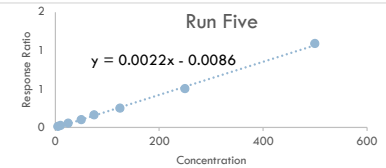
Slope 0.00215
 Intercept -0.00842
 R² 0.99917



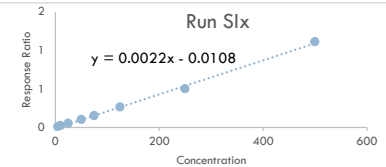
Slope 0.00230
 Intercept -0.00080
 R² 0.99913



Slope 0.00213
 Intercept -0.00736
 R² 0.99971



Slope 0.00216
 Intercept -0.00861
 R² 0.99843



Slope 0.00221
 Intercept -0.01075
 R² 0.99769

Max %RE = 10.48

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

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

LINEARITY

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

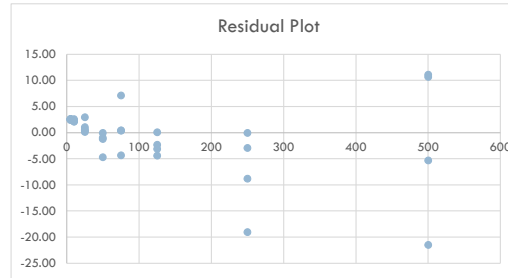
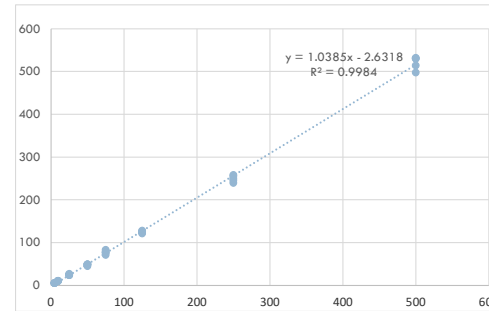
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	5.05	2.50	2.55
	10	10.02	7.72	2.30
	25	23.51	23.38	0.13
	50	49.41	49.48	-0.07
	75	75.96	75.58	0.38
	125	124.63	127.78	-3.15
	250	255.31	258.28	-2.97
	500	513.95	519.28	-5.33
OPI1_20220418B_JR	5	5.09	2.50	2.60
	10	9.84	7.72	2.12
	25	23.96	23.38	0.58
	50	48.22	49.48	-1.26
	75	75.98	75.58	0.40
	125	125.45	127.78	-2.32
	250	249.43	258.28	-8.84
	500	530.36	519.28	11.09
OPI1_20220418B_KMY	5	4.89	2.50	2.39
	10	10.31	7.72	2.59
	25	26.33	23.38	2.95
	50	44.76	49.48	-4.72
	75	82.68	75.58	7.10
	125	123.37	127.78	-4.41
	250	239.17	258.28	-19.11
	500	497.72	519.28	-21.56
OPI1_20220421B_CD	5	5.06	2.50	2.57
	10	10.03	7.72	2.32
	25	23.75	23.38	0.37
	50	48.48	49.48	-1.00
	75	71.19	75.58	-4.38
	125	127.82	127.78	0.04
	250	258.21	258.28	-0.07
	500	529.92	519.28	10.65
OPI1_20220421B_CLR	5	5.09	2.50	2.59
	10	9.77	7.72	2.05
	25	24.44	23.38	1.06
	50	48.18	49.48	-1.30
	75	78.63	75.58	3.05
	125	121.29	127.78	-6.48
	250	245.60	258.28	-12.68
	500	531.86	519.28	12.59
OPI1_20220427B_CD	5	5.04	2.50	2.55
	10	10.04	7.72	2.32
	25	23.98	23.38	0.60
	50	49.13	49.48	-0.35
	75	73.01	75.58	-2.56
	125	128.13	127.78	0.35
	250	242.41	258.28	-15.87
	500	538.92	519.28	19.64

Slope	1.0434
Std err in slope, S _b	0.0066
Degrees freedom	46
Confidence level	95%
Student t	2.0129
Confidence interval	0.013
Slope	1.043 ± 0.013
Range	1.0302 - 1.0566

Intercept	-3.0309
Std err in Intercept	1.3476
Degrees freedom	46
Confidence Level	95%
Student t	2.0129
Confidence interval	2.712
Intercept	-3.031 ± 2.712
Lower	-5.7433 - -0.3184

NO

NO



Comments: The linearity of the method is acceptable because the individual calibration curves on all six 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

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	100	400	
OPI1_20220414B_JR	1-1	16.10	106.32	409.35	
	1-2	16.16	104.06	409.65	
	1-3	16.13	106.88	411.80	
	1-4	16.28	103.99	414.82	
	Within Run	Mean	16.17	105.31	411.41
		SD	0.08	1.50	2.52
		%CV	0.49%	1.43%	0.61%
	% Bias	7.78%	5.31%	2.85%	
OPI1_20220418B_JR	2-1	16.26	101.41	412.01	
	2-2	16.02	101.23	405.07	
	2-3	15.99	102.91	411.77	
	Within Run	Mean	16.09	101.85	409.62
		SD	0.15	0.92	3.94
		%CV	0.91%	0.90%	0.96%
		% Bias	7.25%	1.85%	2.40%
OPI1_2022048B_KMY	3-1	15.39	95.28	383.43	
	3-2	15.31	93.30	387.88	
	3-3	13.45	98.64	408.27	
	3-4	15.95	99.19	372.60	
	Within Run	Mean	15.03	96.60	388.05
		SD	1.09	2.80	14.93
		%CV	7.24%	2.90%	3.85%
	% Bias	0.17%	-3.40%	-2.99%	
OPI1_20220421B_CD	4-1	16.11	106.84	421.81	
	4-2	15.68	106.18	428.35	
	4-3	16.32	106.37	423.54	
	Within Run	Mean	16.04	106.47	424.56
		SD	0.32	0.34	3.39
		%CV	2.02%	0.32%	0.80%
		% Bias	6.93%	6.47%	6.14%
OPI1_20220421B_CLR	5-1	15.97	99.79	411.27	
	5-2	15.90	102.72	418.30	
	5-3	16.24	101.11	420.80	
	Within Run	Mean	16.04	101.21	416.79
		SD	0.18	1.47	4.94
		%CV	1.11%	1.45%	1.19%
		% Bias	6.91%	1.21%	4.20%
OPI1_20220427B_CD	6-1	15.77	102.08	411.43	
	6-2	15.57	104.45	376.99	
	6-3	15.84	101.00	404.43	
	Within Run	Mean	15.72	102.51	397.62
		SD	0.14	1.76	18.20
		%CV	0.88%	1.72%	4.58%
		% Bias	4.83%	2.51%	-0.60%

Mean		15.82	102.19	407.18
SD		0.63	3.73	15.33
Precision (%CV)	Max Within-Run	7.24%	2.90%	4.58%
	Between-Run	3.96%	3.65%	3.76%
% Bias		5.65%	2.32%	2.00%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Tramadol
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 296.34 1/x Weighting: = 196.41 1/x2 Weighting: = 191.28	1/x2 weighting will be used.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9488 - 0.9844 95% CI of Intercept = -0.0720 - 2.2066	The linearity of the method is acceptable because the individual calibration curves on all six 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 $\pm 20\%$ of target	Max %RE of Calibrators = 10.20	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ 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	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = 5.55% Max Within-Run Precision = 6.89% Max Between-Run Precision = 4.12%	N/A

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	$\Sigma \%RE $	C_{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE	
Unweighted:	296.34	5.0	0.889	1	4.444255	5	0.88885093	25	0.790056	3.933521	-21.3296	21.32958	
1/x Weighting:	196.41	10	1.687	1	16.8709	10	1.68708955	100	2.846271	9.038964	-9.61036	9.610358	
1/x ² Weighting:	191.28	25	3.894	1	97.35183	25	3.89407316	625	15.16381	23.15458	-7.38168	7.381682	
		50	7.855	1	392.7417	50	7.85483445	2500	61.69842	48.48716	-3.02569	3.025686	
		75	11.910	1	893.2706	75	11.910275	5625	141.8547	74.42529	-0.76628	0.766278	
		125	19.115	1	2389.408	125	19.1152632	15625	365.3933	120.5076	-3.59394	3.593941	
		5.0	0.852	1	4.260368	5	0.85207368	25	0.72603	3.698298	-26.034	26.03404	
		10	1.668	1	16.68182	10	1.66818177	100	2.78283	8.918032	-10.8197	10.81968	
		25	3.963	1	99.0665	25	3.96265988	625	15.70267	23.59325	-5.62699	5.62699	
		50	7.695	1	384.7705	50	7.69540957	2500	59.21933	47.46749	-5.06501	5.065013	
		75	12.042	1	903.1407	75	12.0418762	5625	145.0068	75.287	0.355997	0.355997	
		125	18.783	1	2347.848	125	18.7827835	15625	352.793	118.3811	-5.29514	5.295142	
		5.0	0.93485	1	4.67424	5	0.934848	25	0.873941	4.227713	-15.4457	15.44574	
		10	1.95516	1	19.55158	10	1.955158	100	3.822643	10.7535	7.534994	7.534994	
		25	4.98975	1	124.7438	25	4.989751	625	24.89762	30.16241	20.64964	20.64964	
		50	8.46085	1	423.0427	50	8.460853	2500	71.58603	52.36318	4.726365	4.726365	
		75	15.04520	1	1128.39	75	15.0452	5625	226.358	94.47591	25.96789	25.96789	
		125	22.76608	1	2845.76	125	22.76608	15625	518.2944	143.8578	15.08623	15.08623	
		5.0	0.91812	1	4.590625	5	0.91812497	25	0.842953	4.120754	-17.5849	17.58491	
		10	1.78039	1	17.80388	10	1.780388	100	3.169781	9.63569	-3.8431	3.843095	
		25	4.12195	1	103.0487	25	4.12194687	625	16.99045	24.61203	-1.55186	1.551865	
		50	8.02737	1	401.3687	50	8.02737413	2500	64.43874	49.5907	-0.8186	0.818598	
		75	11.66249	1	874.6868	75	11.6624904	5625	136.0137	72.84049	-2.87935	2.879347	
		125	18.53500	1	2316.875	125	18.5350039	15625	343.5464	116.7963	-6.56296	6.562958	
		5.0	0.891	1	4.45441	5	0.89088201	25	0.793671	3.946512	-21.0698	21.06977	
		10	1.755	1	17.55489	10	1.75548852	100	3.08174	9.476436	-5.23564	5.235638	
		25	4.225	1	105.6175	25	4.22469834	625	17.84808	25.26922	1.078882	1.078882	
		50	8.180	1	409.0141	50	8.18028261	2500	68.91702	50.58869	1.137372	1.137372	
		75	12.844	1	963.2968	75	12.8439575	5625	164.9672	80.39702	7.196025	7.196025	
		125	19.165	1	2395.592	125	19.1647373	15625	367.2872	120.824	-3.3408	3.340796	
		5.0	0.893	1	4.466626	5	0.89332529	25	0.79803	3.962139	-20.7572	20.75723	
		10	1.798	1	17.98148	10	1.79814796	100	3.233336	9.749281	-2.50719	2.507189	
		25	4.082	1	102.04	25	4.08159881	625	16.65945	24.35397	-2.58411	2.584111	
		50	8.171	1	408.5695	50	8.17139006	2500	66.77162	50.51181	1.023621	1.023621	
		75	11.542	1	865.6633	75	11.542177	5625	133.2218	72.07098	-3.90536	3.905362	
		125	18.811	1	2351.369	125	18.8109521	15625	353.8519	118.5612	-5.15101	5.151012	
		Sum	1740.0		281.9082		30	23460.01	1740	281.908227	147000	3770.243	296.341

Comments: 1/x² weighting will be used.

Slope	0.15635051
Intercept	0.27384292
R ²	0.98396368

Acceptance Criteria: The least complex weighting scheme that minimizes $\Sigma | \%RE |$

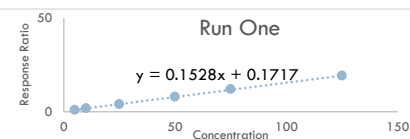
Validation Study 1

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

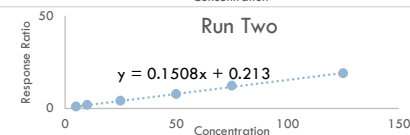
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

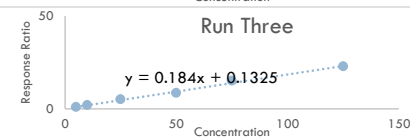
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	5	4.98	0.40	0.889
	10	10.16	1.60	1.687
	25	24.48	2.08	3.894
	50	50.19	0.38	7.855
	75	76.51	2.01	11.910
	125	123.27	1.38	19.115
OPI1_20220418B_JR	5	4.94	1.14	0.852
	10	10.23	2.27	1.668
	25	25.08	0.33	3.963
	50	49.25	1.49	7.695
	75	77.40	3.19	12.042
	125	121.04	3.17	18.783
OPI1_20220418B_KMY	5	4.90	2.00	0.935
	10	10.32	3.20	1.955
	25	26.45	5.80	4.990
	50	44.90	10.20	8.461
	75	79.89	6.52	15.045
	125	120.92	3.26	22.766
OPI1_20220421B_CD	5	4.87	2.60	0.918
	10	10.45	4.45	1.780
	25	25.58	2.34	4.122
	50	50.84	1.67	8.027
	75	74.34	0.88	11.662
	125	118.78	4.98	18.535
OPI1_20220421B_CLR	5	4.93	1.48	0.891
	10	10.24	2.36	1.755
	25	25.40	1.59	4.225
	50	49.69	0.62	8.180
	75	78.33	4.44	12.844
	125	117.14	6.29	19.165
OPI1_20220427B_CD	5	4.83	3.40	0.893
	10	10.63	6.30	1.798
	25	25.26	1.02	4.082
	50	51.45	2.90	8.171
	75	73.04	2.61	11.542
	125	119.60	4.32	18.811



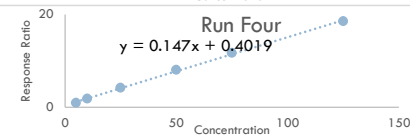
Slope 0.15283
 Intercept 0.17169
 R² 0.99953



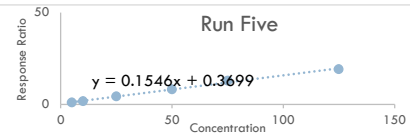
Slope 0.15077
 Intercept 0.21304
 R² 0.99846



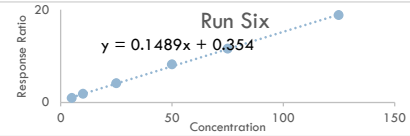
Slope 0.18399
 Intercept 0.13250
 R² 0.99380



Slope 0.14701
 Intercept 0.40194
 R² 0.99891



Slope 0.15462
 Intercept 0.36991
 R² 0.99543



Slope 0.14888
 Intercept 0.35397
 R² 0.99911

Max %RE = 10.20

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1 **LINEARITY**

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

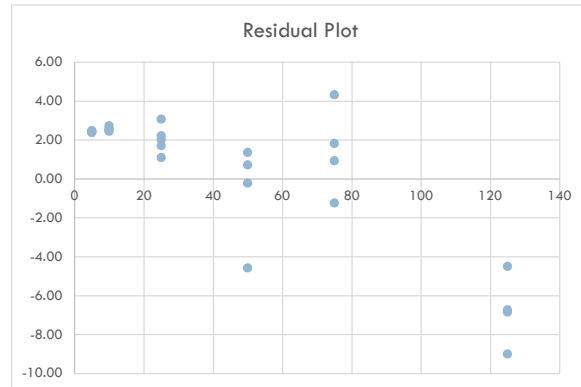
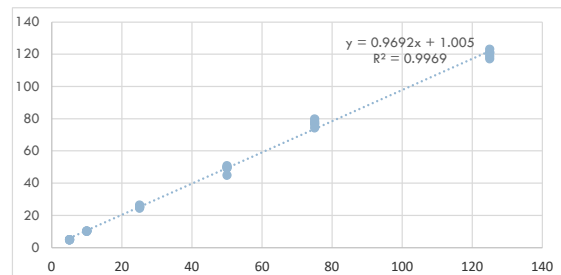
Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	5	4.98	2.50	2.48
	10	10.16	7.72	2.44
	25	24.48	23.38	1.10
	50	50.19	49.48	0.71
	125	123.27	127.78	-4.51
OPI1_20220418B_JR	5	4.94	2.50	2.45
	10	10.23	7.72	2.51
	25	25.08	23.38	1.71
	50	49.25	49.48	-0.22
	125	77.40	75.58	1.82
OPI1_20220418B_KMY	5	4.90	2.50	2.40
	10	10.32	7.72	2.60
	25	26.45	23.38	3.07
	50	44.90	49.48	-4.58
	125	79.89	75.58	4.31
OPI1_20220421B_CD	5	4.87	2.50	2.37
	10	10.45	7.72	2.73
	25	25.58	23.38	2.21
	50	50.84	49.48	1.36
	125	74.34	75.58	-1.24
OPI1_20220421B_CLR	5	4.93	2.50	2.43
	10	10.24	7.72	2.52
	25	25.40	23.38	2.02
	50	49.69	49.48	0.21
	125	78.33	75.58	2.75
OPI1_20220427B_CD	5	4.83	2.50	2.33
	10	10.63	7.72	2.91
	25	25.26	23.38	1.88
	50	51.45	49.48	1.97
	125	73.04	75.58	-2.54
125	119.60	127.78	-8.18	

Slope	0.9666
Std err in slope, S_b	0.0088
Degrees freedom	34
Confidence level	95%
Student t	2.0322
Confidence interval	0.018
Slope	0.967 ± 0.018
Range	0.9488 - 0.9844

Intercept	1.0673
Std err in Intercept	0.5606
Degrees freedom	34
Confidence Level	95%
Student t	2.0322
Confidence interval	1.139
Intercept	1.067 ± 1.139
Lower	-0.0720 - 2.2066

NO **YES**



Comments: The linearity of the method is acceptable because the individual calibration curves on all six 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

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		15	30	100	
OPI1_20220414B_JR	1-1	15.72	31.01	92.12	
	1-2	15.75	31.02	92.89	
	1-3	15.65	31.14	91.38	
	1-4	16.08	31.02	94.03	
	Within Run	Mean	15.80	31.05	92.61
		SD	0.19	0.06	1.13
		%CV	1.21%	0.20%	1.22%
	% Bias	5.33%	3.49%	-7.40%	
OPI1_20220418B_JR	2-1	16.11	31.38	99.41	
	2-2	15.91	30.99	100.58	
	2-3	16.22	31.50	100.63	
	Within Run	Mean	16.08	31.29	100.21
		SD	0.16	0.26	0.69
		%CV	0.97%	0.84%	0.69%
		% Bias	7.20%	4.30%	0.21%
OPI1_20220418B_KMY	3-1	15.26	28.39	90.75	
	3-2	14.77	27.28	92.69	
	3-3	13.84	28.56	94.39	
	3-4	16.33	29.77	88.76	
	Within Run	Mean	15.05	28.50	91.65
		SD	1.04	1.02	2.43
		%CV	6.89%	3.58%	2.65%
	% Bias	0.33%	-5.00%	-8.35%	
OPI1_20220421B_CD	4-1	16.30	31.83	98.86	
	4-2	15.77	31.71	98.45	
	4-3	16.47	32.04	97.27	
	Within Run	Mean	16.18	31.86	98.19
		SD	0.36	0.17	0.83
		%CV	2.25%	0.52%	0.84%
		% Bias	7.87%	6.20%	-1.81%
OPI1_20220421B_CLR	5-1	15.78	30.55	97.71	
	5-2	15.69	30.17	98.82	
	5-3	15.76	30.22	99.51	
	Within Run	Mean	15.74	30.31	98.68
		SD	0.05	0.21	0.91
		%CV	0.29%	0.68%	0.92%
		% Bias	4.96%	1.04%	-1.32%
OPI1_20220427B_CD	6-1	16.09	31.29	96.74	
	6-2	15.83	31.78	90.14	
	6-3	16.49	30.50	92.20	
	Within Run	Mean	16.14	31.19	93.03
		SD	0.33	0.64	3.38
		%CV	2.06%	2.06%	3.63%
		% Bias	7.60%	3.97%	-6.97%

Mean		15.79	30.61	95.37
SD		0.61	1.26	3.82
Precision (%CV)	Max Within-Run	6.89%	3.58%	3.63%
	Between-Run	3.88%	4.12%	4.01%
% Bias		5.55%	2.33%	-4.27%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Norbuprenorphine
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 529.75 1/x Weighting: = 204.09 1/x2 Weighting: = 198.71	1/x2 weighting will be used.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9746 - 0.9928 95% CI of Intercept = -0.1667 - 0.5780	The linearity of the method is acceptable because the individual calibration curves on all six 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 $\pm 20\%$ of target	Max %RE of Calibrators = 9.07	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ of target	N/A	Not performed. All 8 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 8 calibrators used in validation will be used for casework.
2	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = -6.46% Max Within-Run Precision = 6.83% Max Between-Run Precision = 5.13%	N/A

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	$\Sigma \%RE $	C_{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
Unweighted:	529.75	0.5	0.061	1	0.030715	0.5	0.06143094	0.25	0.003774	0.260384	-47.9231	47.92313
1/x Weighting:	204.09	1.0	0.119	1	0.118769	1	0.11876936	1	0.014106	0.735486	-26.4514	26.45136
1/x ² Weighting:	198.71	5.0	0.573	1	2.864363	5	0.57287253	25	0.328183	4.498153	-10.0369	10.03695
		10	1.213	1	12.12921	10	1.21292067	100	1.471177	9.801546	-1.98454	1.984544
		15	1.745	1	26.17391	15	1.74492739	225	3.044772	14.20972	-5.26857	5.268566
		25	2.975	1	74.37648	25	2.97505922	625	8.850977	24.4025	-2.39	2.390003
		50	5.846	1	292.2887	50	5.84577459	2500	34.17308	48.18904	-3.62192	3.621919
		100	11.324	1	1132.391	100	11.3239071	10000	128.2309	93.58045	-6.41955	6.419546
		0.5	0.062	1	0.03085	0.5	0.06170084	0.25	0.003807	0.262621	-47.4759	47.47585
		1.0	0.119	1	0.119397	1	0.11939719	1	0.014256	0.740689	-25.9311	25.93114
		5.0	0.590	1	2.947587	5	0.58951738	25	0.347531	4.636071	-7.27859	7.278586
		10	1.190	1	11.90358	10	1.1903579	100	1.416952	9.614592	-3.85408	3.854079
		15	1.821	1	27.31583	15	1.82105559	225	3.316243	14.84051	-1.06328	1.063281
		25	2.861	1	71.52124	25	2.86084948	625	8.18446	23.45617	-6.17534	6.175338
		50	5.627	1	281.3437	50	5.62687397	2500	31.66171	46.37525	-7.24951	7.249508
		100	11.802	1	1180.244	100	11.80244	10000	139.2976	97.54554	-2.45446	2.454457
		0.5	0.06733	1	0.033666	0.5	0.067332	0.25	0.004534	0.30928	-38.144	38.14398
		1.0	0.12792	1	0.127922	1	0.127922	1	0.016364	0.811324	-18.8676	18.86755
		5.0	0.71553	1	3.577635	5	0.715527	25	0.511979	5.680177	-13.60355	13.60355
		10	1.25573	1	12.55725	10	1.255725	100	1.576845	10.15622	-1.562192	1.562192
		15	2.18375	1	32.7563	15	2.183753	225	4.768777	17.84579	-18.97195	18.97195
		25	3.43983	1	85.9957	25	3.439828	625	11.83242	28.25354	-13.01416	13.01416
		50	6.21714	1	310.8568	50	6.217136	2500	38.65278	51.26611	-2.532229	2.532229
		100	12.81743	1	1281.743	100	12.81743	10000	164.2865	105.9557	-5.95677	5.95677
		0.5	0.06601	1	0.033005	0.5	0.06601075	0.25	0.004357	0.298332	-40.3355	40.33553
		1.0	0.12837	1	0.12837	1	0.12837001	1	0.016479	0.815037	-18.4963	18.49633
		5.0	0.61704	1	3.085205	5	0.61704102	25	0.38074	4.86413	-2.71741	2.717408
		10	1.30989	1	13.09889	10	1.3098885	100	1.715809	10.60502	-6.050169	6.050169
		15	1.82457	1	27.36851	15	1.82456705	225	3.329045	14.8696	-0.86931	0.869309
		25	3.12476	1	78.11908	25	3.12476322	625	9.764145	25.64294	-2.571743	2.571743
		50	6.16397	1	308.1986	50	6.16397266	2500	37.99456	50.82561	-1.651214	1.651214
		100	12.03361	1	1203.361	100	12.0336075	10000	144.8077	99.46098	-0.53902	0.53902
		0.5	0.072	1	0.036021	0.5	0.07204105	0.25	0.00519	0.348299	-30.3402	30.34022
		1.0	0.126	1	0.125913	1	0.12591269	1	0.015854	0.794676	-20.5324	20.53245
		5.0	0.605	1	3.024319	5	0.60486384	25	0.36586	4.76323	-4.73539	4.735393
		10	1.278	1	12.78015	10	1.27801473	100	1.633322	10.34091	-3.409103	3.409103
		15	1.804	1	27.05389	15	1.80359253	225	3.252946	14.69581	-2.02793	2.027932
		25	2.998	1	74.93998	25	2.99759926	625	8.985601	24.58926	-1.64294	1.642943
		50	6.345	1	317.2506	50	6.34501237	2500	40.25918	52.32569	-4.651378	4.651378
		100	12.316	1	1231.566	100	12.3156593	10000	151.6755	101.798	-1.798041	1.798041
		0.5	0.066	1	0.032859	0.5	0.0657187	0.25	0.004319	0.295912	-40.8175	40.81751
		1.0	0.131	1	0.131139	1	0.1311394	1	0.017198	0.837984	-16.2016	16.20164
		5.0	0.635	1	3.173967	5	0.6347933	25	0.402963	5.011224	-0.224475	0.224475
		10	1.250	1	12.50387	10	1.25038656	100	1.563467	10.11199	-1.19853	1.198553
		15	1.774	1	26.60657	15	1.77377115	225	3.146264	14.44871	-3.67525	3.67525
		25	3.207	1	80.163	25	3.20651986	625	10.28177	26.32037	-5.281462	5.281462
		50	6.103	1	305.1546	50	6.10309213	2500	37.24773	50.32116	-0.642311	0.642311
		100	12.242	1	1224.207	100	12.2420674	10000	149.8682	101.1883	-1.188263	1.188263
Sum	1239.0	1239.0	150.9709		48	9795.589	1239	150.970913	80857.5	1188.748		529.7465
Slope	0.12068654											
Intercept	0.03000606											
R ²	0.99716761											

Comments: 1/x² weighting will be used.

Acceptance Criteria: The least complex weighting scheme that minimizes $\Sigma | \%RE |$

Validation Study 1

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

STANDARD CURVE WEIGHT VERIFICATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
0.5	0.061	2	0.061431	1	0.12286189	0.5	0.007548	0.449611	-10.0778	10.07775
1.0	0.119	1	0.118769	1	0.014106	0.9221195	-7.88051	7.880512		
5.0	0.573	0.2	0.572873	1	0.11457451	5	0.065637	4.655997	-6.88007	6.880068
10	1.213	0.1	1.212927	1	0.147118	9.920115	-0.79885	0.798848		
15	1.745	0.066667	1.744927	1	0.11632849	15	0.202985	14.29564	-4.69573	4.695735
25	2.975	0.04	2.975059	1	0.11900237	25	0.354039	24.41294	-2.34824	2.348236
50	5.846	0.02	5.845775	1	0.11691549	50	0.683462	48.02333	-3.95334	3.953339
100	11.324	0.01	11.32391	1	0.11323907	100	1.282309	93.0786	-6.9214	6.921404
0.5	0.062	2	0.061701	1	0.12340169	0.5	0.007614	0.451831	-9.63379	9.63379
1.0	0.119	1	0.119397	1	0.11939719	1	0.014256	0.926359	-7.36415	7.364147
5.0	0.590	0.2	0.589517	1	0.1190346	5	0.069906	4.792893	-4.14213	4.142133
10	1.190	0.1	1.190358	1	0.11903579	10	0.141696	9.734546	-2.65454	2.654539
15	1.821	0.066667	1.821056	1	0.12140371	15	0.221083	14.92176	-0.52159	0.521592
25	2.861	0.04	2.860849	1	0.11443398	25	0.327378	23.47362	-6.10554	6.105539
50	5.627	0.02	5.626874	1	0.11253748	50	0.633234	46.22297	-7.55406	7.554064
100	11.802	0.01	11.80244	1	0.1180244	100	1.392976	97.01432	-2.98568	2.985678
0.5	0.06733	2	0.067332	1	0.134664	0.5	0.009067	0.498145	-0.37103	0.371025
1.0	0.12792	1	0.127922	1	0.016364	0.996471	-0.35286	0.352864		
5.0	0.71553	0.2	0.715527	1	0.1431054	5	0.102396	5.829268	-16.58536	16.58536
10	1.25573	0.1	1.255725	1	0.157685	10.27216	2.721622	2.721622		
15	2.18375	0.066667	2.183753	1	0.14558353	15	0.317918	17.90479	19.36527	19.36527
25	3.43983	0.04	3.439828	1	0.13759312	25	0.473297	28.23546	12.94185	12.94185
50	6.21714	0.02	6.217136	1	0.12434272	50	0.773056	51.07762	2.155234	2.155234
100	12.81743	0.01	12.81743	1	0.1281743	100	1.642865	105.3622	5.362175	5.362175
0.5	0.06601	2	0.066011	1	0.13202151	0.5	0.008715	0.487278	-2.54436	2.544361
1.0	0.12837	1	0.12837	1	0.016479	1.000156	0.015607	0.015607		
5.0	0.61704	0.2	0.617041	1	0.1234082	5	0.076148	5.019263	0.385267	0.385267
10	1.30989	0.1	1.309889	1	0.1309888	10	0.171581	10.71764	7.176364	7.176364
15	1.82457	0.066667	1.824567	1	0.1216378	15	0.221936	14.95064	-0.32906	0.329056
25	3.12476	0.04	3.124763	1	0.12499053	25	0.390566	25.64419	2.576766	2.576766
50	6.16397	0.02	6.163973	1	0.12327945	50	0.759891	50.64037	1.280744	1.280744
100	12.03361	0.01	12.03361	1	0.12033607	100	1.448077	98.91557	-1.08443	1.084426
0.5	0.072	2	0.072041	1	0.14408209	0.5	0.01038	0.536875	7.374947	7.374947
1.0	0.126	1	0.125913	1	0.015854	0.979946	-2.00543	2.005432		
5.0	0.605	0.2	0.604864	1	0.12097277	5	0.073172	4.919111	-1.61777	1.617775
10	1.278	0.1	1.278015	1	0.163332	10.45549	4.54856	4.54856		
15	1.804	0.066667	1.803593	1	0.1202395	15	0.216863	14.77814	-1.4791	1.479099
25	2.998	0.04	2.997599	1	0.11990397	25	0.359424	24.59832	-1.60671	1.606708
50	6.345	0.02	6.345012	1	0.12690025	50	0.805184	52.12934	4.25869	4.25869
100	12.316	0.01	12.31566	1	0.12315659	100	1.516755	101.2353	1.235328	1.235328
0.5	0.066	2	0.065719	1	0.13143741	0.5	0.008638	0.484876	-3.02476	3.024758
1.0	0.131	1	0.131139	1	0.017198	1.022933	2.293307	2.293307		
5.0	0.635	0.2	0.634793	1	0.12695866	5	0.080593	5.165268	3.305363	3.305363
10	1.250	0.1	1.250387	1	0.12503866	10	0.158347	10.22826	2.282558	2.282558
15	1.774	0.066667	1.773771	1	0.11825141	15	0.209751	14.63287	-3.11422	3.114218
25	3.207	0.04	3.20652	1	0.12826079	25	0.411271	26.3186	5.266418	5.266418
50	6.103	0.02	6.103092	1	0.12206184	50	0.744955	50.13966	0.279312	0.279312
100	12.242	0.01	12.24207	1	0.12242067	100	1.498682	100.6301	0.630066	0.630066
Sum	1239.0	150.97091	20.62	150.9709	48	5.97564919	1239	18.43938		204.0942
Slope	0.12158695									
Intercept	0.00676408									
R ²	0.99871628									

C _{nom}	y	w	wxy	wx	wy	wx ²	wy ²	C _{found}	%RE	%RE
0.5	0.061	4	0.12286189	2	0.245724	1	0.015095	0.46749	-6.50204	6.5020327
1.0	0.119	1	0.11876936	1	0.118769	1	0.014106	0.934585	-6.54155	6.54154837
5.0	0.573	0.04	0.11457451	0.2	0.022915	1	0.013127	4.638385	-7.3233	7.32329656
10	1.213	0.01	0.12129207	0.1	0.012129	1	0.014712	9.847845	-1.52155	1.521546915
15	1.745	0.004444	0.11632849	0.066667	0.007755	1	0.013532	14.18172	-5.4552	5.455200614
25	2.975	0.0016	0.11900237	0.04	0.004476	1	0.014162	24.20272	-3.18914	3.189136603
50	5.846	0.0004	0.11691549	0.02	0.002338	1	0.013669	47.58836	-4.82328	4.823277327
100	11.324	0.0001	0.11323907	0.01	0.001132	1	0.012823	92.21475	-7.78525	7.785247791
0.5	0.062	4	0.12340169	2	0.246803	1	0.0152208	0.469688	-0.0623	0.062300527
1.0	0.119	1	0.11939719	1	0.119397	1	0.014256	0.939959	-6.0301	6.03098674
5.0	0.590	0.04	0.11793948	0.2	0.022581	1	0.013901	4.769429	-4.61142	4.611424253
10	1.190	0.01	0.11903579	0.1	0.011904	1	0.01417	9.684043	-3.95957	3.959573542
15	1.821	0.004444	0.12140371	0.066667	0.008094	1	0.014739	14.80188	-1.32079	1.320790615
25	2.861	0.0016	0.11443398	0.04	0.004577	1	0.013095	23.27233	-6.91067	6.910674064
50	5.627	0.0004	0.11253748	0.02	0.002251	1	0.012665	45.80514	-8.38973	8.389727888
100	11.802	0.0001	0.1180244	0.01	0.00118	1	0.01393	96.11301	-3.88699	3.886986008
0.5	0.06733	4	0.134664	2	0.269328	1	0.018134	0.515561	3.112293	3.112293107
1.0	0.12792	1	0.127922	1	0.016364	1.009144	0.914445	0.914445	0.914445	0.914445093
5.0	0.71553	0.04	0.1431054	0.2	0.028621	1	0.020479	5.795938	15.91876	15.91876297
10	1.25573	0.01	0.1255725	0.1	0.012557	1	0.015768	10.19654	1.965412	1.965412437
15	2.18375	0.004444	0.14558353	0.066667	0.009706	1	0.021195	17.75652	18.37677	18.37676896
25	3.43983	0.0016	0.13759312	0.04	0.005504	1	0.018932	27.98855	11.95541	11.95540605
50	6.21714	0.0004	0.12434272	0.02	0.002487	1	0.015461	50.61357	1.227149	1.227149269
100	12.81743	0.0001	0.1281743	0.01	0.001282	1	0.016429	104.3814	4.381405	4.38140459
0.5	0.06601	4	0.13202151	2	0.264043	1	0.01743	0.504798	0.99646	0.99645523
1.0	0.12837	1	0.12837001	1	0.016479	1.012794	1.279408	1.279408	1.279408	1.279408435
5.0	0.61704	0.04	0.1234082	0.2	0.024682	1	0.01523	4.939644	-0.12712	0.127119756
10	1.30989	0.01	0.1309888	0.1	0.013099	1	0.017158	10.63777	6.37775	6.377749945
15	1.82457	0.004444	0.1216378	0.066667	0.008109	1	0.014796	14.83049	-1.13009	1.130088272
25	3.12476	0.0016	0.12499053	0.04	0.005	1	0.015623	25.42225	1.688985	1.688984915
50	6.16397	0.0004	0.12327945	0.02	0.002466	1	0.015198	50.18049	0.369893	0.369892537
100	12.03361	0.0001	0.12033607	0.01	0.001203	1	0.014481	97.99617	-2.00383	2.003831773
0.5	0.072	4	0.14408209	2	0.288164	1	0.02076	0.539232	10.78453	10.78453241
1.0	0.126	1	0.12591269	1	0.015854	0.972239	-0.72239	0.722392865		
5.0	0.605	0.04	0.12097277	0.2	0.024195	1	0.014634	4.894445	-2.11109	2.111094463
10	1.278	0.01	0.12780147	0.1	0.01278	1	0.016333	10.37812	2.781196	3.781196171
15	1.804	0.004444	0.1202395	0.066667	0.008016	1	0.014458	14.65962	-2.26918	2.269183511
25	2.998	0.0016	0.11990397	0.04	0.004796	1	0.014377	24.38633	-2.45467	2.454666973
50	6.345	0.0004	0.12690025	0.02	0.002538	1	0.016104	51.65529	3.310582	3.310582129
100	12.316	0.0001	0.12315659	0.01	0.001232	1	0.015168	100.2938	0.293841	0.293841107
0.5	0.066	4	0.13143741	2	0.262875	1	0.017276	0.502419	0.483822	0.483821557
1.0	0.131	1	0.1311394	1	0.017198	1.035354	3.535428	3.53542769		
5.0	0.635	0.04	0.12695866	0.2	0.025392	1	0.016119	5.138259	2.76518	2.765180137
10	1.250	0.01	0.12503866	0.1	0.012504	1	0.015635	10.15305	1.530528</	

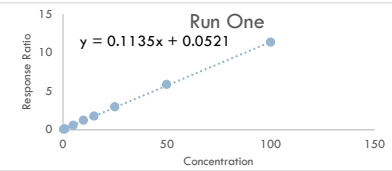
Validation Study 1

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

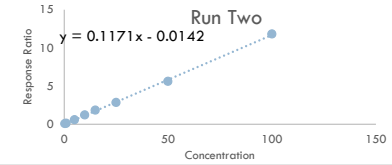
VALIDATION CURVE CALIBRATION

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

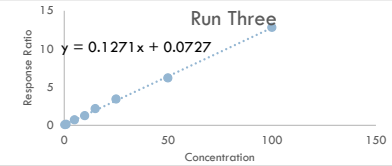
Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	0.5	0.50	0.00	0.061
	1.0	0.99	1.00	0.119
	5.0	4.89	2.20	0.573
	10	10.38	3.80	1.213
	15	14.94	0.40	1.745
	25	25.49	1.96	2.975
	50	50.10	0.20	5.846
	100	97.07	2.93	11.324
OPI1_20220418B_JR	0.5	0.50	0.14	0.062
	1.0	0.99	0.58	0.119
	5.0	5.02	0.31	0.590
	10	10.16	1.55	1.190
	15	15.55	3.67	1.821
	25	24.44	2.22	2.861
	50	48.10	3.79	5.627
	100	100.93	0.93	11.802
OPI1_20220418B_KMY	0.5	0.51	2.00	0.067
	1.0	0.96	4.00	0.128
	5.0	5.36	7.20	0.716
	10	9.41	5.90	1.256
	15	16.36	9.07	2.184
	25	25.77	3.08	3.440
	50	46.58	6.84	6.217
	100	96.03	3.97	12.817
OPI1_20220421B_CD	0.5	0.50	0.00	0.066
	1.0	1.00	0.00	0.128
	5.0	4.95	1.00	0.617
	10	10.55	5.50	1.310
	15	14.70	2.00	1.825
	25	25.21	0.84	3.125
	50	49.76	0.48	6.164
	100	97.16	2.84	12.034
OPI1_20220421B_CLR	0.5	0.51	2.42	0.072
	1.0	0.95	4.62	0.126
	5.0	4.88	2.43	0.605
	10	10.40	3.96	1.278
	15	14.70	1.98	1.804
	25	24.49	2.05	2.998
	50	51.92	3.85	6.345
	100	100.85	0.85	12.316
OPI1_20220427B_CD	0.5	0.49	1.26	0.066
	1.0	1.02	2.27	0.131
	5.0	5.09	1.88	0.635
	10	10.07	0.70	1.250
	15	14.30	4.66	1.774
	25	25.88	3.53	3.207
	50	49.30	1.40	6.103
	100	98.93	1.08	12.242



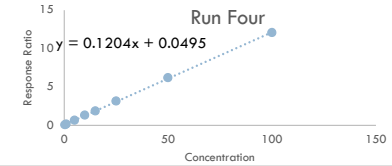
Slope 0.11350
 Intercept 0.05214
 R² 0.99967



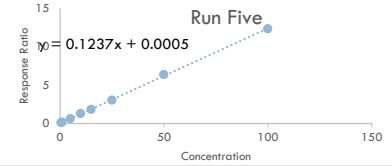
Slope 0.11712
 Intercept -0.01416
 R² 0.99939



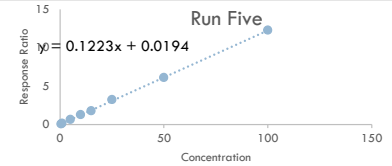
Slope 0.12709
 Intercept 0.07268
 R² 0.99893



Slope 0.12045
 Intercept 0.04949
 R² 0.99979



Slope 0.12368
 Intercept 0.00047
 R² 0.99966



Slope 0.12229
 Intercept 0.01942
 R² 0.99980

Max %RE = 9.07

Comments: N/A

Acceptance Criteria: %RE Calibrators ±20% of target

Validation Study 1

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

LINEARITY

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

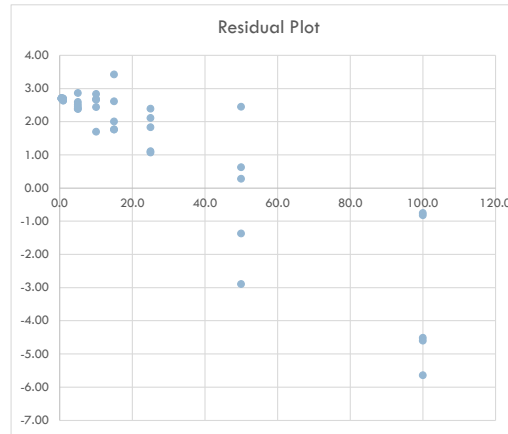
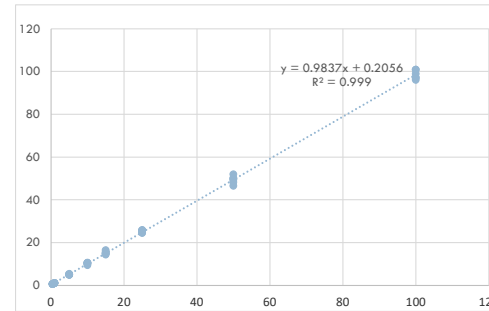
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	0.5	0.50	-2.20	2.70
	1.0	0.99	-1.68	2.67
	5.0	4.89	2.50	2.39
	10	10.38	7.72	2.66
	15	14.94	12.94	2.00
	25	25.49	23.38	2.11
	50	50.10	49.48	0.62
	100	97.07	101.68	-4.61
OPI1_20220418B_JR	0.5	0.50	-2.20	2.70
	1.0	0.99	-1.68	2.67
	5.0	5.02	2.50	2.52
	10	10.16	7.72	2.44
	15	15.55	12.94	2.61
	25	24.44	23.38	1.07
	50	48.10	49.48	-1.37
	100	100.93	101.68	-0.75
OPI1_20220418B_KMY	0.5	0.51	-2.20	2.71
	1.0	0.96	-1.68	2.64
	5.0	5.36	2.50	2.86
	10	9.41	7.72	1.69
	15	16.36	12.94	3.42
	25	25.77	23.38	2.39
	50	46.58	49.48	-2.90
	100	96.03	101.68	-5.65
OPI1_20220421B_CD	0.5	0.50	-2.20	2.70
	1.0	1.00	-1.68	2.68
	5.0	4.95	2.50	2.45
	10	10.55	7.72	2.83
	15	14.70	12.94	1.76
	25	25.21	23.38	1.83
	50	49.76	49.48	0.28
	100	97.16	101.68	-4.52
OPI1_20220421B_CLR	0.5	0.51	-2.20	2.71
	1.0	0.95	-1.68	2.63
	5.0	4.88	2.50	2.38
	10	10.40	7.72	2.68
	15	14.70	12.94	1.77
	25	24.49	23.38	1.11
	50	51.92	49.48	2.45
	100	100.85	101.68	-0.83
OPI1_20220427B_CD	0.5	0.49	-2.20	2.69
	1.0	1.02	-1.68	2.70
	5.0	5.09	2.50	2.60
	10	10.07	7.72	2.35
	15	14.30	12.94	1.36
	25	25.88	23.38	2.51
	50	49.30	49.48	-0.18
	100	98.93	101.68	-2.75

Slope	0.9837
Std err in slope, S_b	0.0045
Degrees freedom	46
Confidence level	95%
Student t	2.0129
Confidence interval	0.009
Slope	0.984 ± 0.009
Range	0.9746 - 0.9928

Intercept	0.2056
Std err in Intercept	0.1850
Degrees freedom	46
Confidence Level	95%
Student t	2.0129
Confidence interval	0.372
Intercept	0.206 ± 0.372
Lower	-0.1667 - 0.5780

NO

YES



Comments: The linearity of the method is acceptable because the individual calibration curves on all six 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

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		1.5	10	80	
OPI1_20220414B_JR	1-1	1.45	9.99	74.50	
	1-2	1.43	9.73	75.65	
	1-3	1.48	10.01	75.74	
	1-4	1.49	9.77	73.18	
	Within Run	Mean	1.46	9.88	74.77
		SD	0.03	0.15	1.20
		%CV	1.88%	1.47%	1.60%
	% Bias	-2.50%	-1.25%	-6.54%	
OPI1_20220418B_JR	2-1	1.43	10.16	78.24	
	2-2	1.41	10.02	75.48	
	2-3	1.44	9.96	74.25	
	Within Run	Mean	1.43	10.05	75.99
		SD	0.01	0.11	2.05
		%CV	0.86%	1.05%	2.69%
		% Bias	-4.90%	0.47%	-5.01%
OPI1_20220418B_KMY	3-1	1.37	9.91	70.78	
	3-2	1.42	9.18	70.75	
	3-3	1.30	9.36	79.65	
	3-4	1.52	9.84	68.46	
	Within Run	Mean	1.40	9.57	72.41
		SD	0.09	0.36	4.95
		%CV	6.60%	3.74%	6.83%
	% Bias	-6.50%	-4.28%	-9.49%	
OPI1_20220421B_CD	4-1	1.48	9.40	71.57	
	4-2	1.59	10.10	80.29	
	4-3	1.53	10.62	75.31	
	Within Run	Mean	1.53	10.04	75.72
		SD	0.06	0.61	4.37
		%CV	3.59%	6.10%	5.78%
		% Bias	2.22%	0.40%	-5.35%
OPI1_20220421B_CLR	5-1	1.62	9.95	76.74	
	5-2	1.58	9.69	76.90	
	5-3	1.49	9.84	77.65	
	Within Run	Mean	1.56	9.83	77.10
		SD	0.07	0.13	0.48
		%CV	4.19%	1.29%	0.63%
		% Bias	4.23%	-1.74%	-3.63%
OPI1_20220427B_CD	6-1	1.45	9.66	72.18	
	6-2	1.45	10.30	70.57	
	6-3	1.42	9.82	76.20	
	Within Run	Mean	1.44	9.92	72.98
		SD	0.02	0.33	2.90
		%CV	1.19%	3.35%	3.97%
		% Bias	-3.77%	-0.75%	-8.77%

Mean		1.47	9.87	74.70
SD		0.08	0.33	3.22
Precision (%CV)	Max Within-Run	6.60%	6.10%	6.83%
	Between-Run	5.13%	3.31%	4.31%
% Bias		-1.87%	-1.19%	-6.46%

Comments: N/A

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

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Buprenorphine
 Units: ng/mL
 Method: OPI1.M
 Instrument: LCMS-1
 SOP Reference: Toxicology Analytical Manual v3.7

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes $\sum \%RE $	Unweighted: = 337.06 1/x Weighting: = 279.48 1/x2 Weighting: = 279.71	1/x2 weighting will be used. While 1/x weighting gave a lower %RE, the 1/x2 weighting had lower overall bias and within-run precision. Using this weighting, the curve has an acceptable slope and intercept.
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9867 - 1.0112 95% CI of Intercept = -0.4102 - 0.5982	N/A
1	Validation Calibration	%RE Calibrators $\pm 20\%$ of target	Max %RE of Calibrators = 14.00	N/A
1	Case Work Calibration	%RE Calibrators $\pm 20\%$ of target	N/A	Not performed. All 8 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 8 calibrators used in validation will be used for casework.
2	Bias & Precision	%Bias $\leq 20\%$ Within-Run %CV $\leq 20\%$ Between-Run %CV $\leq 20\%$	Max Bias = 2.84% Max Within-Run Precision = 7.31% Max Between-Run Precision = 4.82%	N/A

Validation Study 1

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

VALIDATION CURVE CALIBRATION

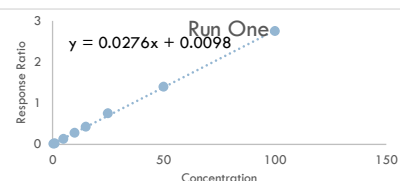
Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Batch Name	Target	Calculated Result	% RE	y (Response Ratio)
OPI1_20220414B_JR	0.5	0.54	8.00	0.015
	1.0	0.86	14.00	0.024
	5.0	4.64	7.20	0.128
	10	10.20	2.00	0.282
	15	15.37	2.47	0.425
	25	27.14	8.56	0.750
	50	50.64	1.28	1.399
	100	99.58	0.42	2.751
OPI1_20220418B_JR	0.5	0.50	0.14	0.014
	1.0	1.00	0.43	0.029
	5.0	5.02	0.33	0.145
	10	9.82	1.81	0.284
	15	15.70	4.69	0.455
	25	25.11	0.45	0.727
	50	48.55	2.91	1.405
	100	99.54	0.46	2.882
OPI1_20220418B_KMY	0.5	0.52	4.00	0.017
	1.0	0.90	10.00	0.029
	5.0	5.57	11.40	0.180
	10	9.24	7.60	0.299
	15	16.20	8.00	0.524
	25	25.33	1.32	0.821
	50	48.50	3.00	1.572
	100	95.99	4.01	3.111
OPI1_20220421B_CD	0.5	0.50	0.62	0.018
	1.0	1.03	2.95	0.033
	5.0	4.68	6.42	0.138
	10	9.47	5.33	0.276
	15	14.60	2.70	0.424
	25	26.19	4.76	0.759
	50	52.09	4.17	1.506
	100	103.20	3.20	2.980
OPI1_20220427B_CD	0.5	0.49	1.40	0.019
	1.0	1.03	3.32	0.035
	5.0	4.85	3.09	0.151
	10	10.24	2.45	0.315
	15	14.31	4.57	0.439
	25	26.52	6.06	0.810
	50	47.70	4.59	1.454
	100	101.83	1.83	3.099

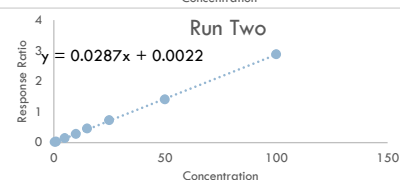
Max %RE = 14.00

Comments: N/A

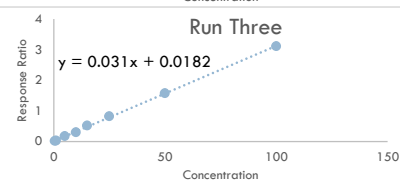
Acceptance Criteria: %RE Calibrators ±20% of target



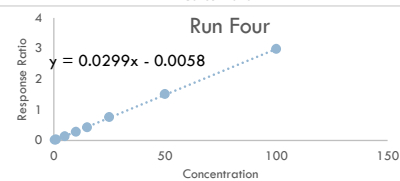
Slope 0.02758
 Intercept 0.00979
 R² 0.99942



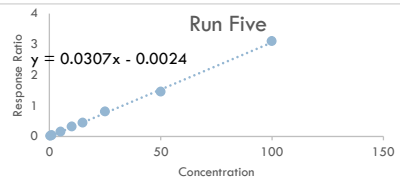
Slope 0.02868
 Intercept 0.00221
 R² 0.99975



Slope 0.03104
 Intercept 0.01817
 R² 0.99948



Slope 0.02993
 Intercept -0.00581
 R² 0.99981



Slope 0.03071
 Intercept -0.00241
 R² 0.99873

Validation Study 1

LINEARITY

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

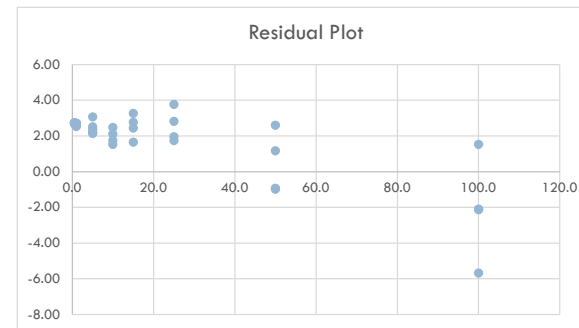
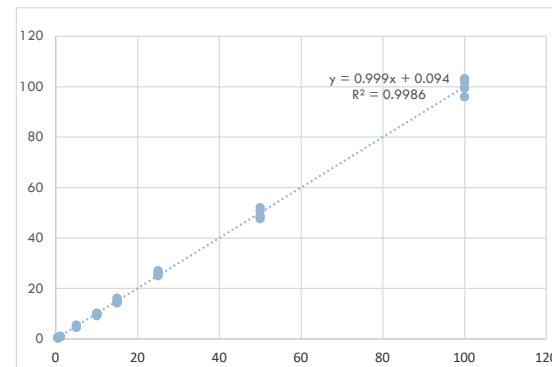
Study Date	Target (x)	Calculated (y)	Predicted	Residual
OPI1_20220414B_JR	0.5	0.54	-2.20	2.74
	1.0	0.86	-1.68	2.54
	5.0	4.64	2.50	2.14
	10	10.20	7.72	2.48
	15	15.37	12.94	2.43
	25	27.14	23.38	3.76
	50	50.64	49.48	1.16
OPI1_20220418B_JR	0.5	0.50	-2.20	2.70
	1.0	1.00	-1.68	2.67
	5.0	5.02	2.50	2.52
	10	9.82	7.72	2.10
	15	15.70	12.94	2.77
	25	25.11	23.38	1.74
	50	48.55	49.48	-0.93
OPI1_20220418B_KMY	0.5	0.52	-2.20	2.72
	1.0	0.90	-1.68	2.58
	5.0	5.57	2.50	3.07
	10	9.24	7.72	1.52
	15	16.20	12.94	3.26
	25	25.33	23.38	1.95
	50	48.50	49.48	-0.98
OPI1_20220421B_CD	0.5	0.50	-2.20	2.70
	1.0	1.03	-1.68	2.71
	5.0	4.68	2.50	2.18
	10	9.47	7.72	1.75
	15	14.60	12.94	1.66
	25	26.19	23.38	2.81
	50	52.09	49.48	2.61
OPI1_20220427B_CD	0.5	0.49	-2.20	2.69
	1.0	1.03	-1.68	2.71
	5.0	4.85	2.50	2.35
	10	10.24	7.72	2.53
	15	14.31	12.94	1.38
	25	26.52	23.38	3.14
	50	47.70	49.48	-1.77
	100	101.83	101.68	0.16

Slope	0.9990
Std err in slope, S_b	0.0061
Degrees freedom	38
Confidence level	95%
Student t	2.0244
Confidence interval	0.012
Slope	0.999 ± 0.012
Range	$0.9867 - 1.0112$

YES

Intercept	0.0940
Std err in Intercept	0.2491
Degrees freedom	38
Confidence Level	95%
Student t	2.0244
Confidence interval	0.504
Intercept	0.094 ± 0.504
Lower	$-0.4102 - 0.5982$

YES



Comments: N/A

Acceptance Criteria: 95% CI of slope includes 1
 95% CI of intercept includes 0

Validation Study 2

BIAS AND PRECISION

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

Analyst: JR, KMY, CD, CLR
 Study Dates: 4/14/2022 to 4/27/2022
 Matrix: Blood

Run Date	Run Order	LQC	MQC	HQC	
<i>Target Concentration (ng/mL):</i>		1.5	10	80	
OPI1_20220414B_JR	1-1	1.50	10.83	78.65	
	1-2	1.56	10.57	79.01	
	1-3	1.49	10.78	78.53	
	1-4	1.56	10.33	79.09	
	Within Run	Mean	1.53	10.63	78.82
		SD	0.04	0.23	0.27
		%CV	2.47%	2.15%	0.35%
	% Bias	1.83%	6.27%	-1.47%	
OPI1_20220418B_JR	2-1	1.56	9.91	76.76	
	2-2	1.56	10.22	77.82	
	2-3	1.53	10.08	77.45	
	Within Run	Mean	1.55	10.07	77.34
		SD	0.02	0.15	0.54
		%CV	1.21%	1.53%	0.70%
		% Bias	3.29%	0.67%	-3.32%
OPI1_20220418B_KMY	3-1	1.50	9.96	75.20	
	3-2	1.52	9.25	75.48	
	3-3	1.39	9.75	80.96	
	3-4	1.66	10.40	72.41	
	Within Run	Mean	1.52	9.84	76.01
		SD	0.11	0.48	3.58
		%CV	7.31%	4.85%	4.71%
	% Bias	1.17%	-1.60%	-4.98%	
OPI1_20220421B_CD	4-1	1.41	10.47	81.55	
	4-2	1.42	10.52	82.12	
	4-3	1.51	11.25	81.22	
	Within Run	Mean	1.45	10.75	81.63
		SD	0.06	0.44	0.45
		%CV	3.91%	4.09%	0.55%
		% Bias	-3.40%	7.46%	2.04%
OPI1_20220427B_CD	5-1	1.47	9.69	75.84	
	5-2	1.39	10.53	74.55	
	5-3	1.57	10.20	76.80	
	Within Run	Mean	1.48	10.14	75.73
		SD	0.09	0.43	1.13
		%CV	6.05%	4.20%	1.49%
		% Bias	-1.52%	1.39%	-5.33%

Mean		1.51	10.28	77.85
SD		0.07	0.48	2.70
Precision (%CV)	Max Within-Run	7.31%	4.85%	4.71%
	Between-Run	4.82%	4.70%	3.47%
% Bias		0.28%	2.84%	-2.62%

Comments: N/A

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

Morphine**1/x Weighting:**

		LQC	MQC	HQC
Mean		14.75	28.90	90.78
SD		0.71	1.58	4.99
Precision (%CV)	Max Within-Run	6.70%	2.82%	5.12%
	Between-Run	4.82%	5.47%	5.50%
% Bias		-1.32%	-3.21%	-8.65%
Slope	0.9827	Intercept		0.8351
Std err in slope, Sb	0.0119	Std err in Intercept		0.7627
Degrees freedom	28	Degrees freedom		28
Confidence level	95%	Confidence Level		95%
Student t	2.0484	Student t		2.0484
Confidence interval	0.024	Confidence interval		1.562
Slope	0.983 ± 0.024	Intercept		0.835 ± 1.562
Range	0.9583 - 1.0072	Lower		-0.7273 - 2.3975
	YES			YES

Hydromorphone**1/x Weighting:**

		LQC	MQC	HQC
Mean		15.35	99.49	374.31
SD		0.71	2.23	8.92
Precision (%CV)	Max Within-Run	6.21%	2.74%	3.73%
	Between-Run	4.62%	2.24%	2.38%
% Bias		2.75%	-0.46%	-6.37%
Slope	0.9786	Intercept		1.4294
Std err in slope, Sb	0.0095	Std err in Intercept		1.9494
Degrees freedom	38	Degrees freedom		38
Confidence level	95%	Confidence Level		95%
Student t	2.0244	Student t		2.0244
Confidence interval	0.019	Confidence interval		3.946
Slope	0.979 ± 0.019	Intercept		1.429 ± 3.946
Range	0.9594 - 0.9978	Lower		-2.5170 - 5.3758
	NO			YES

1/x2 Weighting:

		LQC	MQC	HQC
Mean		14.77	28.71	89.60
SD		0.69	1.74	6.06
Precision (%CV)	Max Within-Run	6.45%	2.75%	5.14%
	Between-Run	4.67%	6.08%	6.76%
% Bias		-1.19%	-3.81%	-9.74%
Slope	0.9680	Intercept		1.0497
Std err in slope, Sb	0.0132	Std err in Intercept		0.8439
Degrees freedom	28	Degrees freedom		28
Confidence level	95%	Confidence Level		95%
Student t	2.0484	Student t		2.0484
Confidence interval	0.027	Confidence interval		1.729
Slope	0.968 ± 0.027	Intercept		1.05 ± 1.729
Range	0.9409 - 0.9951	Lower		-0.6790 - 2.7784
	NO			YES

1/x2 Weighting:

		LQC	MQC	HQC
Mean		15.39	99.03	372.46
SD		0.63	3.23	12.11
Precision (%CV)	Max Within-Run	5.90%	2.52%	3.74%
	Between-Run	4.12%	3.27%	3.25%
% Bias		2.93%	-0.80%	-6.71%
Slope	0.9946	Intercept		0.2704
Std err in slope, Sb	0.0049	Std err in Intercept		1.0066
Degrees freedom	38	Degrees freedom		38
Confidence level	95%	Confidence Level		95%
Student t	2.0244	Student t		2.0244
Confidence interval	0.010	Confidence interval		2.038
Slope	0.995 ± 0.01	Intercept		0.27 ± 2.038
Range	0.9847 - 1.0045	Lower		-1.7673 - 2.3081
	YES			YES

O-desmethyltramadol**1/x Weighting:**

		LQC	MQC	HQC
Mean		15.15	29.52	100.67
SD		0.51	0.91	3.72
Precision (%CV)	Max Within-Run	6.84%	3.23%	2.70%
	Between-Run	3.39%	3.07%	3.69%
% Bias		1.21%	-1.40%	1.01%
Slope	1.0146	Intercept		-0.7073
Std err in slope, Sb	0.0093	Std err in Intercept		0.5956
Degrees freedom	28	Degrees freedom		28
Confidence level	95%	Confidence Level		95%
Student t	2.0484	Student t		2.0484
Confidence interval	0.019	Confidence interval		1.220
Slope	1.015 ± 0.019	Intercept		-0.707 ± 1.22
Range	0.9955 - 1.0337	Lower		-1.9273 - 0.5127
	YES			YES

Oxycodone**1/x Weighting:**

		LQC	MQC	HQC
Mean		15.53	30.30	95.09
SD		0.70	1.16	3.15
Precision (%CV)	Max Within-Run	8.03%	3.46%	3.22%
	Between-Run	4.48%	3.82%	3.31%
% Bias		3.81%	1.25%	-4.61%
Slope	0.9865	Intercept		0.6541
Std err in slope, Sb	0.0117	Std err in Intercept		0.7495
Degrees freedom	28	Degrees freedom		28
Confidence level	95%	Confidence Level		95%
Student t	2.0484	Student t		2.0484
Confidence interval	0.024	Confidence interval		1.535
Slope	0.986 ± 0.024	Intercept		0.654 ± 1.535
Range	0.9624 - 1.0105	Lower		-0.8812 - 2.1893
	YES			YES

1/x2 Weighting:

		LQC	MQC	HQC
Mean		15.14	29.79	102.32
SD		0.52	1.02	4.38
Precision (%CV)	Max Within-Run	6.87%	3.24%	2.70%
	Between-Run	3.40%	3.44%	4.28%
% Bias		1.15%	-0.48%	2.71%
Slope	1.0346	Intercept		-1.0335
Std err in slope, Sb	0.0099	Std err in Intercept		0.6322
Degrees freedom	28	Degrees freedom		28
Confidence level	95%	Confidence Level		95%
Student t	2.0484	Student t		2.0484
Confidence interval	0.020	Confidence interval		1.295
Slope	1.035 ± 0.02	Intercept		-1.034 ± 1.295
Range	1.0144 - 1.0549	Lower		-2.3285 - 0.2615
	NO			YES

1/x2 Weighting:

		LQC	MQC	HQC
Mean		15.53	30.14	94.20
SD		0.68	1.23	3.29
Precision (%CV)	Max Within-Run	7.87%	3.40%	3.21%
	Between-Run	4.40%	4.09%	3.49%
% Bias		3.83%	0.70%	-5.54%
Slope	0.9751	Intercept		0.8255
Std err in slope, Sb	0.0120	Std err in Intercept		0.7684
Degrees freedom	28	Degrees freedom		28
Confidence level	95%	Confidence Level		95%
Student t	2.0484	Student t		2.0484
Confidence interval	0.025	Confidence interval		1.574
Slope	0.975 ± 0.025	Intercept		0.826 ± 1.574
Range	0.9504 - 0.9997	Lower		-0.7486 - 2.3996
	NO			YES

Buprenorphine

1/x Weighting:

	LQC	MQC	HQC
Mean	1.51	10.26	77.69
SD	0.07	0.39	2.05
Precision (%CV)			
Max Within-Run	7.59%	4.90%	4.71%
Between-Run	4.65%	3.84%	2.64%
% Bias	0.46%	2.59%	-2.90%
Slope	0.9960	Intercept	0.1031
Std err in slope, Sb	0.0043	Std err in Intercept	0.1760
Degrees freedom	38	Degrees freedom	38
Confidence level	95%	Confidence Level	95%
Student t	2.0244	Student t	2.0244
Confidence interval	0.009	Confidence interval	0.356
Slope	0.996 ± 0.009	Intercept	0.103 ± 0.356
Range	0.9873 - 1.0047	Lower	-0.2532 - 0.4595
	YES		YES

1/x2 Weighting:

	LQC	MQC	HQC
Mean	1.51	10.28	77.85
SD	0.07	0.48	2.70
Precision (%CV)			
Max Within-Run	7.31%	4.85%	4.71%
Between-Run	4.80%	4.70%	3.47%
% Bias	0.30%	2.84%	-2.62%
Slope	0.9990	Intercept	0.0940
Std err in slope, Sb	0.0061	Std err in Intercept	0.2491
Degrees freedom	38	Degrees freedom	38
Confidence level	95%	Confidence Level	95%
Student t	2.0244	Student t	2.0244
Confidence interval	0.012	Confidence interval	0.504
Slope	0.999 ± 0.012	Intercept	0.094 ± 0.504
Range	0.9867 - 1.0112	Lower	-0.4102 - 0.5982
	YES		YES

SUMMARY OF VALIDATION PERFORMANCE

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

Analyst: JR, KMY, CD, CLR
Study Dates: 4/14/2022 to 4/27/2022
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
April 21, 2022	Calibration R2 value for codeine failed to meet criteria; no codeine data were used from this batch. Further, calibrator 2 for buprenorphine failed to meet criteria, so no buprenorphine data were used from this batch.

Deviations from SOP:

The ULOQ for morphine, codeine, o-desmethyltramadol, 6-acetylmorphine, oxycodone, and tramadol were lowered to 125 ng/mL from 500 ng/mL. The MQC and HQC for these analytes were adjusted to 30 ng/mL and 100 ng/mL, respectively. An additional calibration point was added at a concentration of 15 ng/mL for norbuprenorphine and buprenorphine, and 75 ng/mL for all other analytes. The internal standard concentration was lowered from 50 ng/mL to 25 ng/mL for morphine, codeine, o-desmethyltramadol, oxycodone, and tramadol. Due to an interference observed with the m/z 468.3 > m/z 55.1 ion transition for buprenorphine, the quantifier ion was changed from m/z 55.1 to m/z 84.1, with m/z 101.1 as the only qualifier ion. These changes will be reflected in the Toxicology Analytical Manual v3.8.

Other Observations:

Not all validation parameters were assessed for this validation because it was considered to be an extension of the full validation in blood previously performed. Since the only adjustments made were to the regression models and the concentrations of the calibrations and quality controls without changing the extraction procedure or LLOQ, the only validation parameters assessed were weight verification, calibration verification, linearity, and bias and precision.

Working Standards and Reagents Verified in Validation:
Calibrators: 220329C-C-A, 220329C-C-B, 220329C-C-C
Controls: 220414K-Q-MXL, 220414K-Q-MXM, 220414K-Q-MXH
Internal Standard: 220412C-IS-OPI1
Reagent: 220415-PB6A

Sample Preparation Steps:

Refer to Toxicology Analytical Manual v3.7, "Opioids 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 confirmation analysis in blood.