Verification of Quantitative Methods

Purpose	To demonstrate that the newly prepared calibrator and control solutions are acceptable for use in casework.
Analyte	Benzodiazepines (7-aminoclonazepam, zolpidem, α-hydroxyalprazolam, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, temazepam, and diazepam)
Unit of Measure	ng/mL
Analysts Performing Validation Studies	J. Reber, C. Rodgers, E. Cosme
Responsible Supervisor	Dayong Lee
Start Date	July 26, 2022
Completion Date	August 19, 2022
Primary Matrix	Blood
Secondary Matrix	N/A
Lowest Calibrator Concentration	10 ng/mL
Highest Calibrator Concentration	250 ng/mL for zolpidem and 500 ng/mL for 7-aminoclonazepam, α-hydroxyalprazolam, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, temazepam, and diazepam
Equipment/Instrument	LCMS-1
Instrument Serial Number	SG1939G104
Method	BNZ.M

Validation Approval

Analyst: Jami Reber Digitally signed by Jami Reber Date: 2022.09.12 07:26:08 -05'00' 09/12/2022
Date

Analyst: Corissa L. Rodgers, M.S. Digitally signed by Corissa L. Rodgers, M.S. Date: 2022.09.12 08:31:44 -05'00' 09/12/2022
Date

Analyst: Erin Cosme Digitally signed by Erin Cosme Date: 2022.09.12 07:01:25 -05'00' 9/12/2022
Date

Responsible Supervisor: Dayong Lee Digitally signed by Dayong Lee Date: 2022.09.12 15:08:01 -05'00' 09/12/2022
Date

Verification — LINEARITY

Analyte: 7-aminoclonazepam
Units: ng/mL
Instrument: LCMS-1

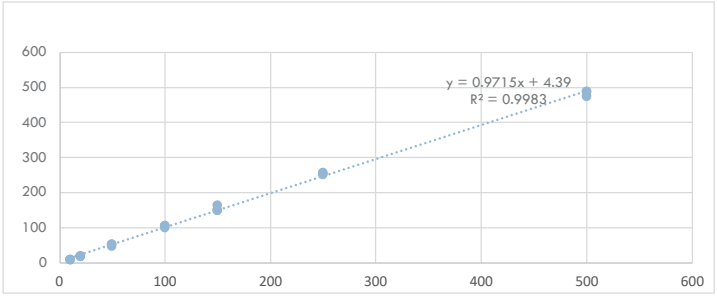
Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.27	-7.27
	20	19.02	-4.91
	50	53.42	6.83
	100	106.35	6.35
	150	149.97	-0.02
	250	253.12	1.25
	500	488.85	-2.23
BNZ_20220810B_EC	10	9.32	-6.85
	20	20.52	2.62
	50	48.47	-3.06
	100	107.23	7.23
	150	150.77	0.51
	250	254.02	1.61
	500	489.67	-2.07
BNZ_20220819B_CLR	10	8.90	-11.05
	20	19.28	-3.58
	50	52.58	5.17
	100	101.19	1.19
	150	165.03	10.02
	250	258.22	3.29
	500	474.80	-5.04

Slope	0.9715
Std err in slope, S_b	0.0091
Degrees freedom	19
Confidence level	95%
Student t	2.09302
Confidence interval	0.019
Slope	0.9715 ± 0.0191
Range	0.9524 - 0.9907

Intercept	4.3900
Std err in Intercept	2.0384
Degrees freedom	19
Confidence Level	95%
Student t	2.09302
Confidence interval	4.266
Intercept	4.39 ± 4.2663
Lower	0.1237 - 8.6564

NO **NO**



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

Verification

BIAS AND IMPRECISION

Analyte: 7-aminoclonazepam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	MQC	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*80*	*N/A*	*400*
BNZ_20220726B_JR	1-1	25.76	72.37	73.31	357.95
	1-2	29.28	74.34	73.25	706.74*
	1-3	26.00	75.14	70.45	0*
	1-4	-	-	76.03	-
	Mean	**27.01**	**73.95**	**73.26**	**357.95**
	SD	**1.97**	**1.43**	**2.28**	**N/A**
	%CV	**7.28%**	**1.93%**	**3.11%**	**N/A**
	% Bias	**-9.96%**	**-7.56%**	**N/A**	**-10.51%**
BNZ_20220805B_CLR	2-1	18.32**	75.89	50.52	343.07
	2-2	23.11**	78.92	47.13**	330.23~
	2-3	21.74**	65.73	46.53**	313.77
	2-4	21.60**	-	48.50**	351.92
	2-5	-	-	-	340.95
	Mean		**73.51**	**50.52**	**337.43**
	SD		**6.91**	**N/A**	**16.47**
	%CV		**9.40%**	**N/A**	**4.88%**
	% Bias		**-8.11%**	**N/A**	**-15.64%**
BNZ_20220810B_EC	3-1	25.89	74.44	58.08	356.31
	3-2	27.32	74.94	59.23	358.70
	3-3	26.81	73.88	57.13	366.27
	3-4	27.35	-	58.98	356.73
	3-5	-	-	-	349.51
Within Run	**Mean**	**26.84**	**74.42**	**58.36**	**357.50**
	SD	**0.68**	**0.53**	**0.95**	**6.00**
	%CV	**2.54%**	**0.72%**	**1.63%**	**1.68%**
	% Bias	**-10.52%**	**-6.98%**	**N/A**	**-10.62%**
BNZ_20220819B_CLR	4-1	28.98	69.98	-	371.98
	4-2	26.14	74.00	-	381.52
	4-3	27.48	75.90	-	388.28
Within Run	**Mean**	**27.53**	**73.29**		**380.59**
	SD	**1.42**	**3.02**		**8.19**
	%CV	**5.16%**	**4.12%**		**2.15%**
	% Bias	**-8.22%**	**-8.38%**		**-4.85%**

Mean		**27.10**	**73.79**	**64.11**	**356.69**
SD		**1.25**	**3.31**	**9.15**	**18.92**
Precision (%CV)	***Max Within-Run***	**7.28%**	**9.40%**	**3.11%**	**4.88%**
	Between-Run	**4.60%**	**4.49%**	**14.28%**	**5.30%**
% Bias		**-9.57%**	**-7.76%**	**N/A**	**-10.41%**

Comments: Values in red outside of acceptable range. Note: UTAK will not be used for 7-aminoclonazepam.
*HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation. **Indicates value outside calibration curve. ~Indicates ion ratio failure.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification — LINEARITY

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

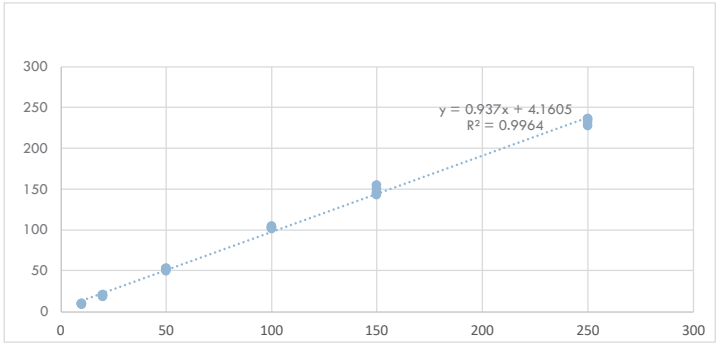
Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.93	-0.69
	20	19.73	-1.34
	50	53.37	6.74
	100	105.19	5.19
	150	143.51	-4.33
	250	236.06	-5.58
BNZ_20220805B_CLR	10	10.17	1.70
	20	18.68	-6.60
	50	53.60	7.20
	100	102.47	2.47
	150	155.71	3.81
	250	228.59	-8.56
BNZ_20220810B_EC	10	9.69	-3.13
	20	21.16	5.78
	50	50.54	1.09
	100	103.94	3.94
	150	146.24	-2.50
	250	237.06	-5.17
BNZ_20220819B_CLR	10	9.82	-1.85
	20	20.47	2.33
	50	51.66	3.31
	100	102.67	2.67
	150	150.41	0.27
	250	233.13	-6.75

Slope	0.9370
Std err in slope, S_b	0.0119
Degrees freedom	22
Confidence level	95%
Student t	2.07387
Confidence interval	0.025
Slope	0.937 ± 0.0248
Range	0.9123 - 0.9618

Intercept	4.1605
Std err in Intercept	1.5267
Degrees freedom	22
Confidence Level	95%
Student t	2.07387
Confidence interval	3.166
Intercept	4.1605 ± 3.1661
Lower	0.9944 - 7.3266

NO **NO**



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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*87.18*	*82*	*200*
BNZ_20220726B_JR	1-1	30.70	81.36	81.87	192.36
	1-2	31.73	84.16	83.21	362.64*
	1-3	30.76	83.95	84.31	0*
	1-4	-	-	84.25	-
Within Run	**Mean**	**31.06**	**83.16**	**83.41**	**192.36**
	SD	**0.58**	**1.56**	**1.14**	**N/A**
	%CV	**1.86%**	**1.88%**	**1.37%**	**N/A**
	% Bias	**3.54%**	**-4.61%**	**1.43%**	**-3.82%**
BNZ_20220805B_CLR	2-1	30.53	84.10	79.29	184.04
	2-2	30.16	81.33	82.73	206.56
	2-3	30.22	82.68	82.32	194.12
	2-4	31.37	-	82.95	184.62
	2-5	-	-	-	182.97
Within Run	**Mean**	**30.57**	**82.70**	**81.82**	**190.46**
	SD	**0.56**	**1.39**	**1.71**	**10.05**
	%CV	**1.82%**	**1.67%**	**2.09%**	**5.28%**
	% Bias	**1.90%**	**-5.13%**	**-0.50%**	**-4.77%**
BNZ_20220810B_EC	3-1	30.30	82.39	81.51	194.27
	3-2	31.93	82.43	83.11	197.62
	3-3	30.76	82.75	82.33	197.37
	3-4	31.55	-	84.11	194.48
	3-5	-	-	-	191.76
Within Run	**Mean**	**31.14**	**82.52**	**82.77**	**195.10**
	SD	**0.74**	**0.19**	**1.11**	**2.44**
	%CV	**2.37%**	**0.24%**	**1.34%**	**1.25%**
	% Bias	**3.79%**	**-5.34%**	**0.65%**	**-2.45%**
BNZ_20220819B_CLR	4-1	32.64	-	-	192.08
	4-2	31.14	-	-	195.77
	4-3	30.91	-	-	191.20
Within Run	**Mean**	**31.56**			**193.02**
	SD	**0.94**			**2.43**
	%CV	**2.98%**			**1.26%**
	% Bias	**5.21%**			**-3.49%**

Mean		**31.05**	**82.79**	**82.67**	**192.80**
SD		**0.72**	**1.08**	**1.40**	**6.16**
Precision (%CV)	***Max Within-Run***	**2.98%**	**1.88%**	**2.09%**	**5.28%**
	Between-Run	**2.32%**	**1.31%**	**1.69%**	**3.20%**
% Bias		**3.61%**	**-5.03%**	**0.53%**	**-3.63%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification — LINEARITY

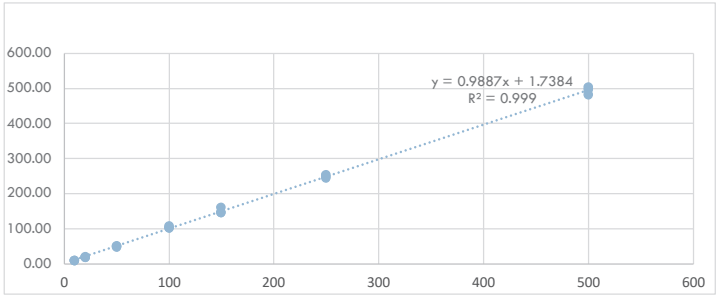
Analyte: alpha-hydroxyalprazolam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	10.14	1.36
	20	19.19	-4.03
	50	50.53	1.07
	100	103.16	3.16
	150	146.04	-2.64
	250	254.42	1.77
	500	496.51	-0.70
BNZ_20220810B_EC	10	10.23	2.27
	20	20.24	1.19
	50	48.25	-3.50
	100	102.18	2.18
	150	146.51	-2.32
	250	248.27	-0.69
	500	504.32	0.86
BNZ_20220819B_CLR	10	8.79	-12.07
	20	19.29	-3.53
	50	51.89	3.78
	100	109.05	9.05
	150	161.52	7.68
	250	245.97	-1.61
	500	483.48	-3.30

Slope	0.9887
Std err in slope, S_b	0.0072
Degrees freedom	19
Confidence level	95%
Student t	2.09302
Confidence interval	0.015
Slope	0.9887 ± 0.0151
Range	0.9736 - 1.0039
	YES

Intercept	1.7384
Std err in Intercept	1.6120
Degrees freedom	19
Confidence Level	95%
Student t	2.09302
Confidence interval	3.374
Intercept	1.7384 ± 3.3739
Lower	-1.6355 - 5.1123
	YES



Comments: N/A

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

Verification

BIAS AND IMPRECISION

Analyte: alpha-hydroxyalprazolam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*85.13*	*85.52*	*400*
BNZ_20220726B_JR	1-1	30.61	86.71	86.52	426.91
	1-2	33.79	91.36	88.92	832.25*
	1-3	30.15	87.71	86.08	0*
	1-4	-	-	87.23	-
Within Run	**Mean**	**31.52**	**88.59**	**87.19**	**426.91**
	SD	**1.98**	**2.45**	**1.25**	**N/A**
	%CV	**6.29%**	**2.76%**	**1.43%**	**N/A**
	% Bias	**5.06%**	**4.07%**	**1.95%**	**6.73%**
BNZ_20220805B_CLR	2-1	31.24	90.94	93.15	409.61
	2-2	30.03	92.25	87.84	398.62
	2-3	30.58	89.74	84.30	351.86
	2-4	32.34	-	88.14	422.98
	2-5	-	-	-	408.86
Within Run	**Mean**	**31.05**	**90.98**	**88.36**	**398.39**
	SD	**0.99**	**1.26**	**3.64**	**27.41**
	%CV	**3.20%**	**1.38%**	**4.12%**	**6.88%**
	% Bias	**3.49%**	**6.87%**	**3.32%**	**-0.40%**
BNZ_20220810B_EC	3-1	30.55	87.93	84.35	412.46
	3-2	32.07	87.84	87.72	424.13
	3-3	30.97	87.73	85.99	419.68
	3-4	31.64	-	87.69	421.18
	3-5	-	-	-	419.14
Within Run	**Mean**	**31.31**	**87.83**	**86.44**	**419.32**
	SD	**0.68**	**0.10**	**1.61**	**4.29**
	%CV	**2.17%**	**0.12%**	**1.86%**	**1.02%**
	% Bias	**4.35%**	**3.18%**	**1.07%**	**4.83%**
BNZ_20220819B_CLR	4-1	33.26	-	-	433.36
	4-2	35.18	-	-	437.62
	4-3	31.53	-	-	435.20
Within Run	**Mean**	**33.32**			**435.39**
	SD	**1.83**			**2.14**
	%CV	**5.48%**			**0.49%**
	% Bias	**11.08%**			**8.85%**

Mean		**31.71**	**89.13**	**87.33**	**415.83**
SD		**1.50**	**1.98**	**2.33**	**21.38**
Precision	***Max Within-Run***	**6.29%**	**2.76%**	**4.12%**	**6.88%**
(%CV)	***Between-Run***	**4.73%**	**2.22%**	**2.67%**	**5.14%**
% Bias		**5.99%**	**4.70%**	**2.11%**	**5.00%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification

LINEARITY

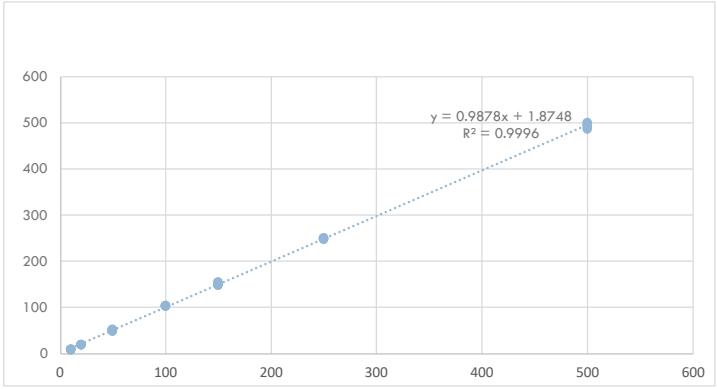
Analyte: Oxazepam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.68	-3.23
	20	19.26	-3.68
	50	51.52	3.04
	100	104.72	4.72
	150	150.26	0.18
	250	250.34	0.14
	500	494.21	-1.16
BNZ_20220805B_CLR	10	9.19	-8.12
	20	20.07	0.34
	50	52.52	5.03
	100	104.57	4.57
	150	149.24	-0.51
	250	249.02	-0.39
	500	495.40	-0.92
BNZ_20220810B_EC	10	10.00	0.01
	20	20.15	0.75
	50	48.63	-2.73
	100	103.66	3.66
	150	148.07	-1.29
	250	248.48	-0.61
	500	501.00	0.20
BNZ_20220819B_CLR	10	9.17	-8.32
	20	19.59	-2.04
	50	51.67	3.33
	100	104.96	4.96
	150	156.17	4.11
	250	251.35	0.54
	500	487.10	-2.58

Slope	0.9878
Std err in slope, S_b	0.0037
Degrees freedom	26
Confidence level	95%
Student t	2.05553
Confidence interval	0.008
Slope	0.9878 ± 0.0076
Range	0.9802 - 0.9955
	NO

Intercept	1.8748
Std err in Intercept	0.8267
Degrees freedom	26
Confidence Level	95%
Student t	2.05553
Confidence interval	1.699
Intercept	1.8748 ± 1.6994
Lower	0.1754 - 3.5742
	NO



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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*80.55*	*82.17*	*400*
BNZ_20220726B_JR	1-1	29.77	82.44	81.71	400.69
	1-2	36.43	85.83	83.58	789.06*
	1-3	30.37	84.47	84.11	0*
	1-4	-	-	85.04	-
Within Run	**Mean**	**32.19**	**84.25**	**83.61**	**400.69**
	SD	**3.68**	**1.71**	**1.40**	**N/A**
	%CV	**11.45%**	**2.03%**	**1.68%**	**N/A**
	% Bias	**7.30%**	**4.59%**	**1.75%**	**0.17%**
BNZ_20220805B_CLR	2-1	29.91	89.62	83.48	395.78
	2-2	29.98	85.08	85.82	406.19
	2-3	30.31	86.89	86.32	357.37
	2-4	32.42	-	87.62	396.44
	2-5	-	-	-	393.15
Within Run	**Mean**	**30.66**	**87.20**	**85.81**	**389.79**
	SD	**1.19**	**2.29**	**1.73**	**18.78**
	%CV	**3.88%**	**2.62%**	**2.01%**	**4.82%**
	% Bias	**2.18%**	**8.25%**	**4.43%**	**-2.55%**
BNZ_20220810B_EC	3-1	30.39	82.54	82.13	396.43
	3-2	31.77	83.66	81.62	403.07
	3-3	30.87	84.44	81.39	405.00
	3-4	31.81	-	84.45	396.40
	3-5	-	-	-	390.07
Within Run	**Mean**	**31.21**	**83.55**	**82.40**	**398.19**
	SD	**0.70**	**0.95**	**1.40**	**5.97**
	%CV	**2.24%**	**1.14%**	**1.70%**	**1.50%**
	% Bias	**4.03%**	**3.72%**	**0.28%**	**-0.45%**
BNZ_20220819B_CLR	4-1	33.97	-	-	416.76
	4-2	32.40	-	-	415.51
	4-3	31.84	-	-	407.96
Within Run	**Mean**	**32.74**			**413.41**
	SD	**1.10**			**4.76**
	%CV	**3.37%**			**1.15%**
	% Bias	**9.12%**			**3.35%**

Mean		**31.59**	**85.00**	**83.94**	**398.63**
SD		**1.85**	**2.25**	**2.02**	**14.28**
Precision (%CV)	***Max Within-Run***	**11.45%**	**2.62%**	**2.01%**	**4.82%**
	Between-Run	**5.85%**	**2.65%**	**2.40%**	**3.58%**
% Bias		**5.66%**	**5.52%**	**2.15%**	**0.13%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification

LINEARITY

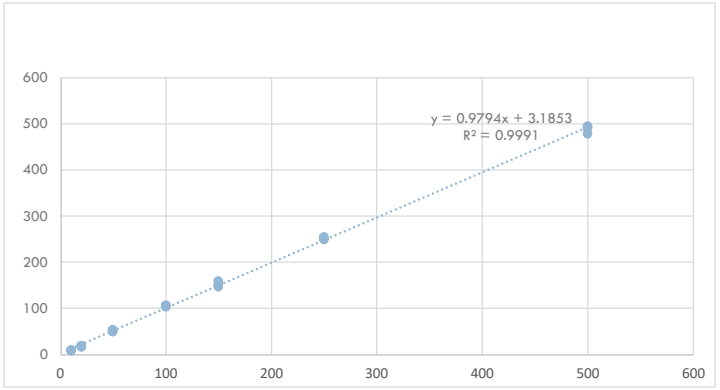
Analyte: Nordiazepam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.84	-1.56
	20	18.78	-6.11
	50	52.54	5.09
	100	104.41	4.41
	150	147.75	-1.50
	250	251.69	0.68
	500	494.98	-1.00
BNZ_20220805B_CLR	10	10.33	3.26
	20	16.60	-16.99
	50	53.84	7.69
	100	107.38	7.38
	150	150.62	0.42
	250	249.99	0.00
	500	491.24	-1.75
BNZ_20220810B_EC	10	9.50	-5.03
	20	20.09	0.47
	50	49.85	-0.30
	100	104.35	4.35
	150	152.17	1.45
	250	251.27	0.51
	500	492.76	-1.45
BNZ_20220819B_CLR	10	8.79	-12.14
	20	20.04	0.18
	50	50.35	0.69
	100	106.57	6.57
	150	160.29	6.86
	250	255.25	2.10
	500	478.72	-4.26

Slope	0.9794
Std err in slope, S_b	0.0057
Degrees freedom	26
Confidence level	95%
Student t	2.05553
Confidence interval	0.012
Slope	0.9794 ± 0.0118
Range	0.9676 - 0.9911
	NO

Intercept	3.1853
Std err in Intercept	1.2764
Degrees freedom	26
Confidence Level	95%
Student t	2.05553
Confidence interval	2.624
Intercept	3.1853 ± 2.6237
Lower	0.5616 - 5.8091
	NO



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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*84.33*	*86.29*	*400*
BNZ_20220726B_JR	1-1	29.22	80.62	84.39	408.57
	1-2	32.27	83.87	87.12	775.80*
	1-3	29.59	84.18	87.44	0*
	1-4	-	-	87.57	-
Within Run	**Mean**	**30.36**	**82.89**	**86.63**	**408.57**
	SD	**1.66**	**1.97**	**1.51**	**N/A**
	%CV	**5.48%**	**2.38%**	**1.74%**	**N/A**
	% Bias	**1.20%**	**-1.71%**	**0.39%**	**2.14%**
BNZ_20220805B_CLR	2-1	30.41	89.49	86.78	399.29
	2-2	30.48	85.53	89.98	391.57
	2-3	30.81	87.55	91.30	360.72
	2-4	32.51	-	90.75	400.71
	2-5	-	-	-	397.19
Within Run	**Mean**	**31.05**	**87.52**	**89.70**	**389.90**
	SD	**0.99**	**1.98**	**2.02**	**16.68**
	%CV	**3.18%**	**2.26%**	**2.25%**	**4.28%**
	% Bias	**3.51%**	**3.79%**	**3.95%**	**-2.53%**
BNZ_20220810B_EC	3-1	29.39	84.05	82.99	403.78
	3-2	30.82	84.08	86.51	405.73
	3-3	30.04	84.56	84.61	406.20
	3-4	30.70	-	88.21	401.91
	3-5	-	-	-	391.91
Within Run	**Mean**	**30.24**	**84.23**	**85.58**	**401.91**
	SD	**0.66**	**0.29**	**2.27**	**5.84**
	%CV	**2.19%**	**0.34%**	**2.65%**	**1.45%**
	% Bias	**0.78%**	**-0.12%**	**-0.82%**	**0.48%**
BNZ_20220819B_CLR	4-1	33.12	-	-	412.09
	4-2	31.18	-	-	414.70
	4-3	30.96	-	-	410.31
Within Run	**Mean**	**31.75**			**412.37**
	SD	**1.19**			**2.21**
	%CV	**3.74%**			**0.54%**
	% Bias	**5.84%**			**3.09%**

Mean		**30.82**	**84.88**	**87.30**	**400.33**
SD		**1.16**	**2.50**	**2.54**	**13.35**
Precision	***Max Within-Run***	**5.48%**	**2.38%**	**2.65%**	**4.28%**
(%CV)	***Between-Run***	**3.76%**	**2.94%**	**2.91%**	**3.34%**
% Bias		**2.83%**	**0.65%**	**1.18%**	**0.80%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification

LINEARITY

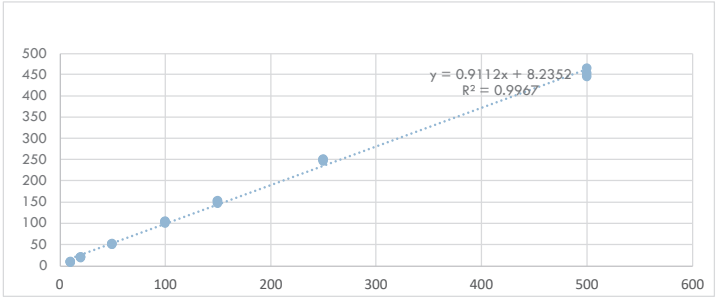
Analyte: Clonazepam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	10.06	0.62
	20	19.14	-4.32
	50	53.07	6.13
	100	105.12	5.12
	150	147.78	-1.48
	250	252.14	0.86
	500	465.31	-6.94
BNZ_20220810B_EC	10	9.65	-3.50
	20	21.00	4.99
	50	51.83	3.66
	100	104.41	4.41
	150	151.61	1.07
	250	246.75	-1.30
	500	453.39	-9.32
BNZ_20220819B_CLR	10	9.52	-4.84
	20	21.58	7.90
	50	52.07	4.13
	100	101.25	1.25
	150	153.34	2.23
	250	250.35	0.14
	500	445.96	-10.81

Slope	0.9112
Std err in slope, S_b	0.0120
Degrees freedom	19
Confidence level	95%
Student t	2.09302
Confidence interval	0.025
Slope	0.9112 ± 0.0251
Range	0.8861 - 0.9363
	NO

Intercept	8.2352
Std err in Intercept	2.6719
Degrees freedom	19
Confidence Level	95%
Student t	2.09302
Confidence interval	5.592
Intercept	8.2352 ± 5.5924
Lower	2.6428 - 13.8275
	NO



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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*79.66*	*81.55*	*400*
BNZ_20220726B_JR	1-1	27.92	81.98	84.91	351.81
	1-2	33.74	85.10	83.69	665.63*
	1-3	28.49	84.19	83.45	0*
	1-4	-	-	82.80	-
Within Run	**Mean**	**30.05**	**83.76**	**83.71**	**351.81**
	SD	**3.21**	**1.60**	**0.88**	**N/A**
	%CV	**10.68%**	**1.92%**	**1.05%**	**N/A**
	% Bias	**0.17%**	**5.14%**	**2.65%**	**-12.05%**
BNZ_20220805B_CLR	2-1	29.05	82.51	77.62	343.22
	2-2	28.08	83.71	82.61	331.88
	2-3	27.83	84.20	81.63	319.18
	2-4	28.96	-	83.89	341.76
	2-5	-	-	-	345.25
Within Run	**Mean**	**28.48**	**83.47**	**81.44**	**336.26**
	SD	**0.62**	**0.87**	**2.71**	**10.85**
	%CV	**2.16%**	**1.04%**	**3.33%**	**3.23%**
	% Bias	**-5.07%**	**4.79%**	**-0.14%**	**-15.94%**
BNZ_20220810B_EC	3-1	29.83	83.52	84.54	354.30
	3-2	30.32	85.62	84.68	359.06
	3-3	28.95	85.09	83.46	369.90
	3-4	30.27	-	84.60	361.90
	3-5	-	-	-	354.00
Within Run	**Mean**	**29.84**	**84.74**	**84.32**	**359.83**
	SD	**0.63**	**1.09**	**0.58**	**6.54**
	%CV	**2.12%**	**1.29%**	**0.68%**	**1.82%**
	% Bias	**-0.53%**	**6.38%**	**3.40%**	**-10.04%**
BNZ_20220819B_CLR	4-1	32.89	-	-	360.31
	4-2	30.21	-	-	362.86
	4-3	29.20	-	-	360.21
Within Run	**Mean**	**30.77**			**361.13**
	SD	**1.91**			**1.50**
	%CV	**6.20%**			**0.42%**
	% Bias	**2.56%**			**-9.72%**

Mean		**29.70**	**83.99**	**83.16**	**351.12**
SD		**1.75**	**1.21**	**2.00**	**13.67**
Precision (%CV)	***Max Within-Run***	**10.68%**	**1.92%**	**3.33%**	**3.23%**
	Between-Run	**5.91%**	**1.44%**	**2.40%**	**3.89%**
% Bias		**-0.72%**	**5.44%**	**1.97%**	**-11.94%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification — LINEARITY

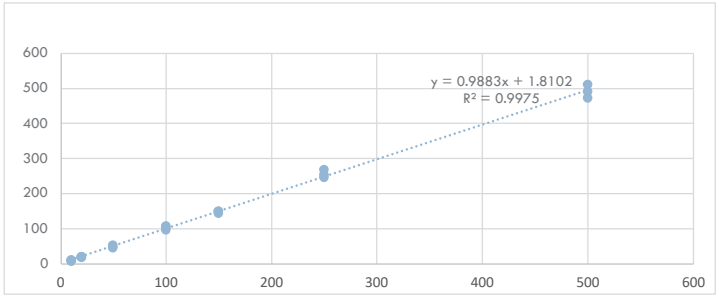
Analyte: Lorazepam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.81	-1.91
	20	18.49	-7.55
	50	52.91	5.82
	100	103.98	3.98
	150	149.86	-0.09
	250	253.87	1.55
	500	491.08	-1.78
BNZ_20220810B_EC	10	10.96	9.61
	20	20.29	1.46
	50	46.76	-6.48
	100	97.72	-2.28
	150	144.13	-3.91
	250	247.92	-0.83
	500	512.22	2.44
BNZ_20220819B_CLR	10	8.49	-15.13
	20	20.72	3.59
	50	50.55	1.10
	100	108.79	8.79
	150	149.31	-0.46
	250	268.36	7.34
	500	473.78	-5.24

Slope	0.9883
Std err in slope, S_b	0.0115
Degrees freedom	19
Confidence level	95%
Student t	2.09302
Confidence interval	0.024
Slope	0.9883 ± 0.024
Range	0.9643 - 1.0122
	YES

Intercept	1.8102
Std err in Intercept	2.5542
Degrees freedom	19
Confidence Level	95%
Student t	2.09302
Confidence interval	5.346
Intercept	1.8102 ± 5.346
Lower	-3.5358 - 7.1561
	YES



Comments: N/A

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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*84.30*	*85.48*	*400*
BNZ_20220726B_JR	1-1	29.35	85.47	83.76	422.51
	1-2	32.31	87.62	88.09	791.68*
	1-3	29.09	85.23	87.54	0*
	1-4	-	-	86.89	-
Within Run	**Mean**	**30.25**	**86.11**	**86.57**	**422.51**
	SD	**1.79**	**1.32**	**1.94**	**N/A**
	%CV	**5.91%**	**1.53%**	**2.24%**	**N/A**
	% Bias	**0.83%**	**2.14%**	**1.28%**	**5.63%**
BNZ_20220805B_CLR	2-1	31.29	95.27	88.80	408.89
	2-2	30.42	91.11	92.23	372.80
	2-3	32.18	88.56	90.00	385.52~
	2-4	32.16	-	91.61	411.73
	2-5	-	-	-	409.83
Within Run	**Mean**	**31.51**	**91.65**	**90.66**	**400.81**
	SD	**0.84**	**3.39**	**1.56**	**18.71**
	%CV	**2.66%**	**3.70%**	**1.72%**	**4.67%**
	% Bias	**5.04%**	**8.71%**	**6.06%**	**0.20%**
BNZ_20220810B_EC	3-1	31.05	84.06	86.53	401.56
	3-2	32.59	82.66	82.96	416.79
	3-3	30.70	85.30	83.84	411.48
	3-4	32.29	-	84.65	410.31
	3-5	-	-	-	392.02
Within Run	**Mean**	**31.66**	**84.01**	**84.50**	**406.43**
	SD	**0.92**	**1.32**	**1.52**	**9.74**
	%CV	**2.91%**	**1.58%**	**1.80%**	**2.40%**
	% Bias	**5.53%**	**-0.35%**	**-1.15%**	**1.61%**
BNZ_20220819B_CLR	4-1	30.49	-	-	400.42
	4-2	31.73	-	-	413.67
	4-3	30.37	-	-	402.29
Within Run	**Mean**	**30.86**			**405.46**
	SD	**0.75**			**7.17**
	%CV	**2.44%**			**1.77%**
	% Bias	**2.88%**			**1.36%**

Mean		**31.14**	**87.25**	**87.24**	**405.72**
SD		**1.12**	**3.93**	**3.08**	**12.63**
Precision (%CV)	***Max Within-Run***	**5.91%**	**3.70%**	**2.24%**	**4.67%**
	Between-Run	**3.61%**	**4.50%**	**3.53%**	**3.11%**
% Bias		**3.57%**	**3.50%**	**2.06%**	**2.20%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. ~IR failure. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification

LINEARITY

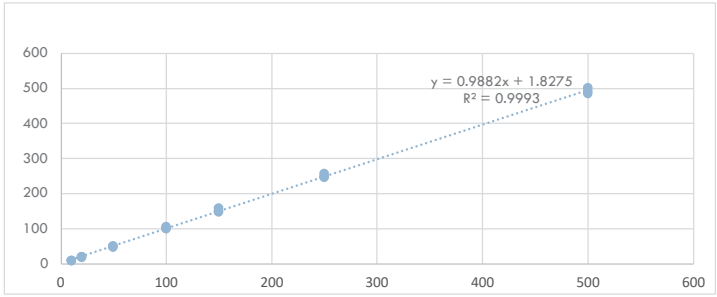
Analyte: Alprazolam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.57	-4.30
	20	19.05	-4.76
	50	51.33	2.66
	100	106.16	6.16
	150	149.38	-0.41
	250	258.70	3.48
	500	485.81	-2.84
BNZ_20220810B_EC	10	9.91	-0.87
	20	20.72	3.59
	50	48.54	-2.92
	100	101.34	1.34
	150	148.04	-1.31
	250	249.37	-0.25
	500	502.08	0.42
BNZ_20220819B_CLR	10	9.01	-9.93
	20	21.30	6.52
	50	49.34	-1.32
	100	101.47	1.47
	150	158.73	5.82
	250	247.05	-1.18
	500	493.09	-1.38

Slope	0.9882
Std err in slope, S_b	0.0062
Degrees freedom	19
Confidence level	95%
Student t	2.09302
Confidence interval	0.013
Slope	0.9882 ± 0.0129
Range	0.9753 - 1.0011
	YES

Intercept	1.8275
Std err in Intercept	1.3747
Degrees freedom	19
Confidence Level	95%
Student t	2.09302
Confidence interval	2.877
Intercept	1.8275 ± 2.8773
Lower	-1.0498 - 4.7047
	YES



Comments: N/A

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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*86.35*	*86.66*	*400*
BNZ_20220726B_JR	1-1	30.28	86.26	88.03	410.57
	1-2	32.18	89.57	89.90	815.87*
	1-3	30.02	88.74	90.07	0*
	1-4	-	-	89.65	-
Within Run	**Mean**	**30.83**	**88.19**	**89.41**	**410.57**
	SD	**1.18**	**1.72**	**0.94**	**N/A**
	%CV	**3.83%**	**1.95%**	**1.05%**	**N/A**
	% Bias	**2.76%**	**2.13%**	**3.18%**	**2.64%**
BNZ_20220805B_CLR	2-1	30.50	91.95	85.38	408.57
	2-2	30.54	88.31	94.05	352.06
	2-3	35.90~	90.97	93.88	369.28
	2-4	32.45	-	94.43	414.91
	2-5	-	-	-	395.73
Within Run	**Mean**	**31.16**	**90.41**	**91.94**	**388.11**
	SD	**1.11**	**1.88**	**4.38**	**26.69**
	%CV	**3.58%**	**2.08%**	**4.76%**	**6.88%**
	% Bias	**3.88%**	**4.70%**	**6.09%**	**-2.97%**
BNZ_20220810B_EC	3-1	30.60	86.53	85.32	396.55
	3-2	32.15	84.45	86.83	398.46
	3-3	31.22	85.37	87.72	402.64
	3-4	32.02	-	87.44	397.38
	3-5	-	-	-	385.19
Within Run	**Mean**	**31.50**	**85.45**	**86.83**	**396.04**
	SD	**0.73**	**1.04**	**1.07**	**6.50**
	%CV	**2.30%**	**1.22%**	**1.23%**	**1.64%**
	% Bias	**4.99%**	**-1.04%**	**0.19%**	**-0.99%**
BNZ_20220819B_CLR	4-1	32.57	-	-	389.58
	4-2	31.88	-	-	407.57
	4-3	30.11	-	-	411.04
Within Run	**Mean**	**31.52**			**402.73**
	SD	**1.27**			**11.52**
	%CV	**4.03%**			**2.86%**
	% Bias	**5.07%**			**0.68%**

Mean		**31.27**	**88.02**	**89.39**	**395.68**
SD		**0.96**	**2.56**	**3.24**	**17.41**
Precision	***Max Within-Run***	**4.03%**	**2.08%**	**4.76%**	**6.88%**
(%CV)	***Between-Run***	**3.07%**	**2.90%**	**3.63%**	**4.40%**
% Bias		**4.17%**	**1.93%**	**3.15%**	**-0.16%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. ~IRs out. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification — LINEARITY

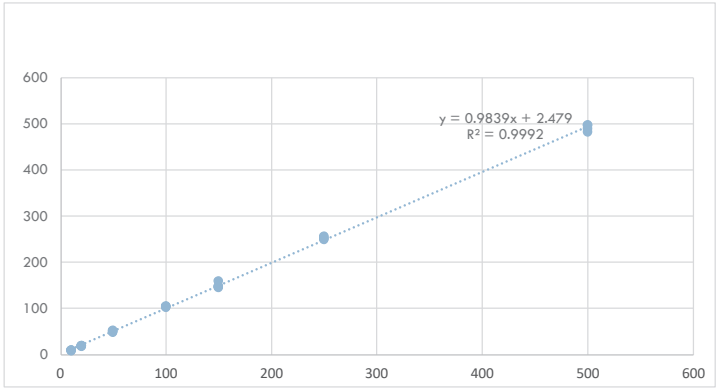
Analyte: Temazepam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.56	-4.39
	20	19.17	-4.16
	50	52.20	4.40
	100	104.83	4.83
	150	148.04	-1.31
	250	256.87	2.75
	500	489.32	-2.14
BNZ_20220805B_CLR	10	9.64	-3.58
	20	18.88	-5.62
	50	53.52	7.04
	100	105.67	5.67
	150	145.46	-3.02
	250	250.79	0.32
	500	496.04	-0.79
BNZ_20220810B_EC	10	9.66	-3.41
	20	20.53	2.67
	50	49.35	-1.29
	100	103.13	3.13
	150	148.54	-0.97
	250	250.53	0.21
	500	498.24	-0.35
BNZ_20220819B_CLR	10	9.16	-8.40
	20	19.56	-2.19
	50	51.01	2.01
	100	104.02	4.02
	150	159.36	6.24
	250	254.71	1.89
	500	482.17	-3.57

Slope	0.9839
Std err in slope, S_b	0.0054
Degrees freedom	26
Confidence level	95%
Student t	2.05553
Confidence interval	0.011
Slope	0.9839 ± 0.0111
Range	0.9728 - 0.9950
	NO

Intercept	2.4790
Std err in Intercept	1.2056
Degrees freedom	26
Confidence Level	95%
Student t	2.05553
Confidence interval	2.478
Intercept	2.479 ± 2.4781
Lower	0.0009 - 4.9571
	NO



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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*84.05*	*83.67*	*400*
BNZ_20220726B_JR	1-1	28.28	83.75	83.93	388.14
	1-2	32.92	85.37	84.79	744.72*
	1-3	28.63	85.31	86.24	0*
	1-4	-	-	86.19	-
Within Run	**Mean**	**29.94**	**84.81**	**85.29**	**388.14**
	SD	**2.58**	**0.92**	**1.13**	**N/A**
	%CV	**8.63%**	**1.08%**	**1.32%**	**N/A**
	% Bias	**-0.19%**	**0.90%**	**1.93%**	**-2.97%**
BNZ_20220805B_CLR	2-1	29.14	85.75	85.33	379.27
	2-2	28.82	86.73	88.60	364.76
	2-3	28.78	87.74	88.90	354.86
	2-4	30.29	-	89.15	384.29
	2-5	-	-	-	383.72
Within Run	**Mean**	**29.26**	**86.74**	**88.00**	**373.38**
	SD	**0.71**	**1.00**	**1.79**	**13.02**
	%CV	**2.42%**	**1.15%**	**2.04%**	**3.49%**
	% Bias	**-2.48%**	**3.20%**	**5.17%**	**-6.66%**
BNZ_20220810B_EC	3-1	28.75	82.01	83.05	377.38
	3-2	29.83	82.57	83.41	383.70
	3-3	28.92	83.41	82.88	385.86
	3-4	29.71	-	85.04	380.49
	3-5	-	-	-	374.24
Within Run	**Mean**	**29.30**	**82.66**	**83.60**	**380.33**
	SD	**0.55**	**0.71**	**0.99**	**4.68**
	%CV	**1.87%**	**0.86%**	**1.18%**	**1.23%**
	% Bias	**-2.32%**	**-1.65%**	**-0.09%**	**-4.92%**
BNZ_20220819B_CLR	4-1	30.87	-	-	392.68
	4-2	29.78	-	-	397.42
	4-3	29.03	-	-	394.11
Within Run	**Mean**	**29.89**			**394.74**
	SD	**0.93**			**2.43**
	%CV	**3.10%**			**0.62%**
	% Bias	**-0.36%**			**-1.32%**

Mean		**29.55**	**84.74**	**85.63**	**381.49**
SD		**1.20**	**1.92**	**2.25**	**11.38**
Precision	***Max Within-Run***	**8.63%**	**1.15%**	**2.04%**	**3.49%**
	Between-Run	**4.08%**	**2.27%**	**2.63%**	**2.98%**
% Bias		**-1.34%**	**0.82%**	**2.34%**	**-3.96%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

Verification

LINEARITY

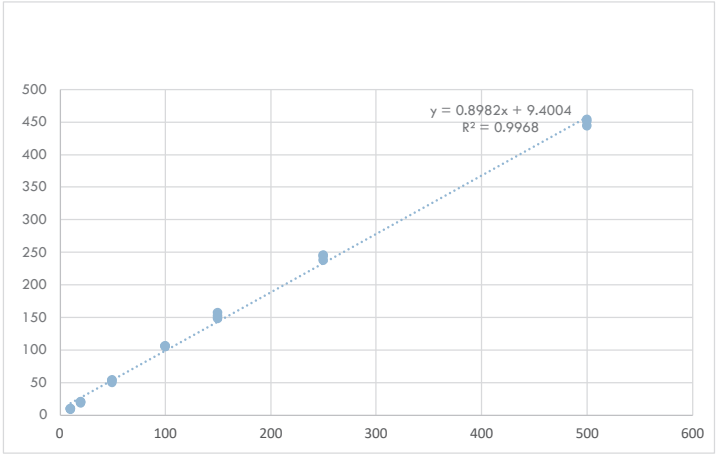
Analyte: Diazepam
Units: ng/mL
Instrument: LCMS-1

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	%Difference
BNZ_20220726B_JR	10	9.88	-1.24
	20	19.81	-0.94
	50	53.28	6.55
	100	106.83	6.83
	150	148.84	-0.77
	250	246.54	-1.38
	500	454.71	-9.06
BNZ_20220805B_CLR	10	9.92	-0.80
	20	19.44	-2.80
	50	54.23	8.47
	100	105.79	5.79
	150	157.52	5.01
	250	238.51	-4.60
	500	444.65	-11.07
BNZ_20220810B_EC	10	9.69	-3.13
	20	20.93	4.66
	50	50.62	1.24
	100	106.92	6.92
	150	153.19	2.13
	250	244.74	-2.10
	500	451.49	-9.70
BNZ_20220819B_CLR	10	9.81	-1.90
	20	20.47	2.33
	50	50.92	1.83
	100	103.15	3.15
	150	155.63	3.75
	250	247.71	-0.92
	500	458.74	-8.25

Slope	0.9032
Std err in slope, S_b	0.0099
Degrees freedom	26
Confidence level	95%
Student t	2.05553
Confidence interval	0.020
Slope	0.9032 ± 0.0203
Range	0.8829 - 0.9235
	NO

Intercept	9.0004
Std err in Intercept	2.2009
Degrees freedom	26
Confidence Level	95%
Student t	2.05553
Confidence interval	4.524
Intercept	9.0004 ± 4.5241
Lower	4.4763 - 13.5244
	NO



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

Verification

BIAS AND IMPRECISION

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
Matrix: Blood

Run Date	Run Order	LQC	Utak C5022	UTAK D1323	HQC
	Target Concentration (ng/mL):	*30*	*83.73*	*85.86*	*400*
BNZ_20220726B_JR	1-1	31.46	86.05	86.37	386.93
	1-2	34.29	88.46	87.52	716.00*
	1-3	31.48	87.79	88.04	0*
	1-4	-	-	87.96	-
Within Run	**Mean**	**32.41**	**87.43**	**87.47**	**386.93**
	SD	**1.63**	**1.24**	**0.77**	**N/A**
	%CV	**5.02%**	**1.42%**	**0.88%**	**N/A**
	% Bias	**8.03%**	**4.42%**	**1.88%**	**-3.27%**
BNZ_20220805B_CLR	2-1	31.31	87.33	85.10	388.74
	2-2	31.14	86.91	88.76	412.67
	2-3	31.30	89.26	88.55	400.39
	2-4	32.88	-	89.65	391.09
	2-5	-	-	-	393.25
Within Run	**Mean**	**31.66**	**87.83**	**88.02**	**397.23**
	SD	**0.82**	**1.25**	**2.00**	**9.67**
	%CV	**2.59%**	**1.43%**	**2.27%**	**2.43%**
	% Bias	**5.53%**	**4.90%**	**2.51%**	**-0.69%**
BNZ_20220810B_EC	3-1	31.57	86.29	86.04	394.53
	3-2	33.27	87.30	87.96	397.62
	3-3	32.27	87.52	86.57	399.99
	3-4	32.84	-	88.69	390.32
	3-5	-	-	-	387.34
Within Run	**Mean**	**32.49**	**87.04**	**87.32**	**393.96**
	SD	**0.74**	**0.66**	**1.22**	**5.17**
	%CV	**2.27%**	**0.76%**	**1.40%**	**1.31%**
	% Bias	**8.29%**	**3.95%**	**1.69%**	**-1.51%**
BNZ_20220819B_CLR	4-1	33.33	-	-	413.99
	4-2	31.52	-	-	420.50
	4-3	31.49	-	-	413.17
Within Run	**Mean**	**32.11**			**415.89**
	SD	**1.05**			**4.02**
	%CV	**3.28%**			**0.97%**
	% Bias	**7.04%**			**3.97%**

Mean		**32.15**	**87.43**	**87.60**	**399.32**
SD		**0.99**	**1.00**	**1.33**	**11.29**
Precision	***Max Within-Run***	**5.02%**	**1.43%**	**2.27%**	**2.43%**
	Between-Run	**3.09%**	**1.15%**	**1.51%**	**2.83%**
% Bias		**7.22%**	**4.42%**	**2.03%**	**-0.37%**

Comments: Values in red outside of acceptable range. HQCs 1-2 and 1-3 fortified incorrectly and will not be used for calculations. HQC SD for day 1 not included in Max Within-Run Precision calculation.

Acceptance Criteria: **Bias: ≤ ±20%; Within-Run Imprecision: CV ≤ ±20%; Between-Run Imprecision: CV ≤ ±20%**

SUMMARY OF VERIFICATION PERFORMANCE

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

Analyst: JR, CLR, EC
Study Dates: 7/26/2022 to 8/19/2022
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 verification.

Failed Runs (include dates/reasons):

Date	Reason
8/5/2022	Due to bubbles in injections, calibration range for 7-aminoclonazepam was limited to 50-500 ng/mL, so data could not be used for linearity or for LQC or UTAK replicates. Calibrator 2 was also dropped for alpha-hydroxyalprazolam, clonazepam, lorazepam and alprazolam so linearity data will not be used for these analytes.
8/19/2022	UTAK replicates outside acceptable range for all analytes, likely due to issue with bottle or lack of full dissolvation. No UTAK data used.

Other Observations: Lot numbers acceptable for use in casework:
Calibrators: 220726C-C-0.1, 220726C-C-1, 220726C-C-5
Quality Controls: 220722K-Q-1, 220722C-Q-1AC, 220726K-Q-5/2.5

Sample Preparation Steps: Refer to Toxicology Analytical Manual v3.8, "Benzodiazepines 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: New calibrator and QC solutions are acceptable for use in BNZ casework.