Table 1 - Transitions for Waters Xevo TQD Mass Spectrometer

Ion transitions (Quantitation ions in **Bold)**					
Compound	Precursor ion	Product Ions	Cone (V)	Collision (eV)	approximate RT (min)
Nordiazepam	271	**140**	18	28	3.93
		165			
D5-Nordiazepam	276	**140**	59	30	3.90
		165		26	
Nitrazepam	282	180	45	36	3.29
		207		34	
7-amino flunitrazepam	284	135	2	28	1.67
		227		24	
Diazepam	285	**154**	2	26	4.19
		193		32	
7-amino clonazepam	286	**121**	14	30	1.25
		222		24	
N-chlordiazepoxide	286	227	34	22	2.48
		232		34	
Oxazepam	287	104	48	40	3.71
		241		24	
Demoxepam	287	104	52	22	3.22
		180		20	
D4-7 amino clonazepam	290	**121**	28	32	1.22
		226		24	

D5-Diazepam	290	154	4	28	4.16
		198		32	
D7-7 amino flunitrazepam	291	230	92	30	1.63
		138	90	26	
D5-Oxazepam	292	**246**	38	18	3.69
		274		14	
Estazolam	295	205	6	38	3.57
		267		24	
Nimetazepam	296	221	40	34	3.50
		268		22	
Chlordiazepoxide	300	192	25	30	2.70
		227		24	
D5-Estazolam	300	210	14	42	3.55
		272		24	
Temazepam	301	177	24	40	3.87
		255	14	16	
Clobazam	301	224	40	34	3.63
		259		20	
Delorazepam	305	140	59	30	4.00
		206		34	
D5-Temazepam	306	**177**	38	40	3.86
		198		34	
Zaleplon	306	236	50	26	2.94
		264		20	

Zolpidem	308	**235**	58	40	1.9
		263		25	
Alprazolam	309	**205**	45	42	3.73
		274		24	
D5-alprazolam	314	210	64	40	3.71
		286		28	
Flunitrazepam	314	183	45	50	3.42
		239		34	
D7-Zolpidem	315	**242**	58	40	1.87
		270		25	
Clonazepam	316	214	52	40	3.36
		270		24	
Bromazepam	316	182	24	32	3.07
		209		26	
Diclazepam	319	154	40	30	4.25
		227		30	
D4-Clonazepam	320	218	52	36	3.35
		274		22	
Lorazepam	321	229	40	30	3.71
		275		20	
8-aminoclonazolam	324.1858	146.1175	54	30	2.1
		220.06	79	38	

a-OH alprazolam	325	216	42	38	3.54
		297		26	
Midazolam	326	223	4	36	2.76
		291		26	
Flualprazolam	327	223	62	40	3.60
		292		26	
D5-a-OH-alprazolam	330	221	62	38	3.52
		302		24	
OH ethyl flurazepam	333	109	52	26	3.71
		211		36	
Flubromazepam	333	184	12	28	3.93
		226		32	
Lormetazepam	335	177	39	42	3.95
		227		36	
Zolpidem COOH*	338.1819	265.2802	88	34	0.9
		293.178		26	
Triazolam	343	239	45	40	3.72
		308		26	
Etizolam	343	138	6	36	3.87
		314		24	
Phenazepam	348.8	179	45	50	4.10
		206		34	

Adinazolam	352.2158	205.1315	46	50	2.51
		295.1714		22	
Bromazolam	353.1258	325.107	40	24	3.83
		205.0881		44	
Pyrazolam	353.92	167	45	34	2.75
		206		30	
Clonazolam	354.2	280	10	34	3.26
		326		26	
a-OH etizolam	359.2	315	2	20	2.80
		282		24	
a-OH triazolam	359.2	277	12	34	3.46
		331		28	
alpha-OH clonazolam	370.1558	296.1534	36	36	2.87
		342.1462		26	
Flubromazolam	371	223	12	44	3.69
		292		26	
alpha-OH flubromazolam	387.1058	223.1588	50	48	3.44
		359.1074		28	
Flurazepam	388	288	40	24	2.85
		315		24	
Zopiclone	389	217	22	36	1.32
		245		14	

*The zolpidem metabolite does not extract

Table 2 - Gradient on H-class Acquity UPLC with Xevo TQD

Time (minutes)	% Water	%MeOH	Formic acid (2%)	Curve
Initial	65	30	5	6
2.50	30	65	5	6
3.25	25	70	5	6
4.50	18	77	5	6
4.51	5	90	5	6
4.80	5	90	5	6
5.91	65	30	5	6
9.00	65	30	5	6

Table 3 - Gradient on I-class Acquity UPLC with Xevo TQs Micro

Time (minutes)	% Water with 0.1% Formic acid	%MeOH with 0.1% Formic acid	Curve
Initial	65	35	6
0.76	65	35	6
3.26	30	70	6
4.01	25	75	6
5.26	18	82	6
5.27	5	95	6
6.56	5	95	6
6.67	65	35	6
9.76	65	35	6

Alprazolam (diagrams 1-3)

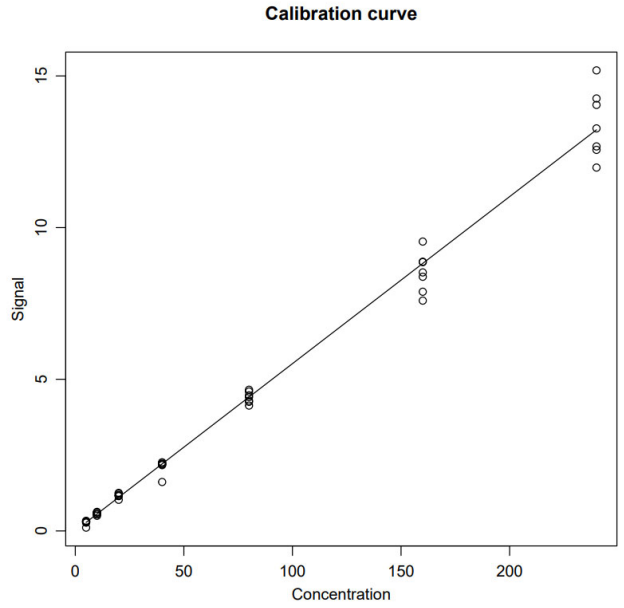
F-test for heteroscedasticity
p-value: 1.019e-06
Weighting needed: Yes

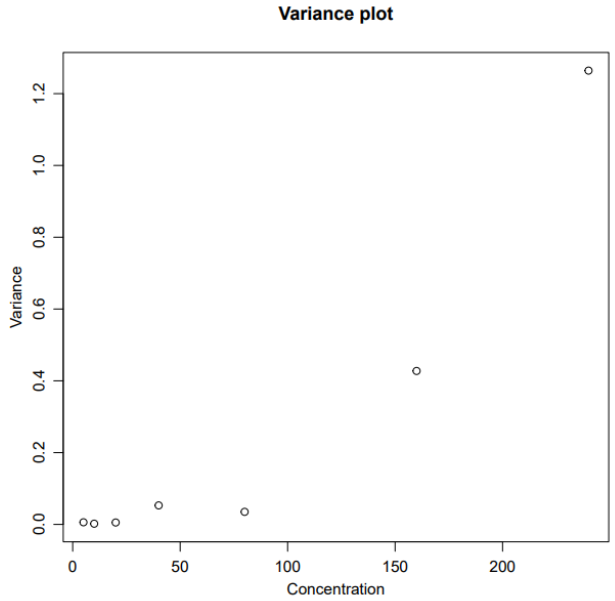
Variance test for weight selection
Scores: No weight: 0.000552 x^(-1): 5.17e-06 x^(-2): 1.66e-06
Selected weight: x^(-2)

Partial F-test for model order selection
p-value: 0.786
Model selected: linear

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.739
Validation test passed: Yes

Model selected: Linear, 1/x^2
Calibration equation:
0.05507 x + 0.007569





7-Amino-clonazepam (diagrams 4-6)

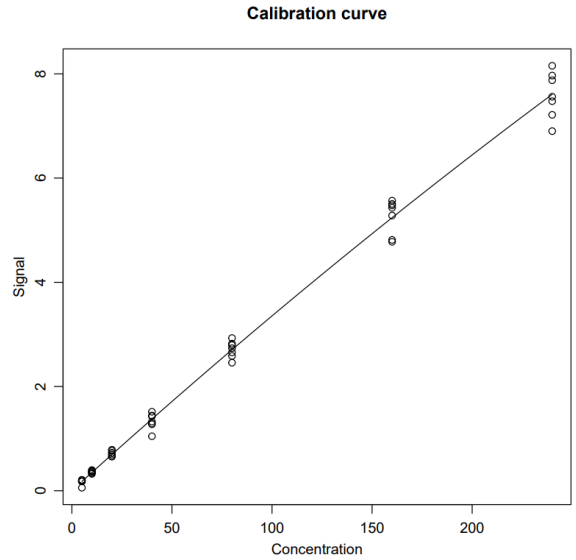
F-test for heteroscedasticity
p-value: 2.397e-05
Weighting needed: Yes

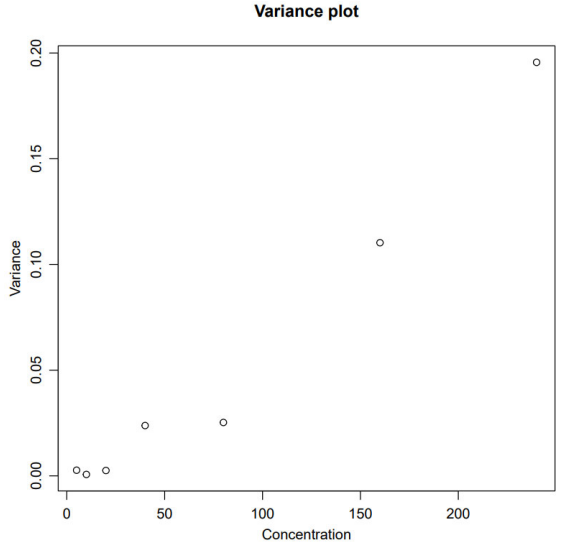
Variance test for weight selection
Scores: No weight: 1.38e-05 x^(-1): 1.27e-07 x^(-2): 3.47e-07
Selected weight: x^(-1)

Partial F-test for model order selection
p-value: 0.01687
Model selected: quadratic

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.994
Validation test passed: Yes

Model selected: Quadratic, 1/x
Calibration equation:
-1.309e-05 x^2 + 0.03482 x + 0.004417





Clonazepam (diagrams 7-9)

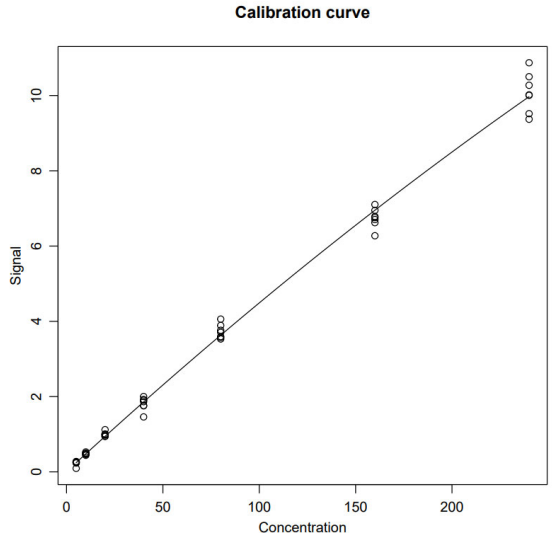
F-test for heteroscedasticity
p-value: 2.624e-05
Weighting needed: Yes

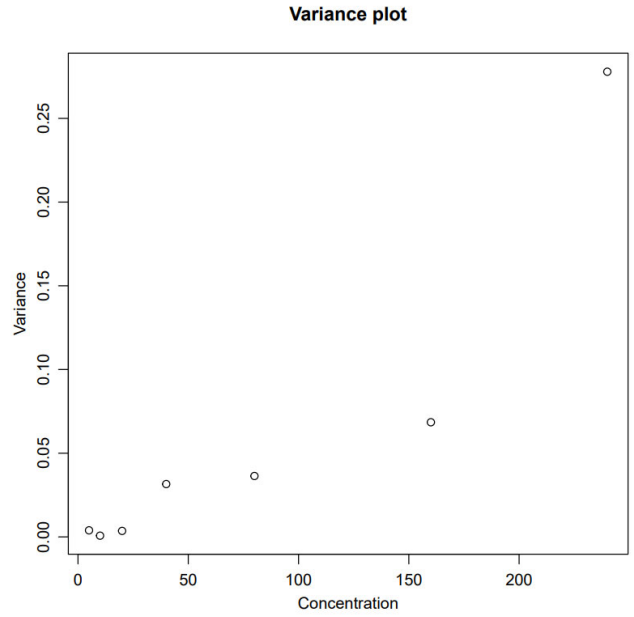
Variance test for weight selection
Scores: No weight: 2.45e-05 x^(-1): 2.26e-07 x^(-2): 7.6e-07
Selected weight: x^(-1)

Partial F-test for model order selection
p-value: 0.0005204
Model selected: quadratic

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.615
Validation test passed: Yes

Model selected: Quadratic, 1/x
Calibration equation:
-2.359e-05 x^2 + 0.04717 x + 0.008825





Diazepam (diagrams 10-12)

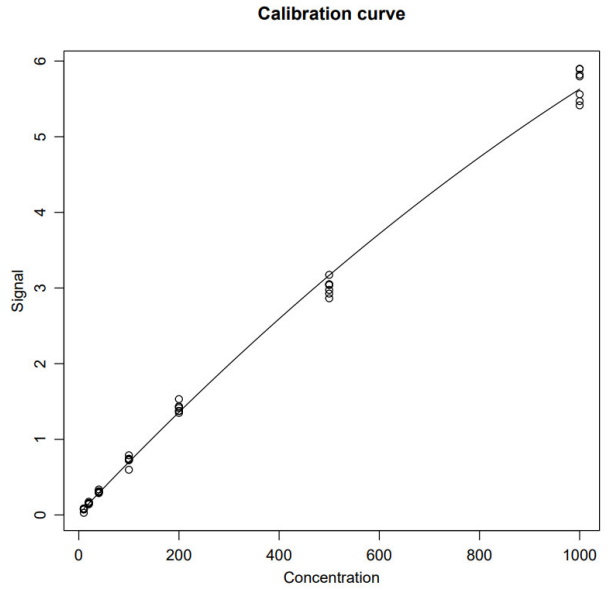
F-test for heteroscedasticity
p-value: 8.61e-06
Weighting needed: Yes

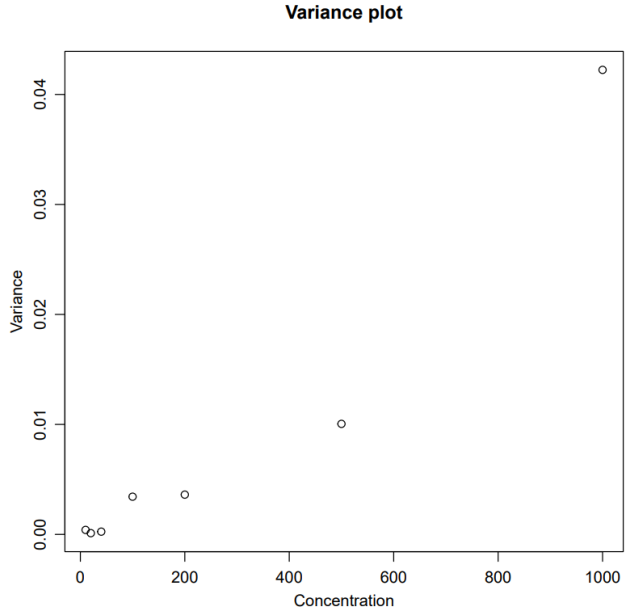
Variance test for weight selection
Scores: No weight: 5.81e-07 x^(-1): 1.78e-09 x^(-2): 9.54e-09
Selected weight: x^(-1)

Partial F-test for model order selection
p-value: 2.27e-10
Model selected: quadratic

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.976
Validation test passed: Yes

Model selected: Quadratic, 1/x
Calibration equation:
-1.39e-06 x^2 + 0.007008 x + 0.01164





Etizolam (diagrams 13-15)

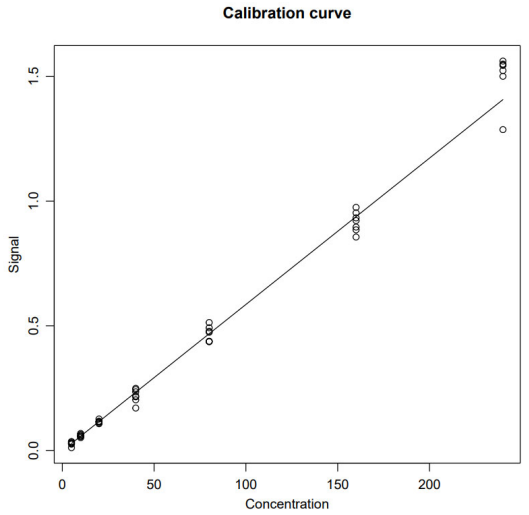
F-test for heteroscedasticity
p-value: 3.552e-06
Weighting needed: Yes

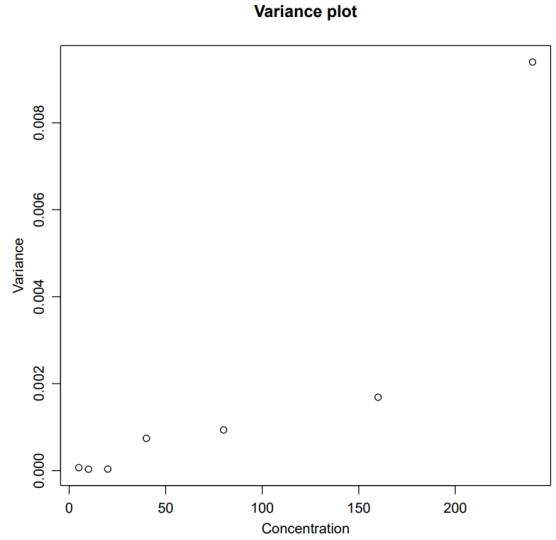
Variance test for weight selection
Scores: No weight: 2.87e-08 x^(-1): 2.44e-10 x^(-2): 2.16e-10
Selected weight: x^(-2)

Partial F-test for model order selection
p-value: 0.2089
Model selected: linear

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.475
Validation test passed: Yes

Model selected: Linear, 1/x^2
Calibration equation:
0.005871 x + -0.001595





Lorazepam (diagrams 16-18)

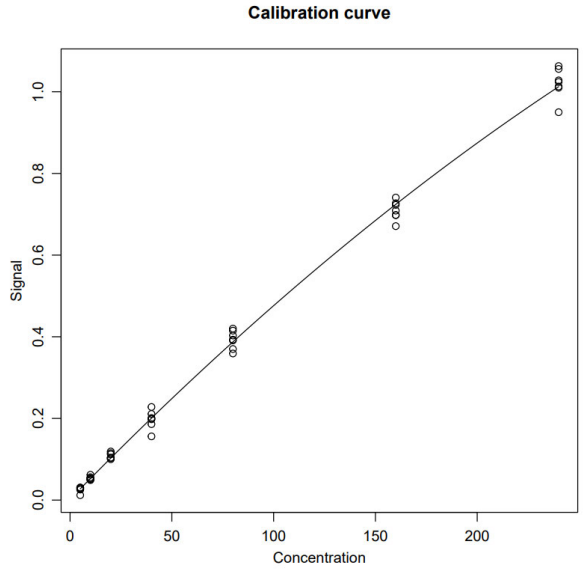
F-test for heteroscedasticity
p-value: 0.0002552
Weighting needed: Yes

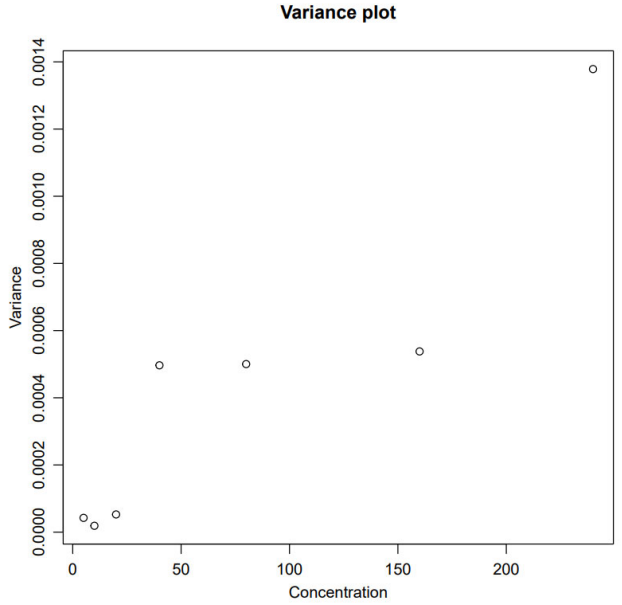
Variance test for weight selection
Scores: No weight: 5.76e-10 x^(-1): 2.14e-11 x^(-2): 8.71e-11
Selected weight: x^(-1)

Partial F-test for model order selection
p-value: 2.213e-07
Model selected: quadratic

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.793
Validation test passed: Yes

Model selected: Quadratic, 1/x
Calibration equation:
-3.797e-06 x^2 + 0.005122 x + 0.00192





Nordiazepam (diagrams 19-21)

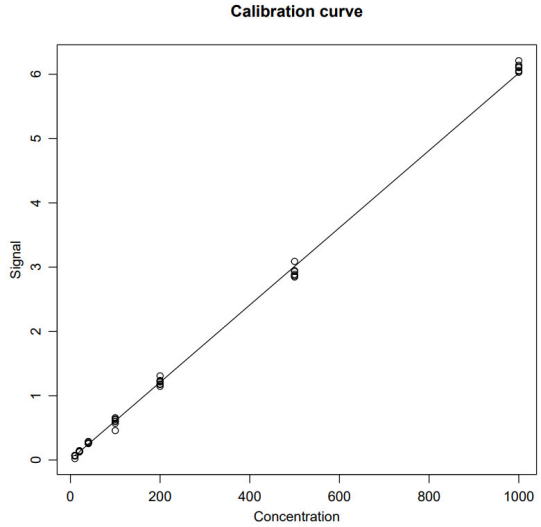
F-test for heteroscedasticity
p-value: 0.00267
Weighting needed: Yes

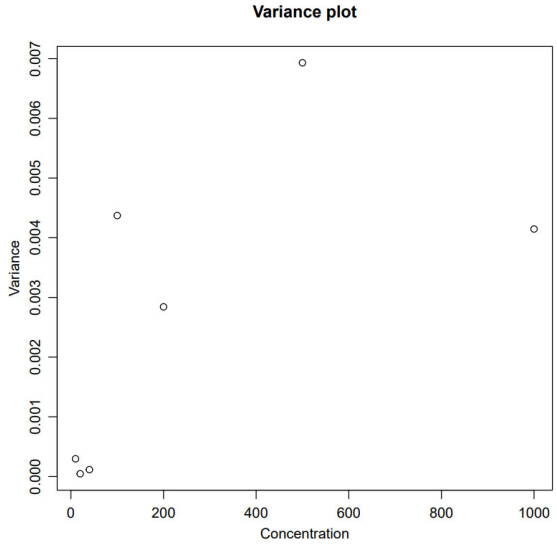
Variance test for weight selection
Scores: No weight: 1.76e-08 x^(-1): 1.83e-09 x^(-2): 5.07e-09
Selected weight: x^(-1)

Partial F-test for model order selection
p-value: 0.1122
Model selected: linear

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.993
Validation test passed: Yes

Model selected: Linear, 1/x
Calibration equation:
0.006011 x + 0.006496





Alpha-OH alprazolam (diagrams 22-24)

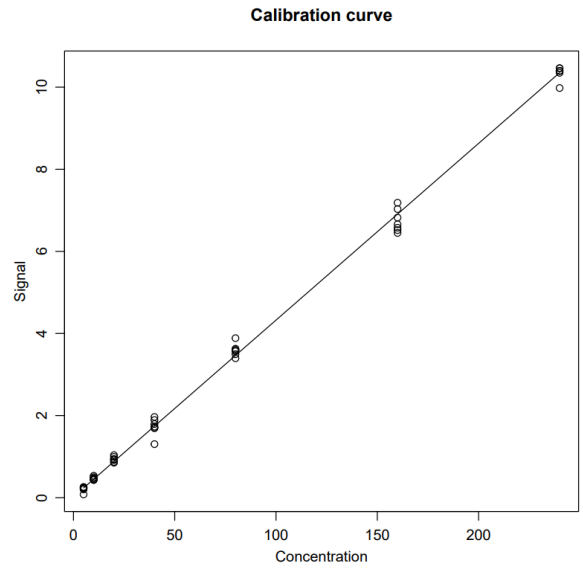
F-test for heteroscedasticity
p-value: 0.01446
Weighting needed: Yes

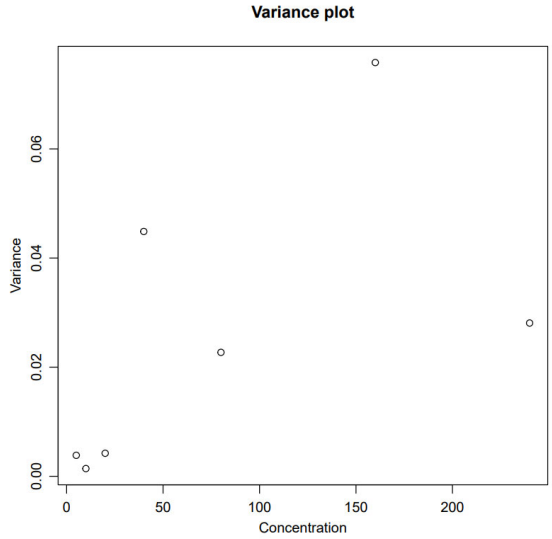
Variance test for weight selection
Scores: No weight: 1.84e-06 x^(-1): 2.19e-07 x^(-2): 7.28e-07
Selected weight: x^(-1)

Partial F-test for model order selection
p-value: 0.2206
Model selected: linear

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.813
Validation test passed: Yes

Model selected: Linear, 1/x
Calibration equation:
0.04305 x + 0.02011





Oxazepam (diagrams 25-27)

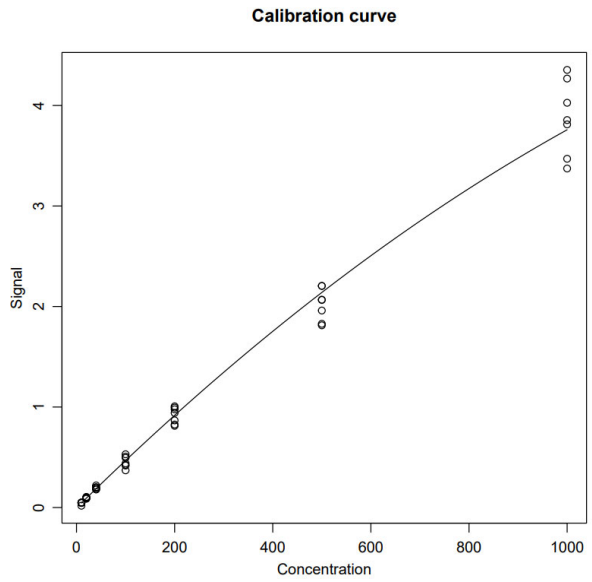
F-test for heteroscedasticity
p-value: 8.72e-09
Weighting needed: Yes

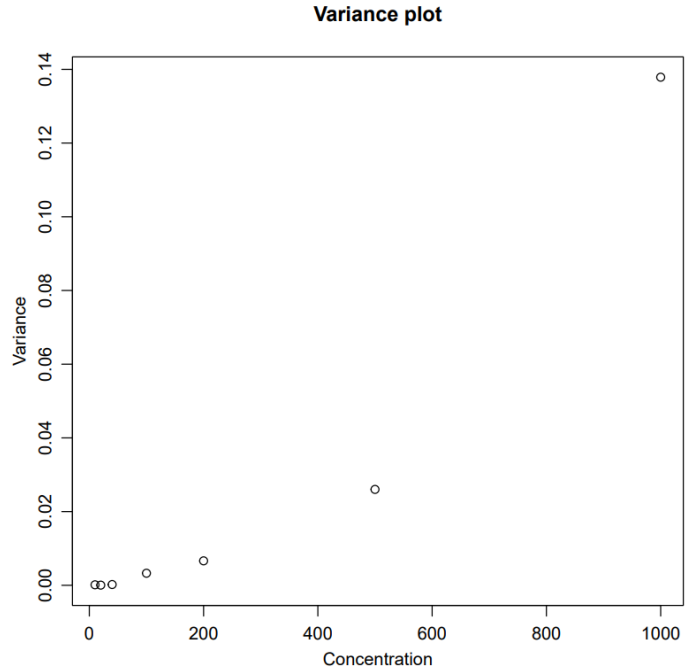
Variance test for weight selection
Scores: No weight: 6.42e-06 x^(-1): 1.65e-08 x^(-2): 8.54e-10
Selected weight: x^(-2)

Partial F-test for model order selection
p-value: 0.0007313
Model selected: quadratic

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.987
Validation test passed: Yes

Model selected: Quadratic, 1/x^2
Calibration equation:
-1.048e-06 x^2 + 0.004809 x + -0.003066





Temazepam (diagrams 28-30)

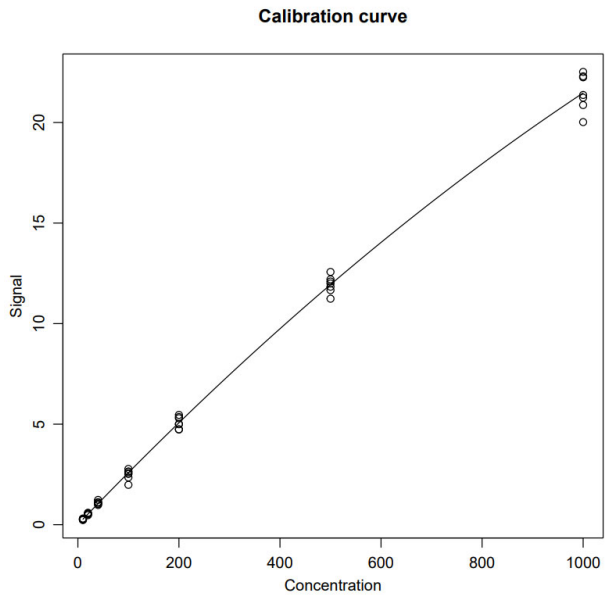
F-test for heteroscedasticity
p-value: 6.209e-09
Weighting needed: Yes

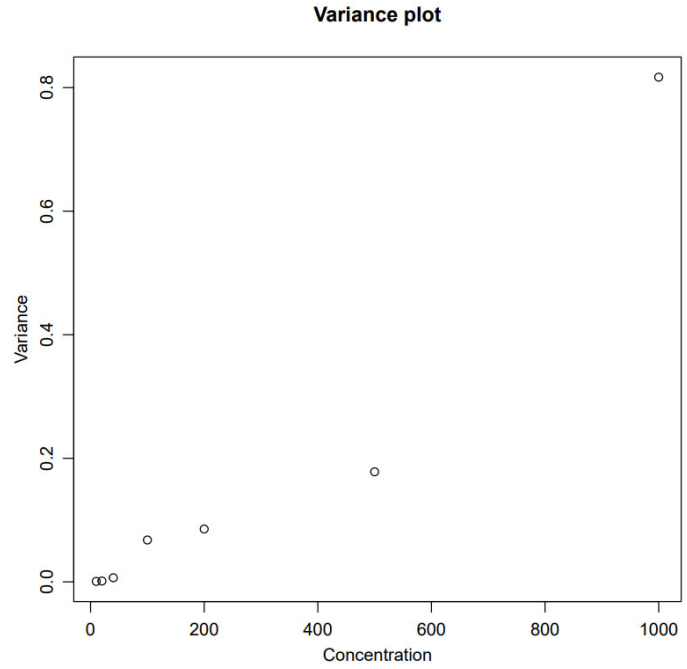
Variance test for weight selection
Scores: No weight: 0.000216 x^(-1): 6.51e-07 x^(-2): 2.89e-08
Selected weight: x^(-2)

Partial F-test for model order selection
p-value: 8.297e-06
Model selected: quadratic

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.904
Validation test passed: Yes

Model selected: Quadratic, 1/x^2
Calibration equation:
-4.792e-06 x^2 + 0.02625 x + 0.01081





Zolpidem (diagrams 31-33)

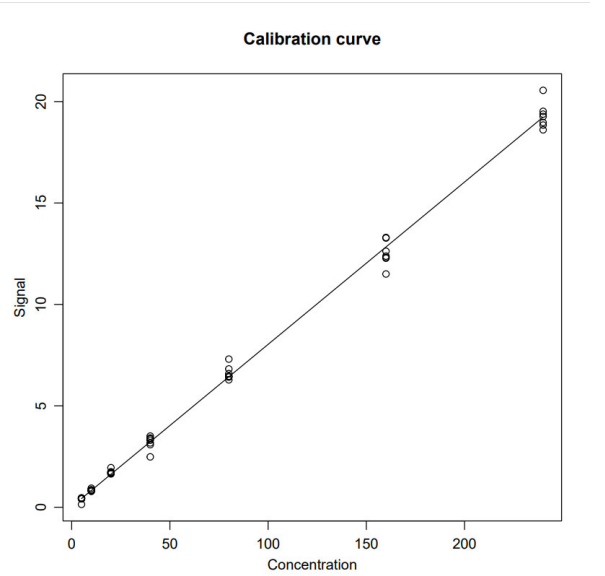
F-test for heteroscedasticity
p-value: 0.0002736
Weighting needed: Yes

Variance test for weight selection
Scores: No weight: 7.7e-05 x^(-1): 1.65e-06 x^(-2): 8.1e-06
Selected weight: x^(-1)

Partial F-test for model order selection
p-value: 0.4847
Model selected: linear

Normality of the standerdized residuals
Test used: Cramer von Mises
p-value: 0.564
Validation test passed: Yes

Model selected: Linear, 1/x
Calibration equation:
0.08003 x + 0.02695



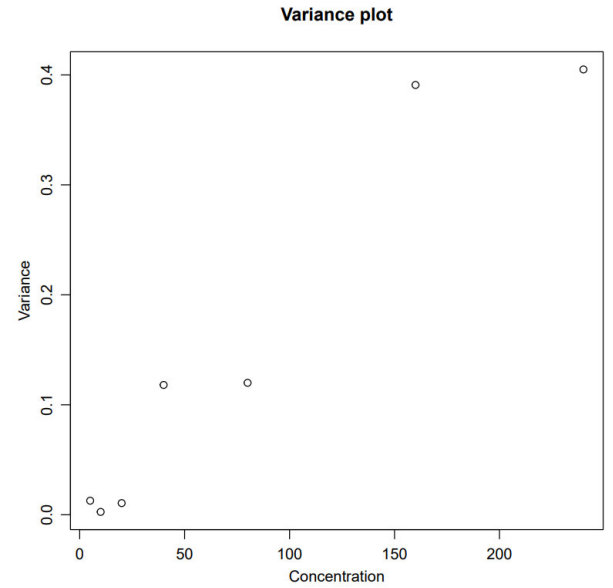


Table 4 – Summary of model order, weighting and calibration points for quantitative results

Drug	Calibration	Weighting	Quantitative range (ng/mL)	LLOQ/LOD (ng/mL)
7-amino clonazepam	quadratic	1/x	5-240	5
Alprazolam	linear	1/x^2		
Clonazepam	quadratic	1/x		
Etizolam	linear	1/x^2		
Lorazepam	quadratic	1/x		
Zolpidem	linear	1/x		
OH-Alprazolam	linear	1/x		
Diazepam	quadratic	1/x	10-1000	10
Nordiazepam	linear	1/x		
Oxazepam	quadratic	1/x^2		
Temazepam	quadratic	1/x^2		

Table 5 – Summary of Bias and Precision experiments. Red/black numbers just show the grouping of three different calculations

	7-amino clonazepam	Alprazolam	Clonazepam	Etizolam	Lorazepam	Zolpidem	a-OH-alprazolam	Diazepam	Nordiazepam	Oxazepam	Temazepam
Bias (low)	6.11	2.14	3.34	2.30	-0.93	4.95	2.75	5.41	13.60	9.07	3.94
Bias (middle)	4.90	1.30	3.47	1.52	-1.60	5.95	5.70	7.48	13.37	7.69	3.42
Bias(high)	1.01	0.56	1.60	7.11	-3.62	0.66	1.39	3.89	5.18	4.04	3.27
Within run CV (low)	4.39	5.52	3.38	5.43	6.35	4.38	5.42	4.23	2.48	4.02	4.93
Within run CV (middle)	4.04	3.39	3.07	5.37	3.56	2.70	4.56	3.42	2.67	2.71	4.84
Within run CV (high)	3.82	4.33	2.94	4.16	4.45	2.00	3.74	2.65	2.02	2.02	3.22
Between run CV (low)	9.79	8.15	5.59	15.64	8.06	9.53	14.40	8.60	4.96	8.30	6.87
Between run CV (middle)	6.48	4.06	5.05	10.24	8.62	7.48	9.43	7.30	5.21	7.35	5.93
Between run CV (high)	6.97	4.43	3.79	8.74	6.67	8.17	10.90	3.98	3.98	19.38	9.45

Table 6 – Summary of calibration, weighting, quantitative range, LOD and LLOQ.

Drug	Calibration	Weighting	Quantitative range (ng/mL)	LLOQ/LOD (ng/mL)
7-amino clonazepam	quadratic	1/x	5-240	5
Alprazolam	linear	1/x^2		
Clonazepam	quadratic	1/x		
Etizolam	quadratic	1/x		
Lorazepam	linear	1/x^2		
Zolpidem	quadratic	1/x		
OH-Alprazolam	linear	1/x		
Diazepam	quadratic	1/x^2	10-1000	10
Nordiazepam	quadratic	1/x^2		
Oxazepam	linear	1/x		
Temazepam	linear	1/x		

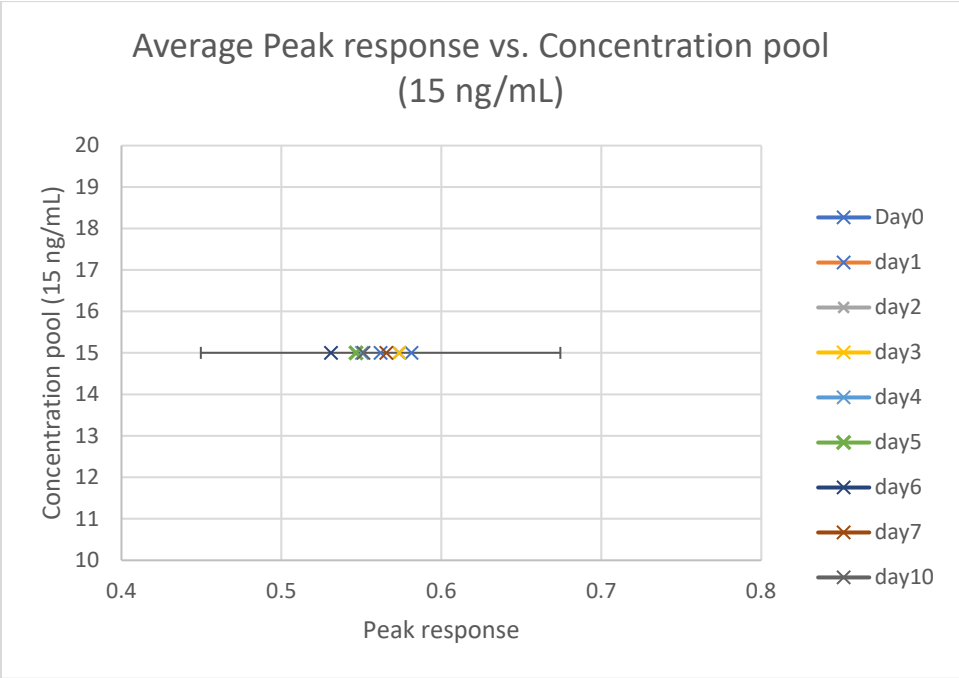
Table 7 - Estimation of ion suppression or enhancement of analytes in blood using post extraction addition approach.

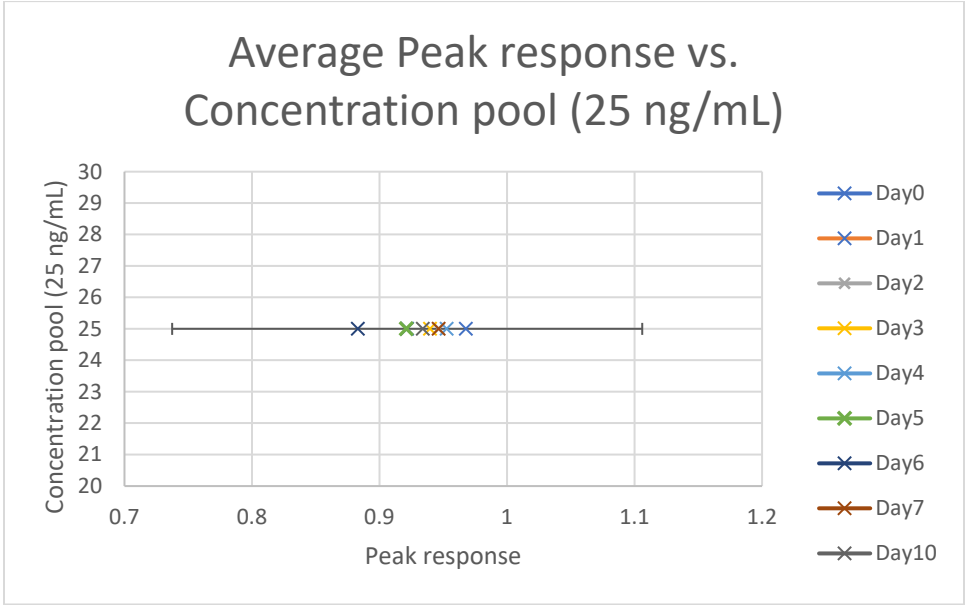
Drug/metabolite	Percent enhancement/suppression (low concentration)	Percent enhancement/suppression (high concentration)
Zopiclone	**-30**	**10**
Zolpidem	**-4**	**-8**
Zaleplon	**4**	**-27**
2-OH ethylflurazepam	**-21**	**-14**
7-aminoclonazepam	**-26**	**-31**
7-aminoflunitrazepam	**-19**	**-16**
Alprazolam	**-10**	**-11**
Bromazepam	**56**	**-16**
Clonazepam	**-24**	**-21**
Clonazolam	**-20**	**-20**
Diazepam	**-20**	**-8**
Estazolam	**-10**	**-13**
Etizolam	**-2**	**-8**
Flualprazolam	**-9**	**-20**
Flubromazepam	**-19**	**-11**
Flubromazolam	**-20**	**-9**
Lorazepam	**-15**	**-10**
Midazolam	**-11**	**-9**
Nordiazepam	**-18**	**-8**

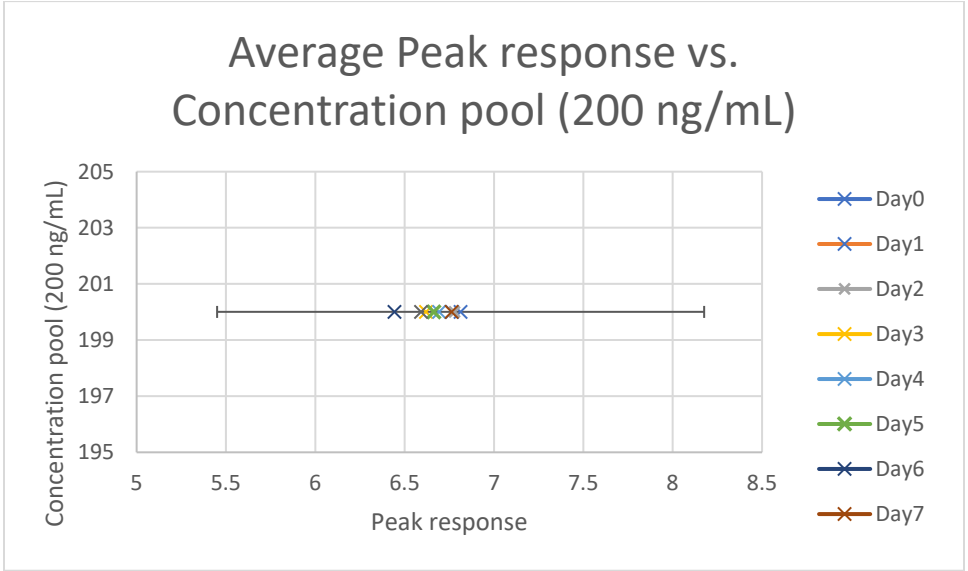
Oxazepam	**-15**	**-10**
Temazepam	**-5**	**-5**
a-OH-etizolam	**-10**	**-17**
a-OH-triazolam	**-15**	**-15**
a-OH-alprazolam	**-8**	**-15**
D7-Zolpidem	**0**	**-11**
D4- 7 aminoclonazepam	**-23**	**-29**
D4- clonazepam	**-15**	**-22**
D5-Diazepam	**-14**	**-17**
D5-Estazolam	**-4**	**-26**
D5-Temazepam	**0**	**-11**
D5-a-OH-alprazolam	**-1**	**-18**
D5- alprazolam	**-3**	**-13**
D7-7 aminoflunitrazepam	**-9**	**-16**
D5-Oxazepam	**-10**	**-18**
D5-Nordiazepam	**-5**	**-10**
Flunitrazepam	**-34**	**-22**
Triazolam	**-13**	**-10**
Chlordiazepoxide	**-10**	**-16**
Clobazam	**-4**	**-17**
Delorazepam	**-13**	**4**
Demoxepam	**0**	**4**
Diclazepam	**-8**	**6**
Flurazepam	**-22**	**-10**
Lormetazepam	**-4**	**8**

Nimetazepam	**-26**	**-23**
Nitrazepam	**-12**	**-11**
Norchlordiazepoxide	**-11**	**-28**
Phenazepam	**-15**	**-2**
Pyrazolam	**40**	**12**

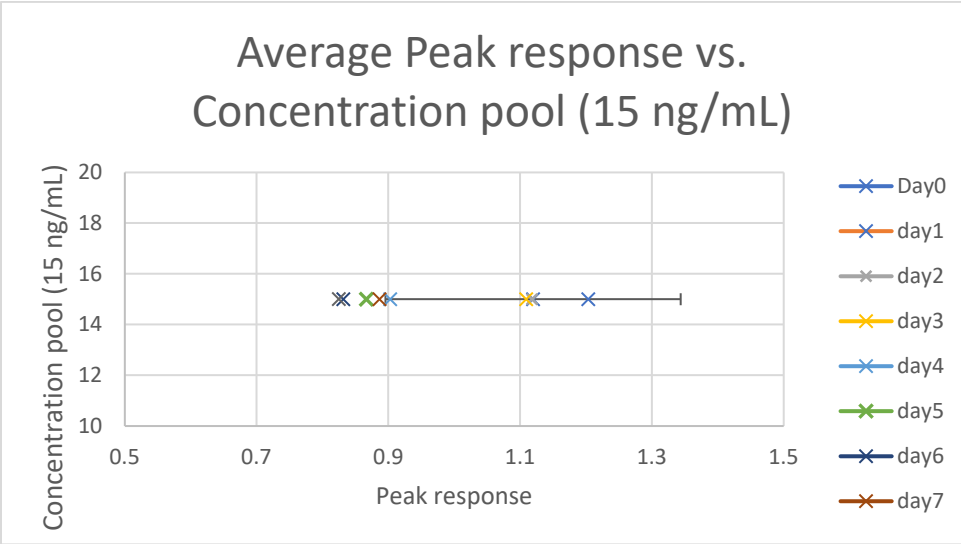
Stability Graphs 1-3 (blood): 7-amino clonazepam

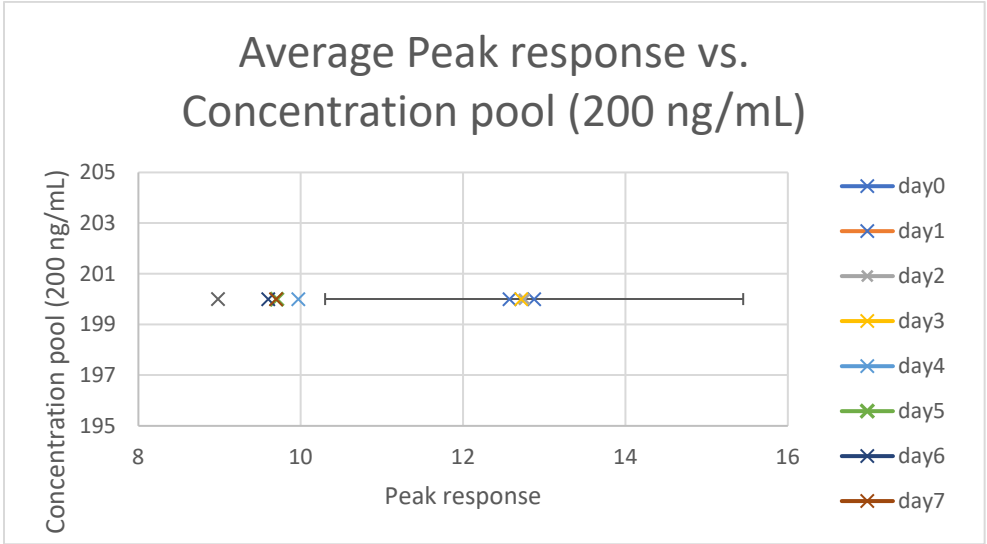


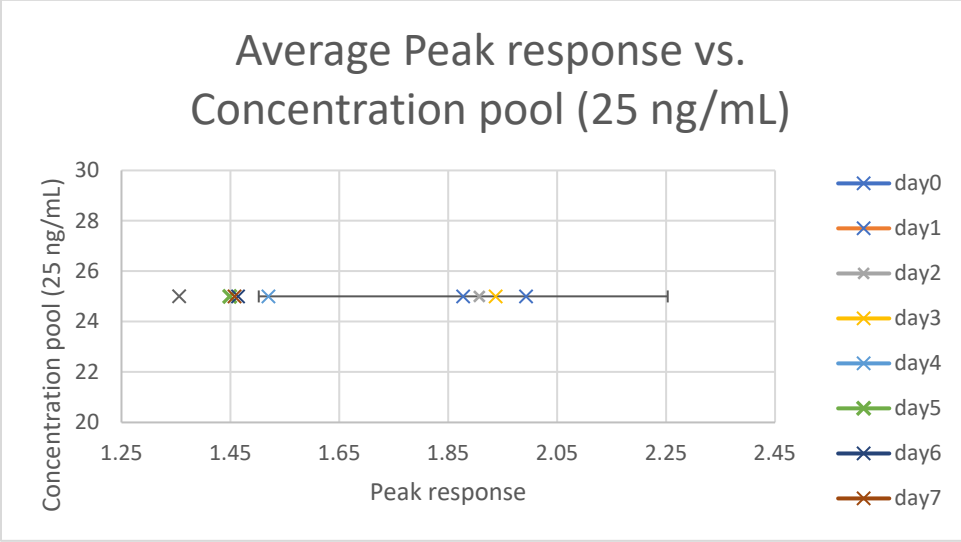




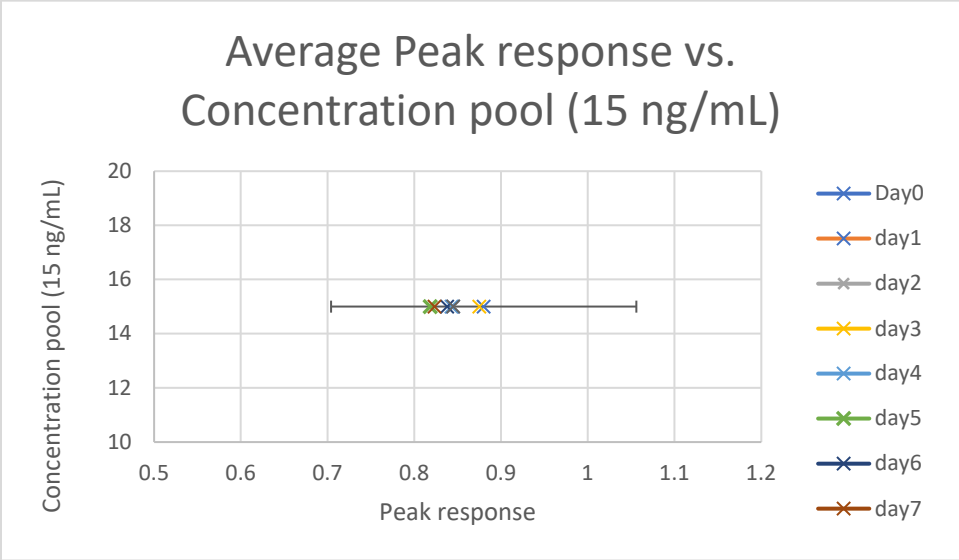
Stability Graphs 4-6 (blood): 7-amino flunitrazepam

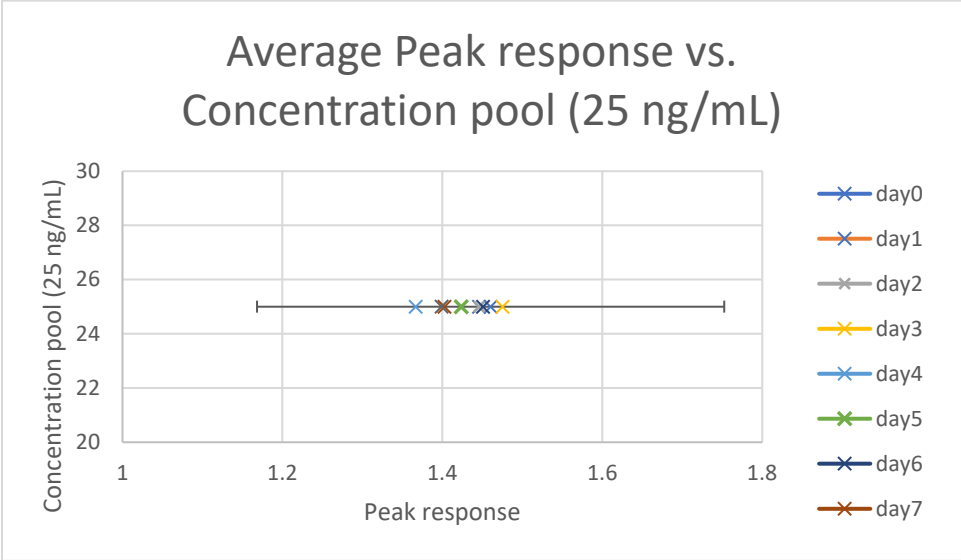


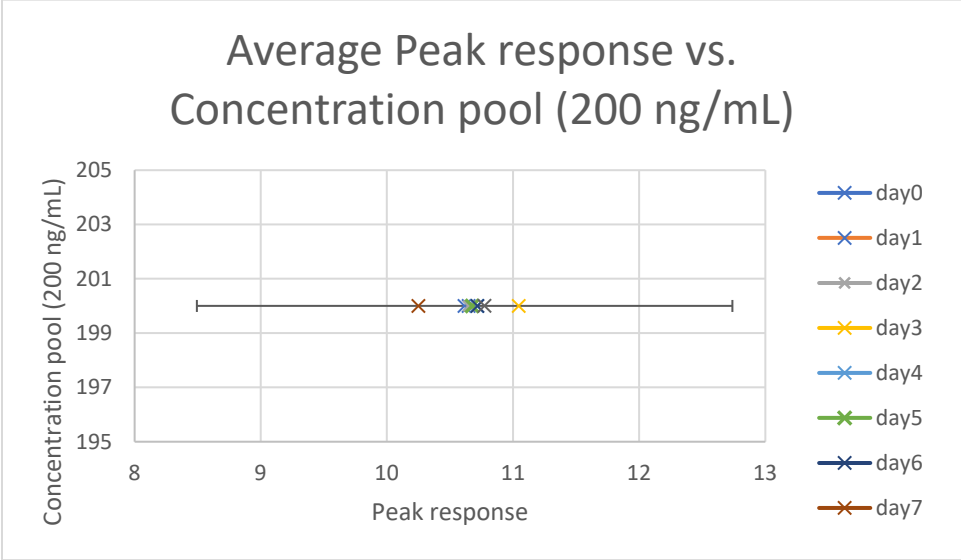




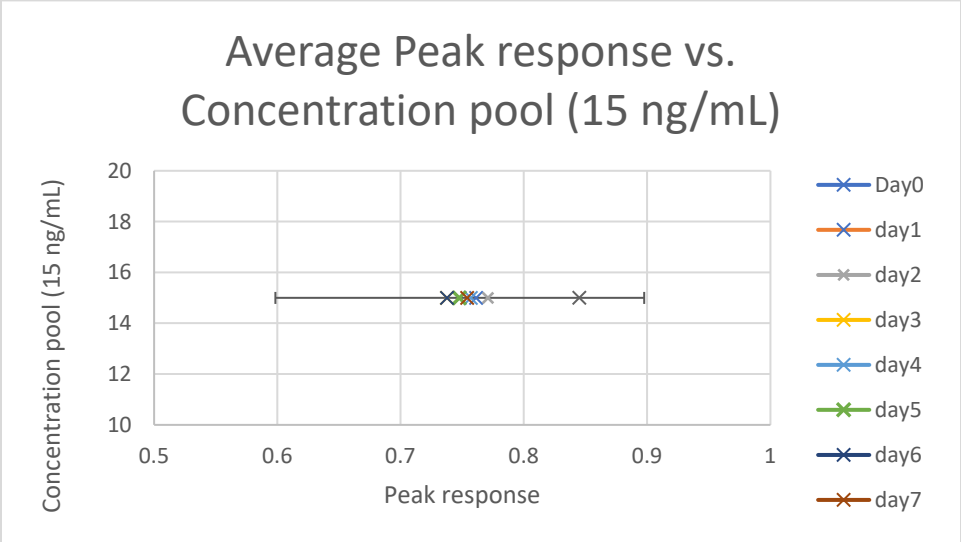
Stability Graphs 7-9 (blood): Alprazolam

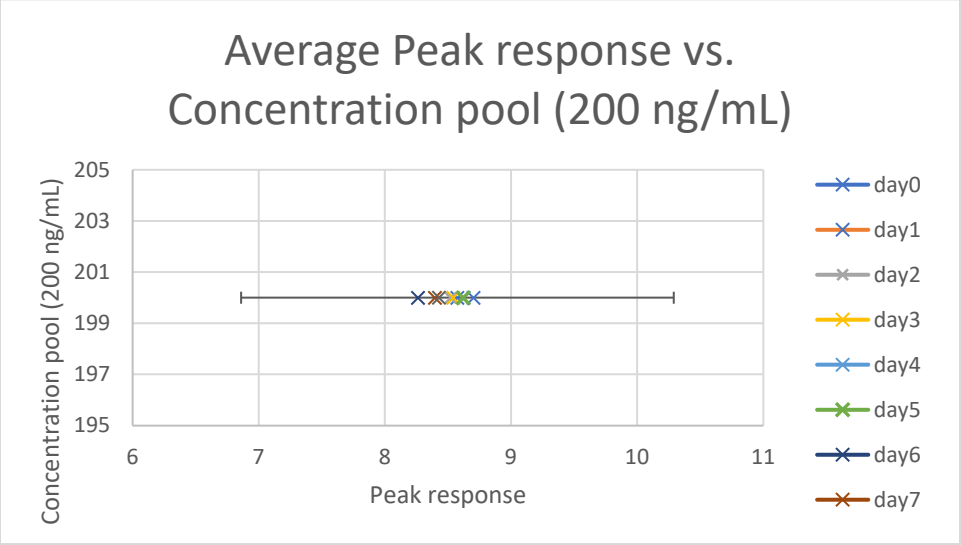


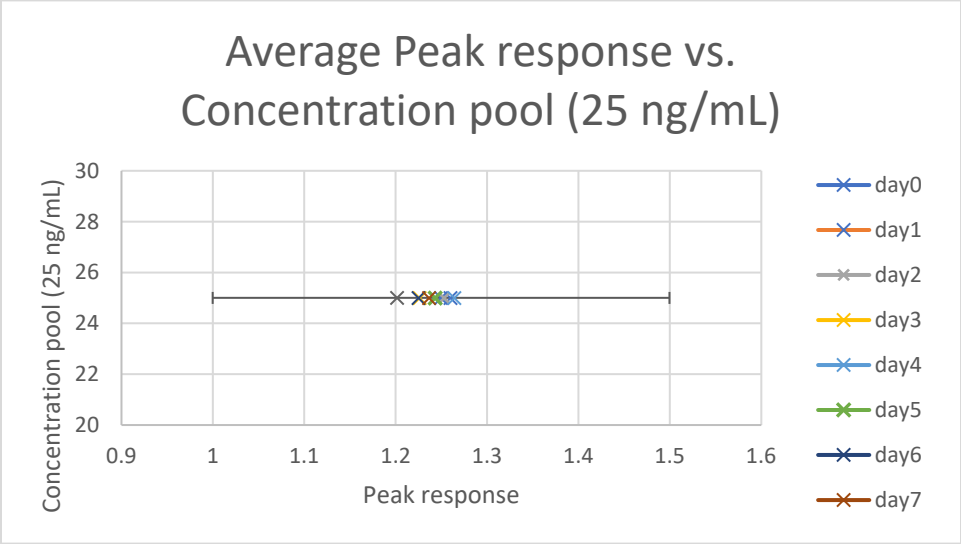




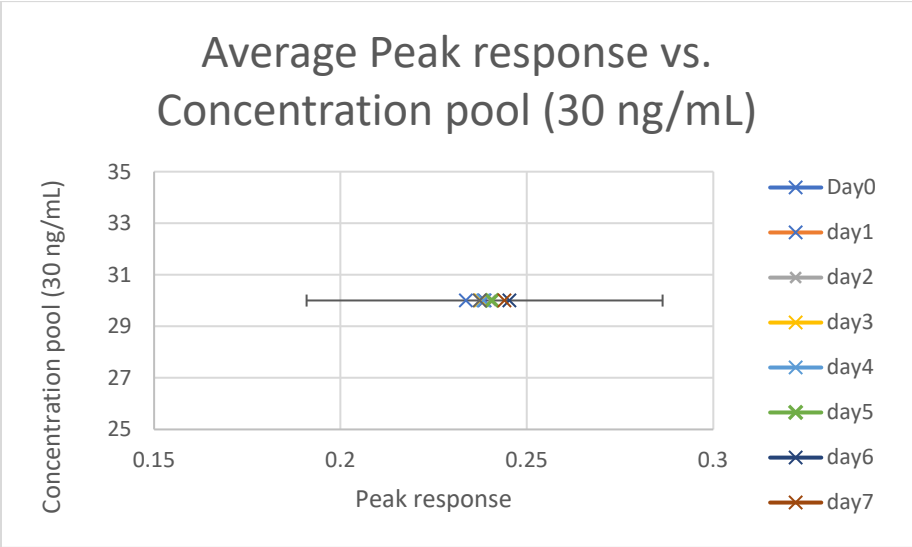
Stability Graphs 10-12 (blood): Clonazepam

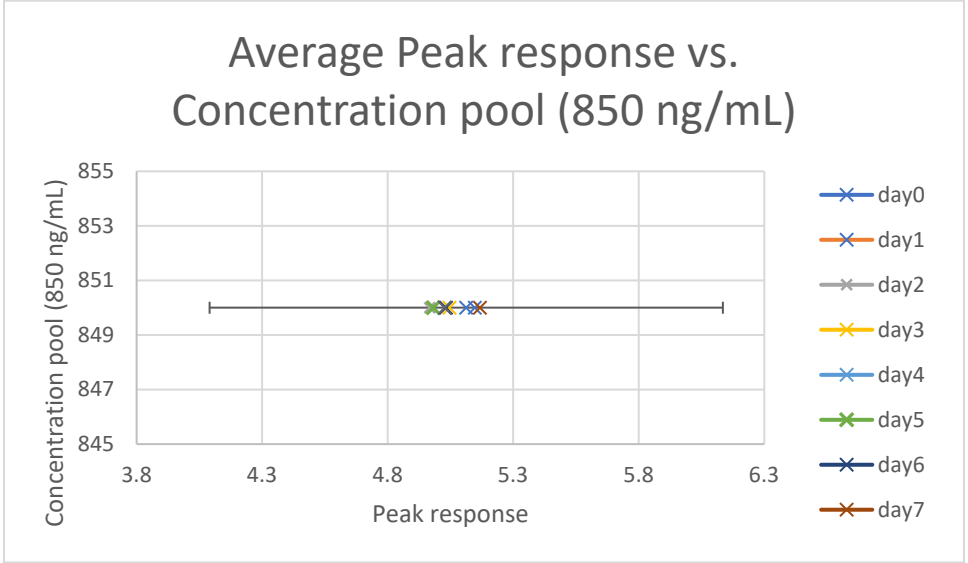


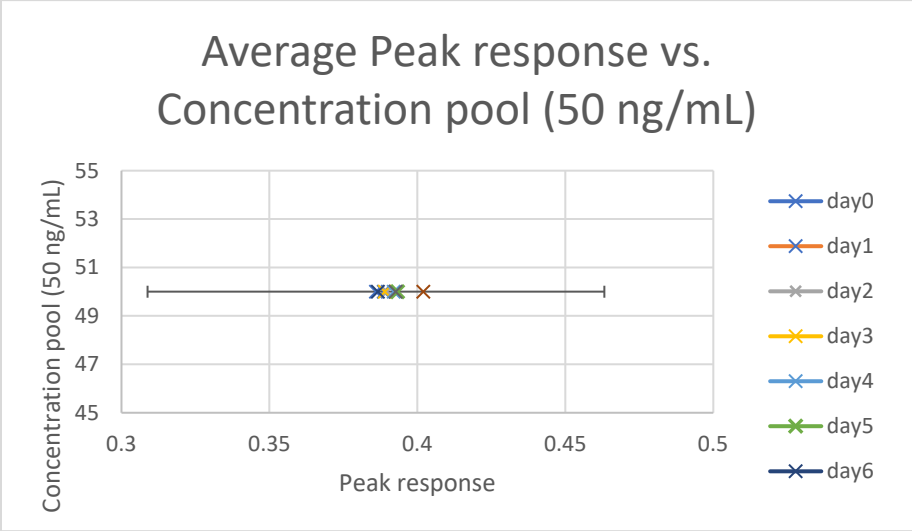




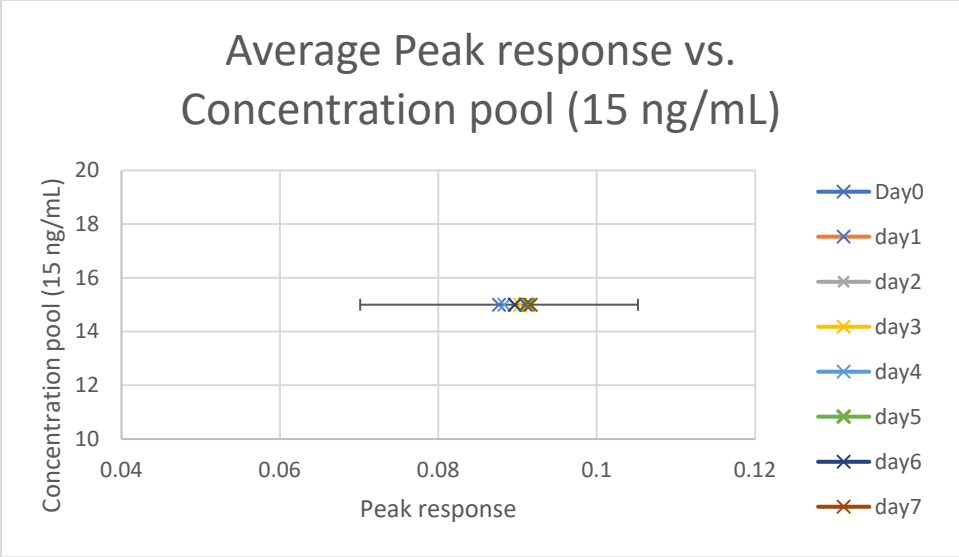
Stability Graphs 13-15 (blood): Diazepam

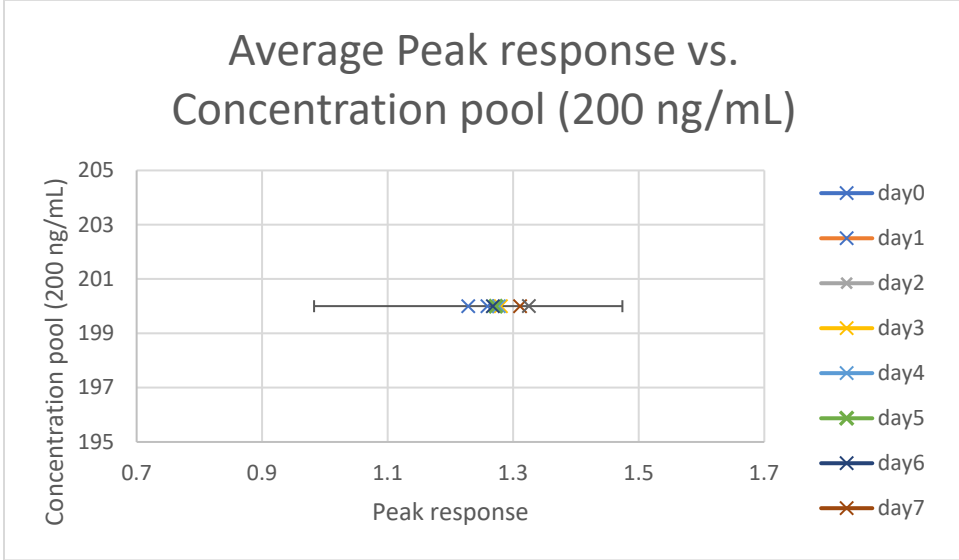


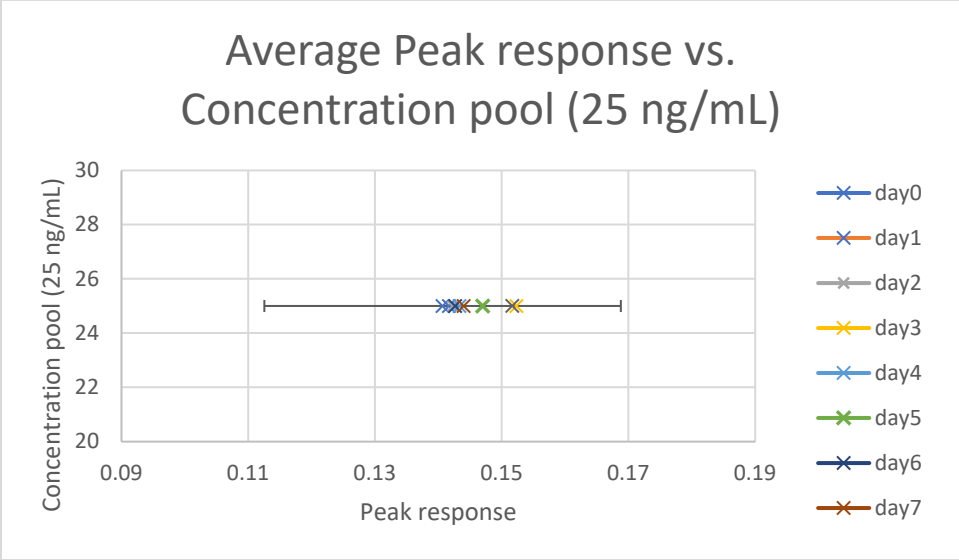




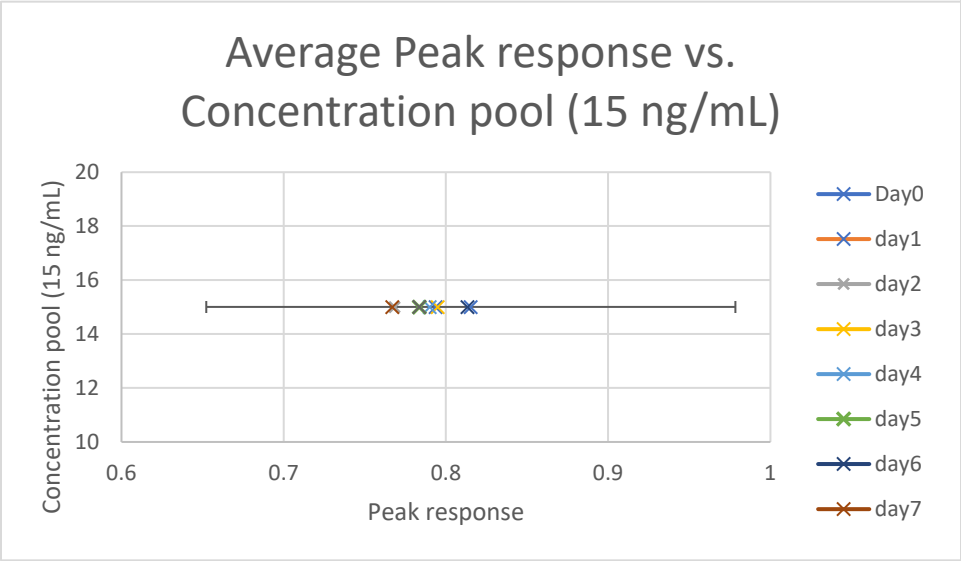
Stability Graphs 16-18 (blood): Etizolam

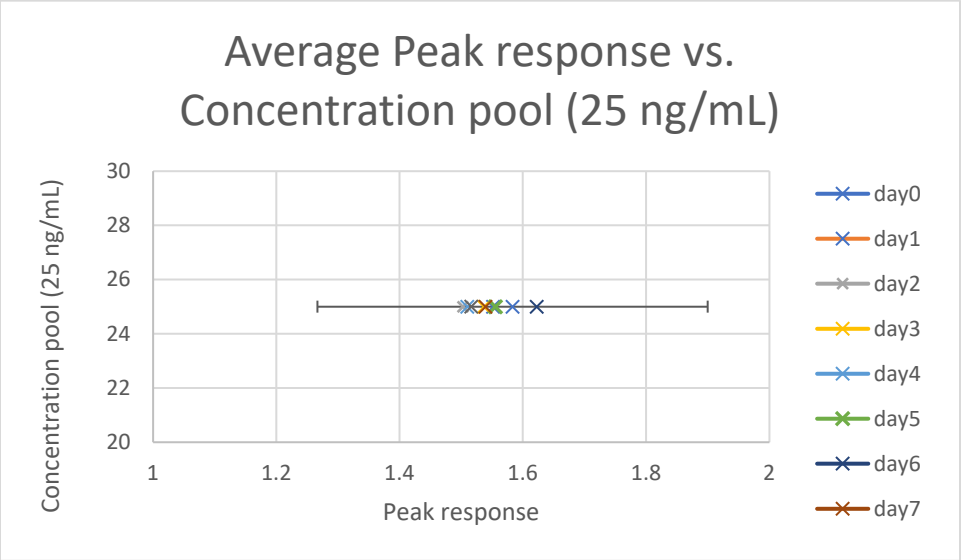


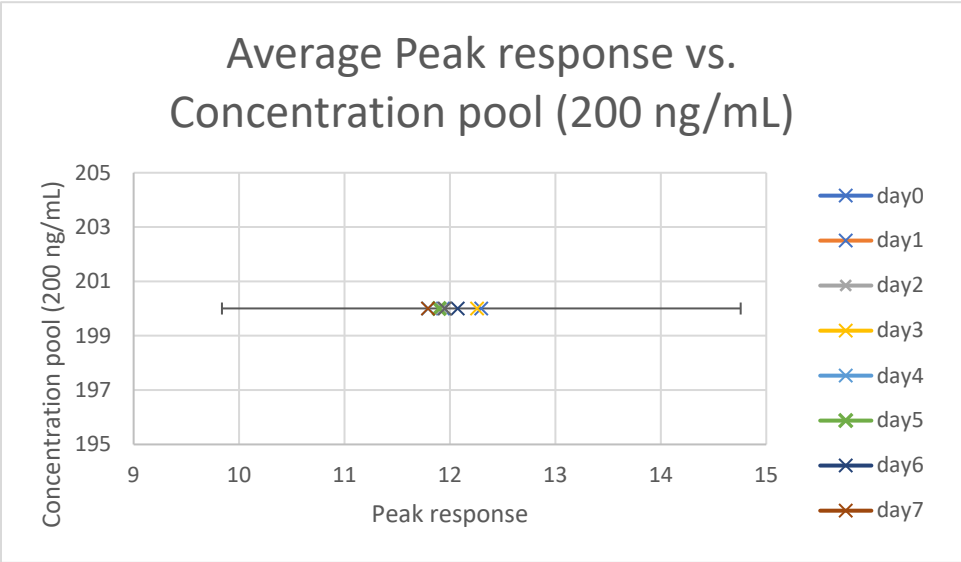




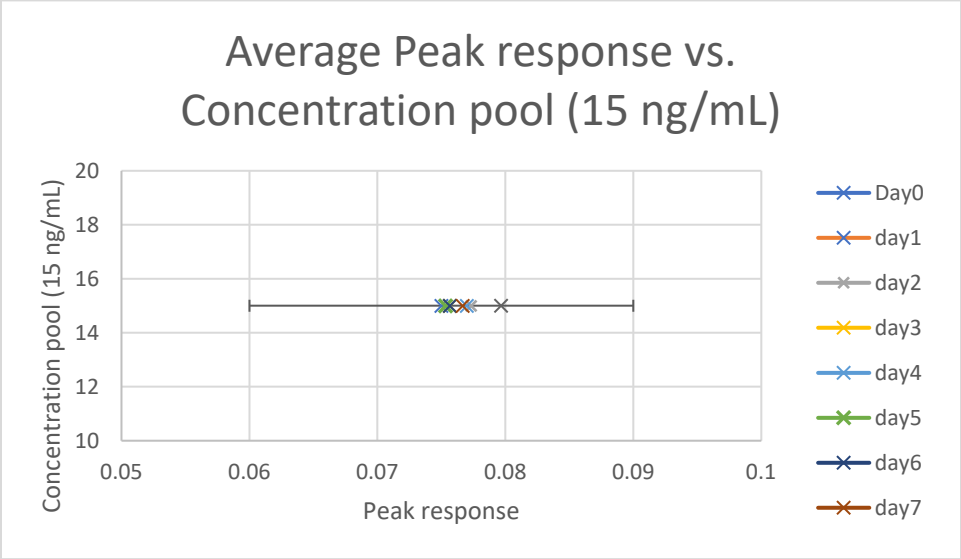
Stability Graphs 19-21 (blood): Flunitrazepam

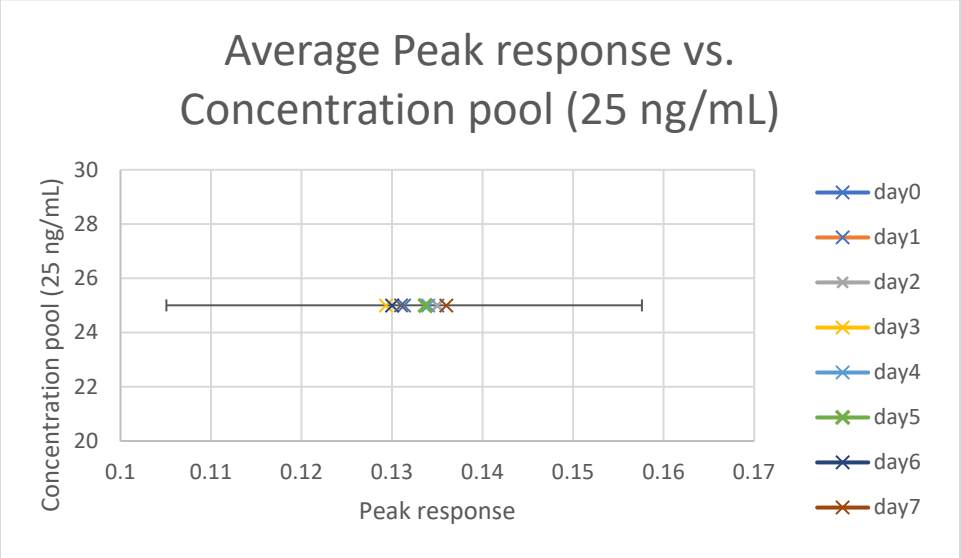


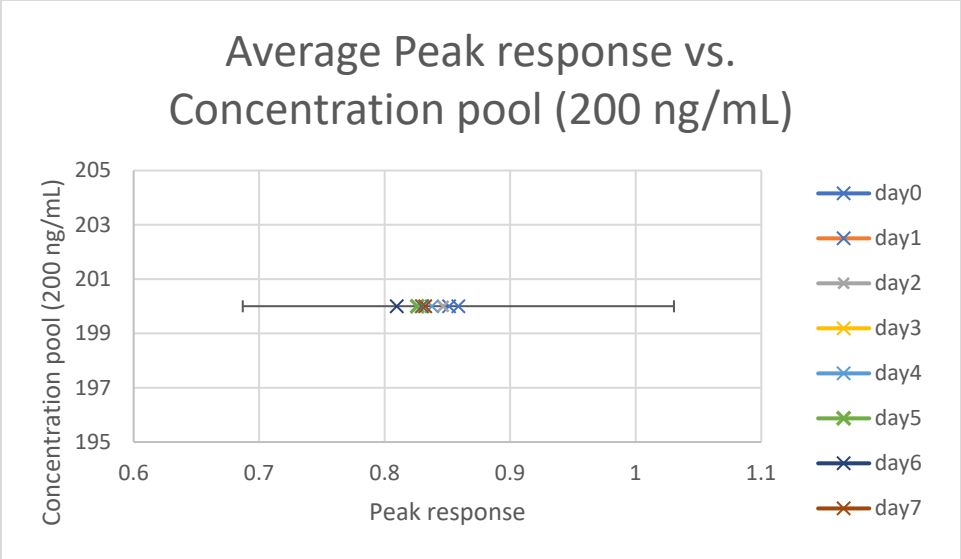




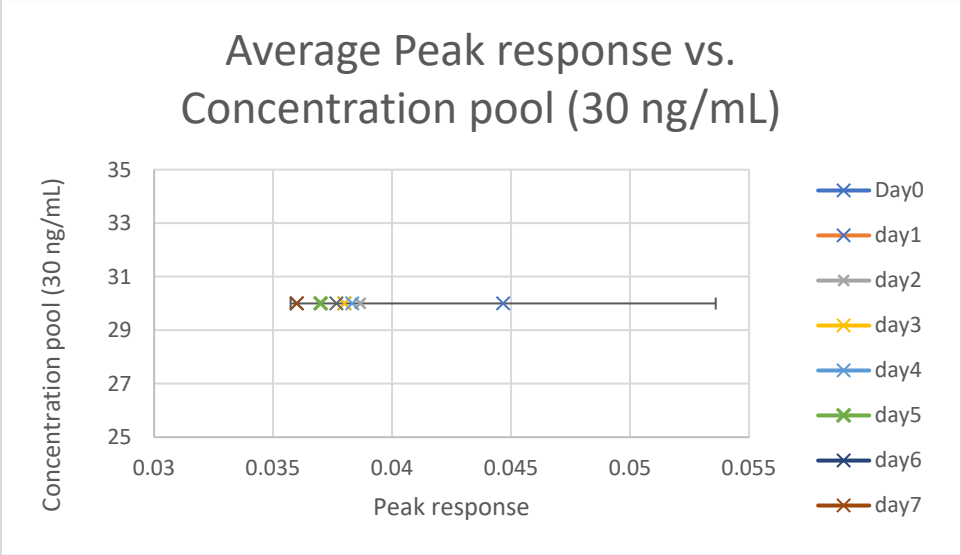
Stability Graphs 22-23 (blood): Lorazepam

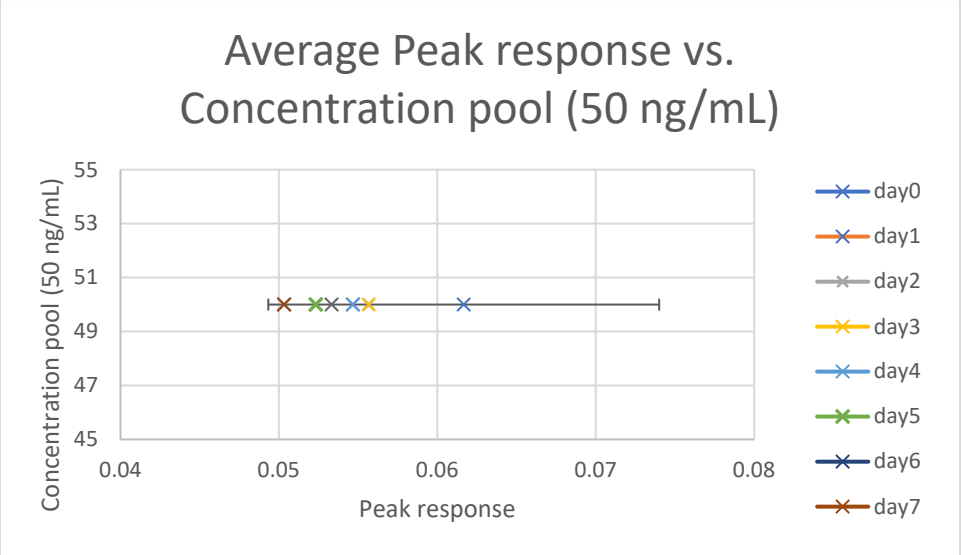


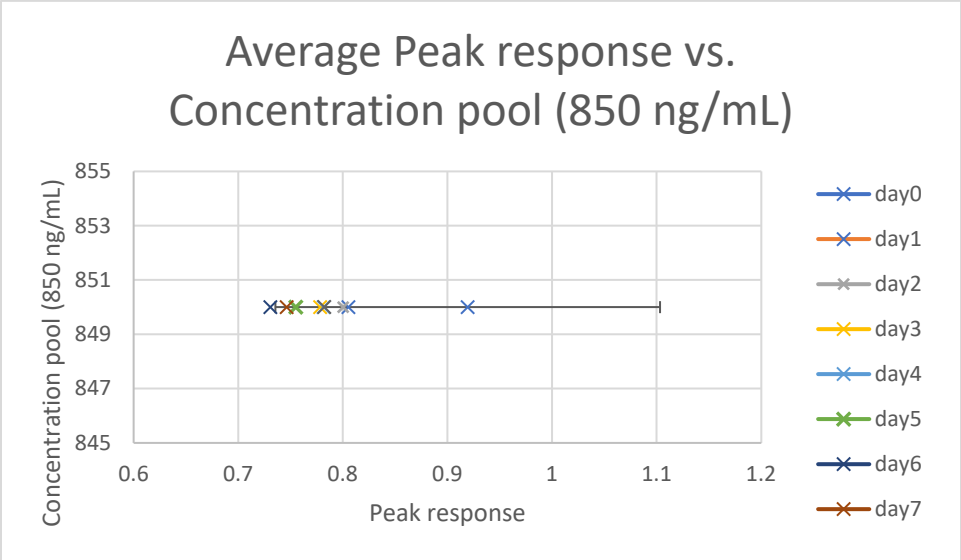




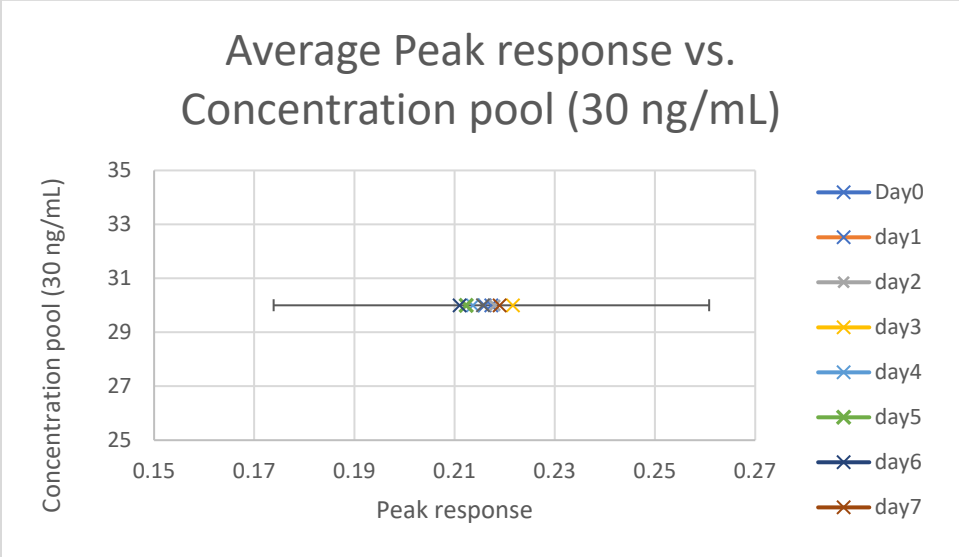
Stability Graphs 24-26 (blood): Midazolam

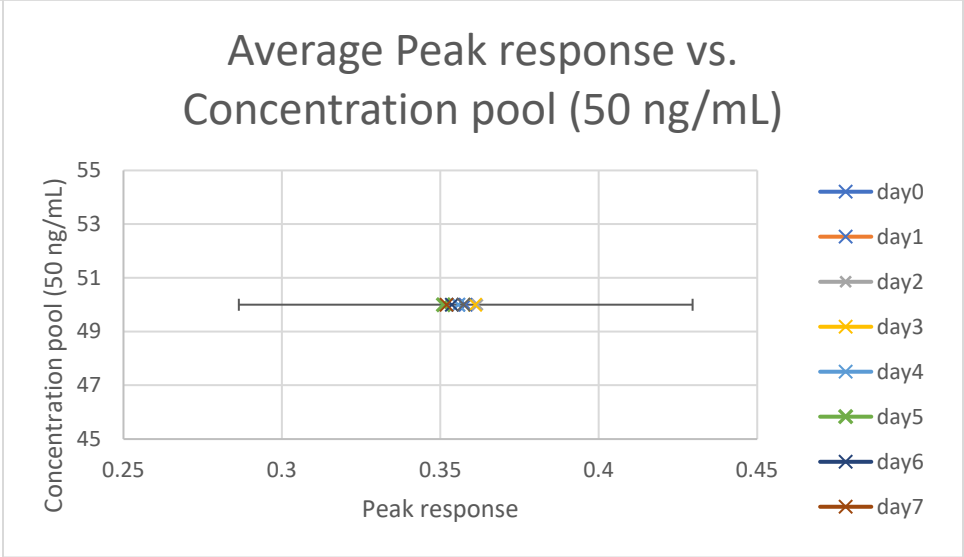


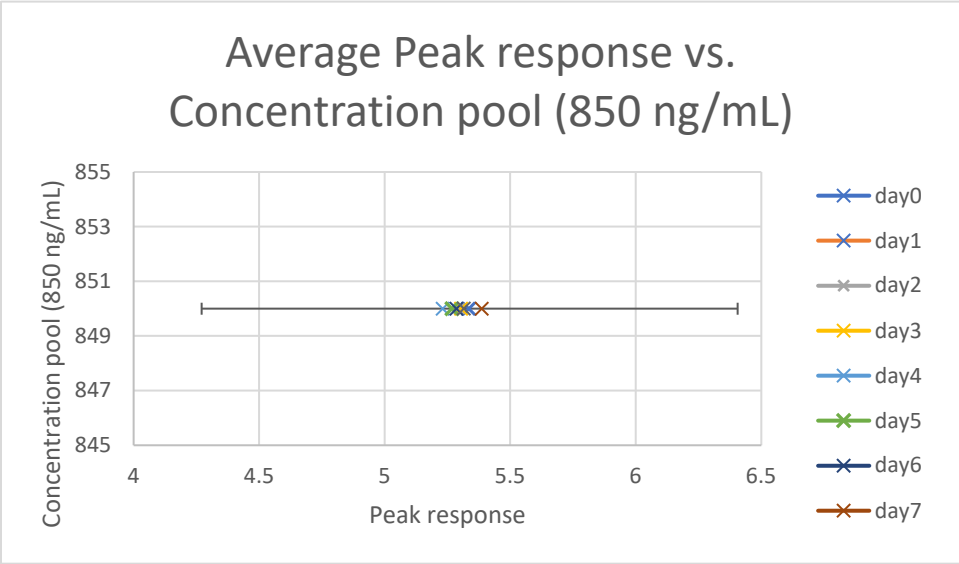




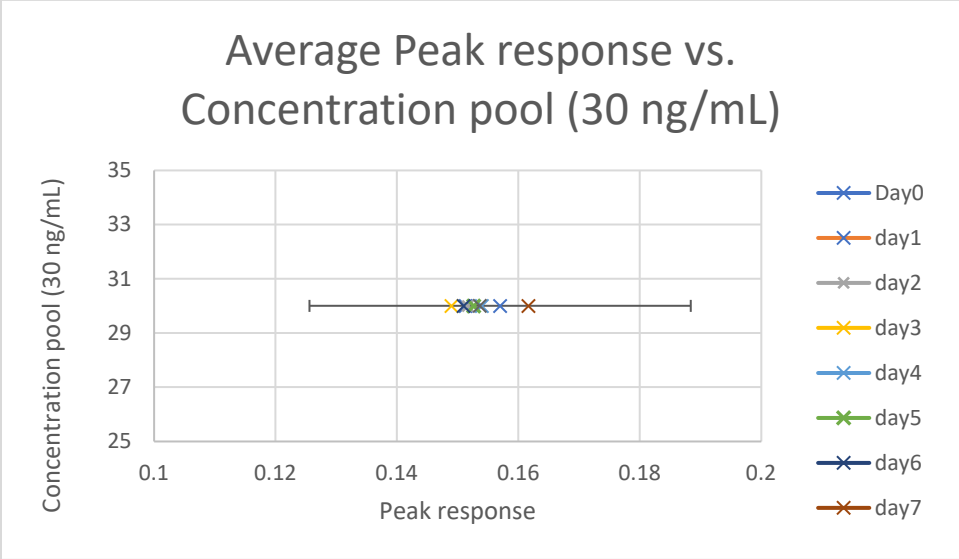
Stability Graphs 27-29 (blood): Nordiazepam

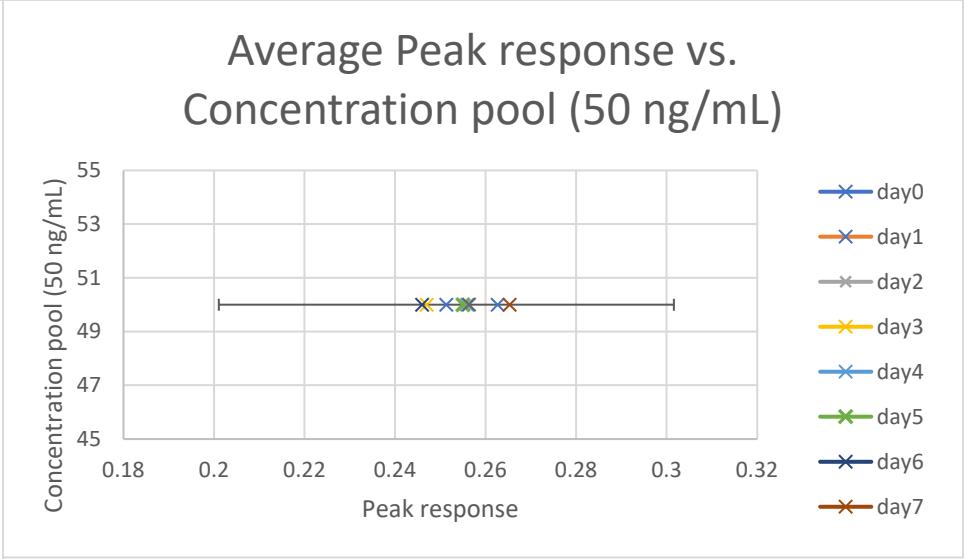


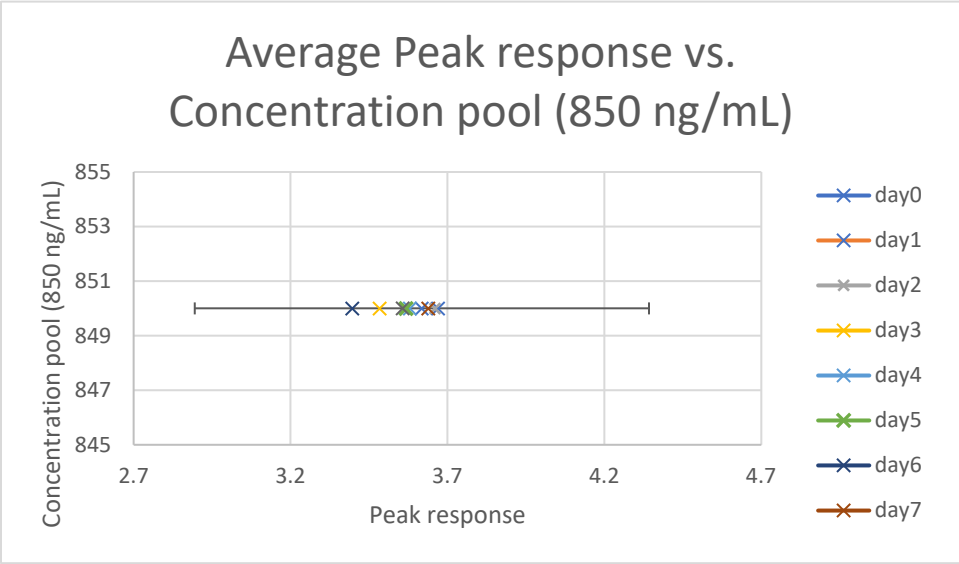




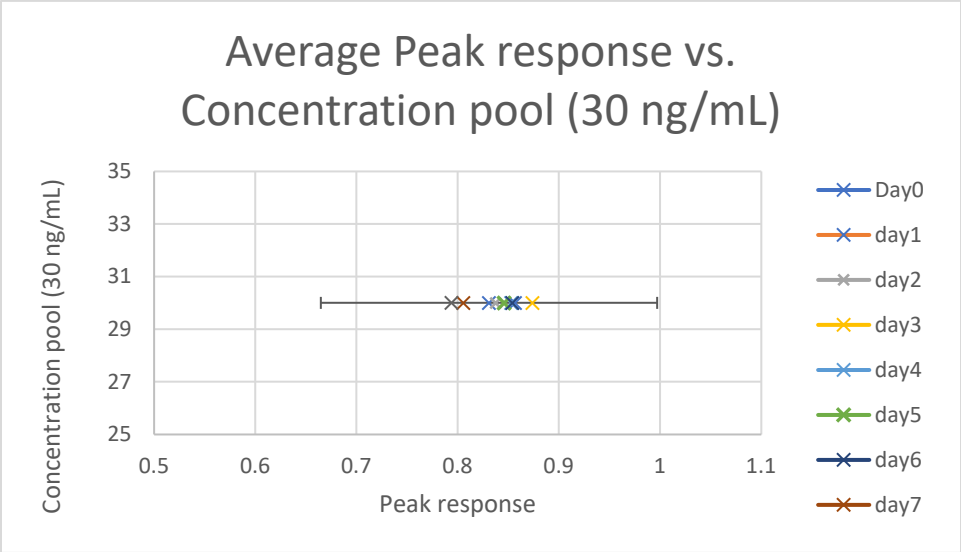
Stability Graphs 30-32 (blood): Oxazepam

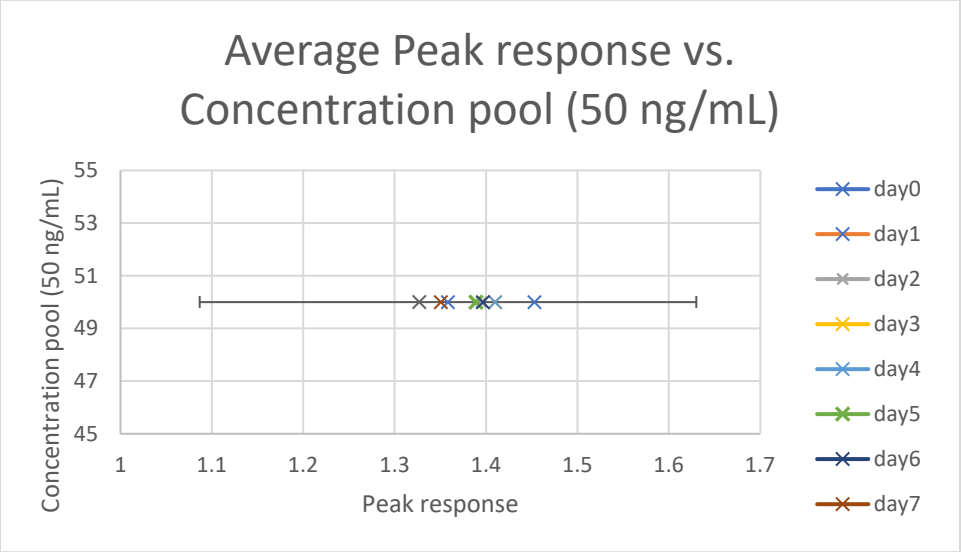


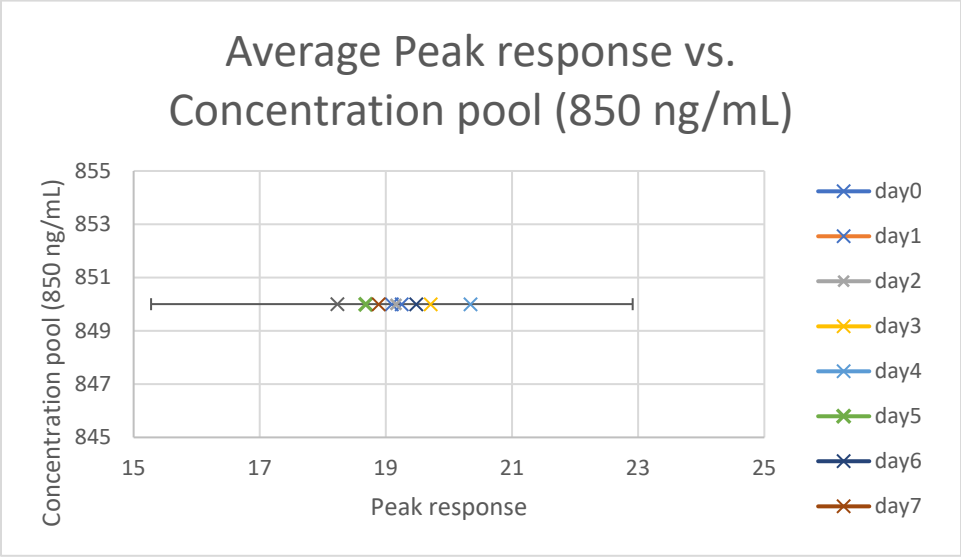




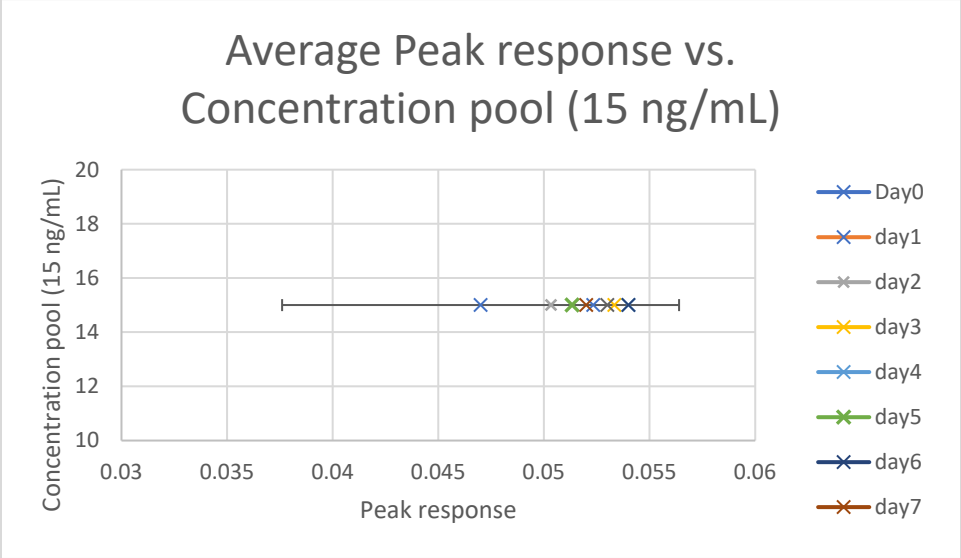
Stability Graphs 33-35 (blood): Temazepam

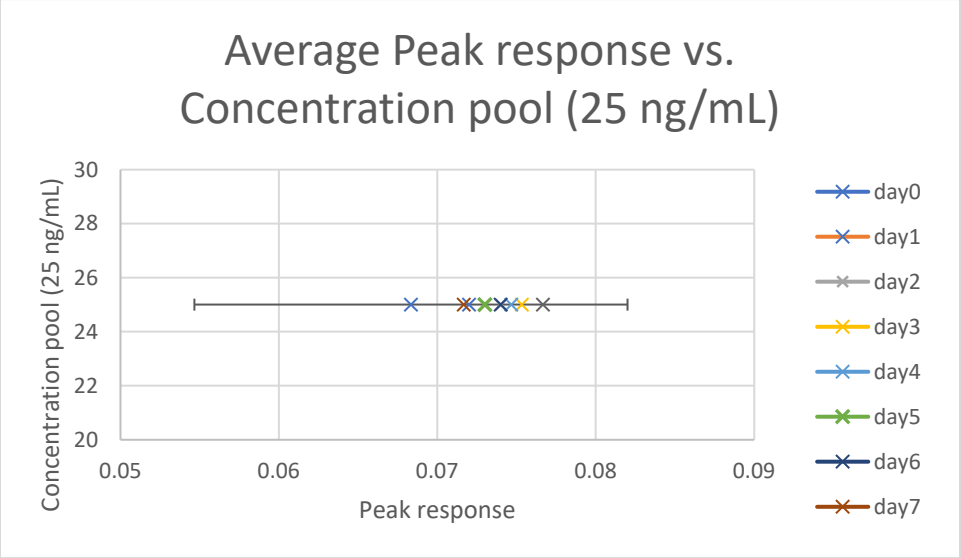


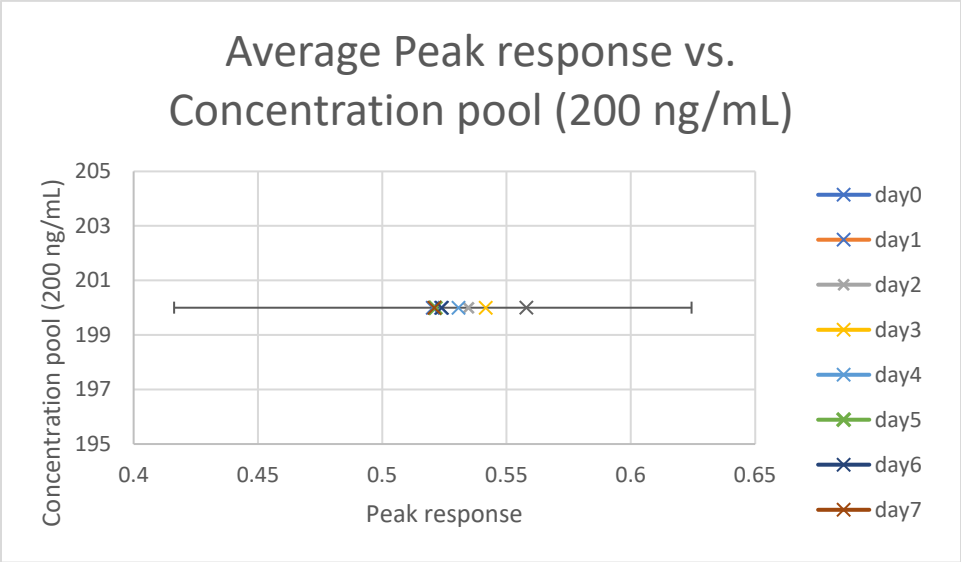




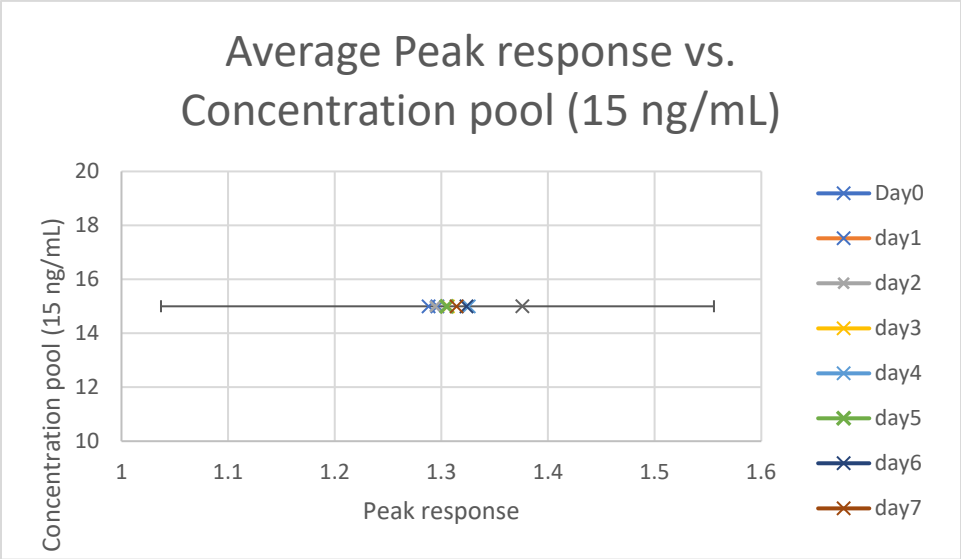
Stability Graphs 36-38 (blood): Triazolam

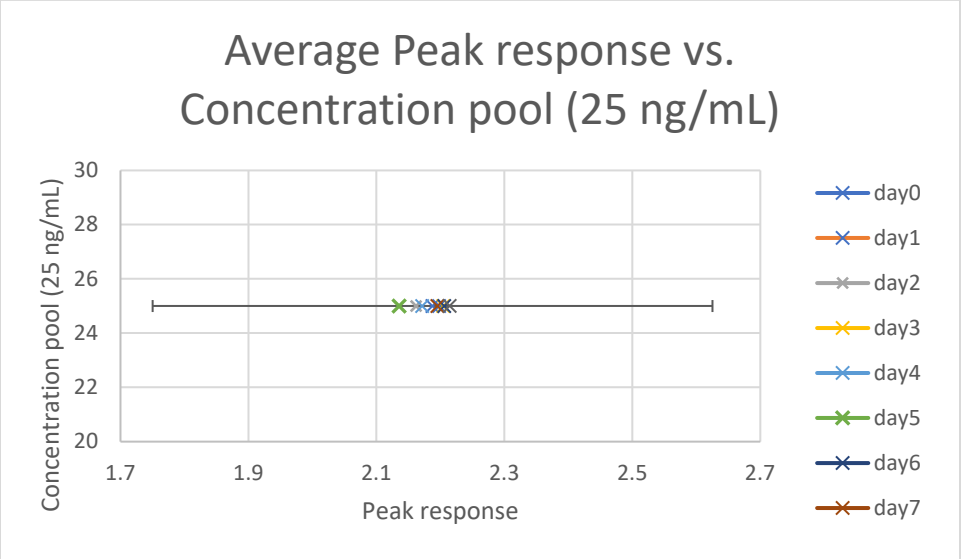


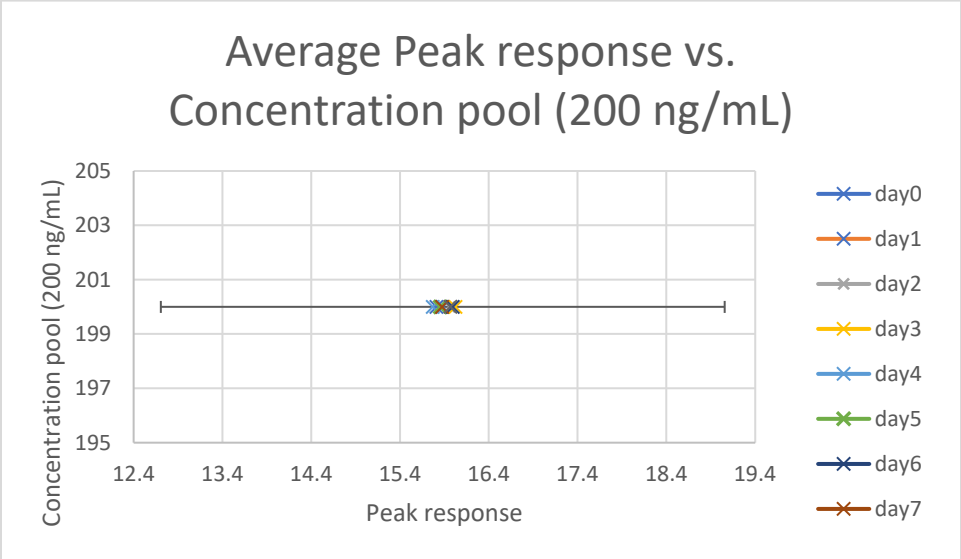




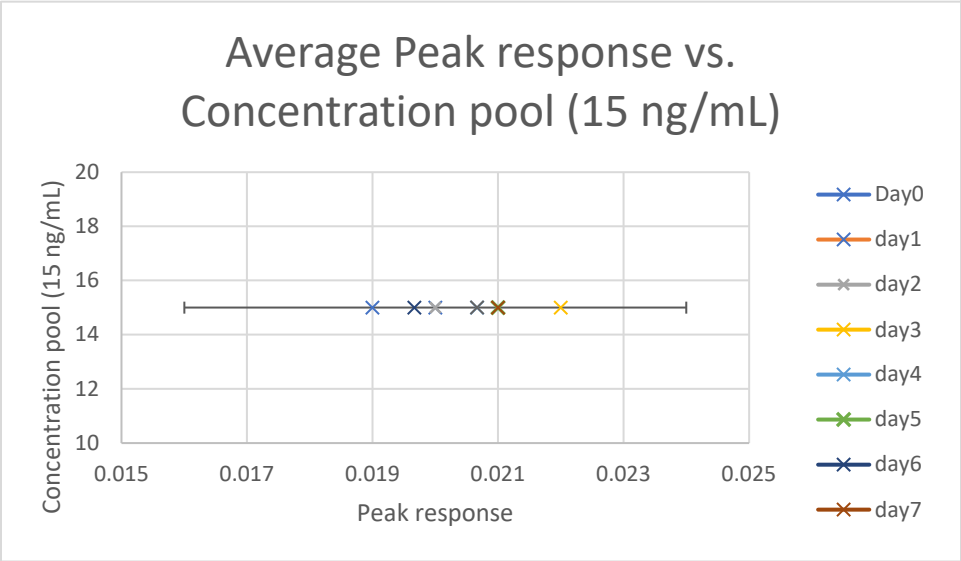
Stability Graphs 39-41 (blood): Zolpidem

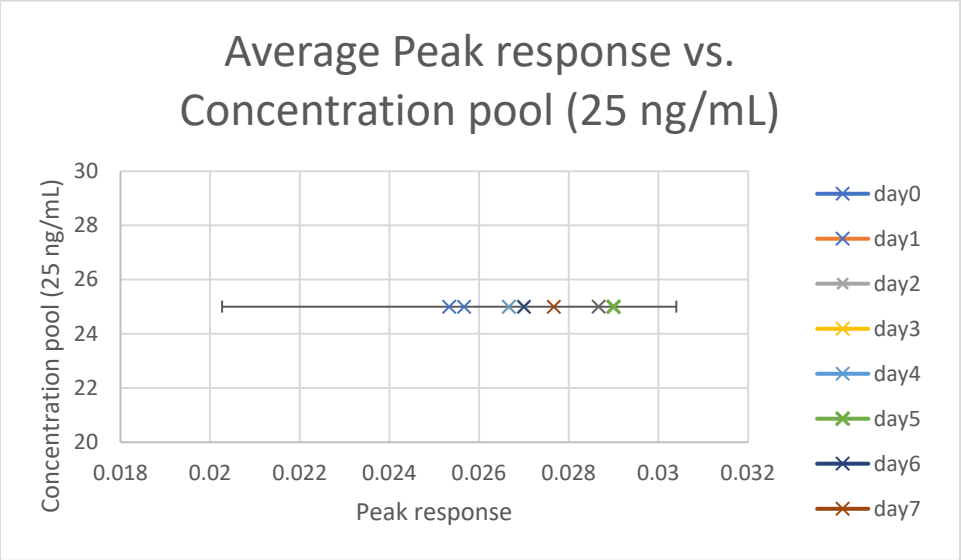


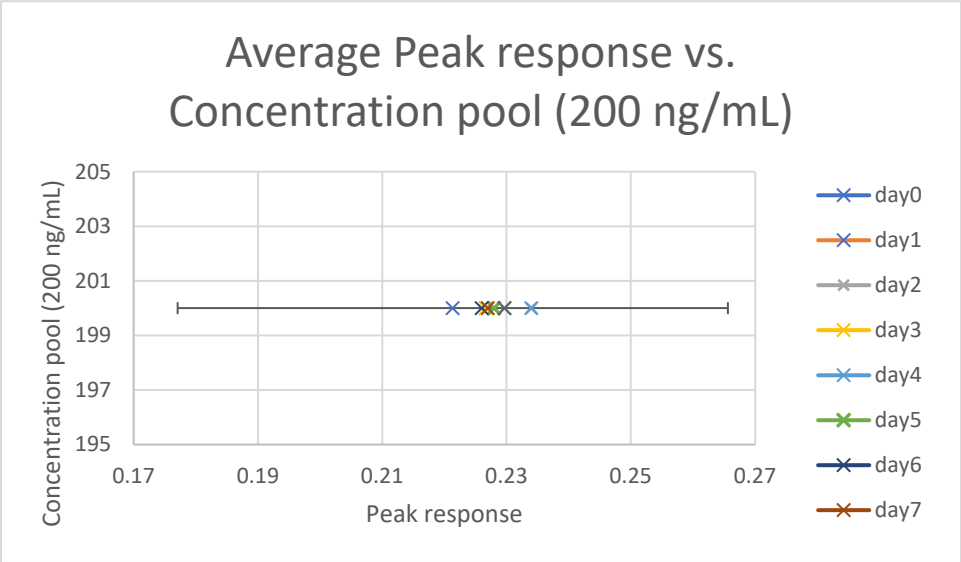




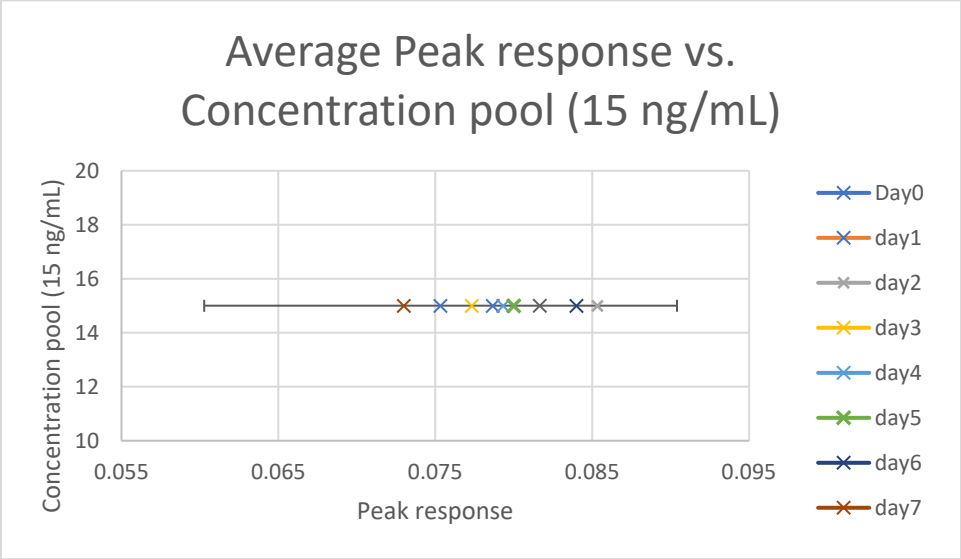
Stability Graphs 42-44 (blood): Alpha-hydroxy etizolam

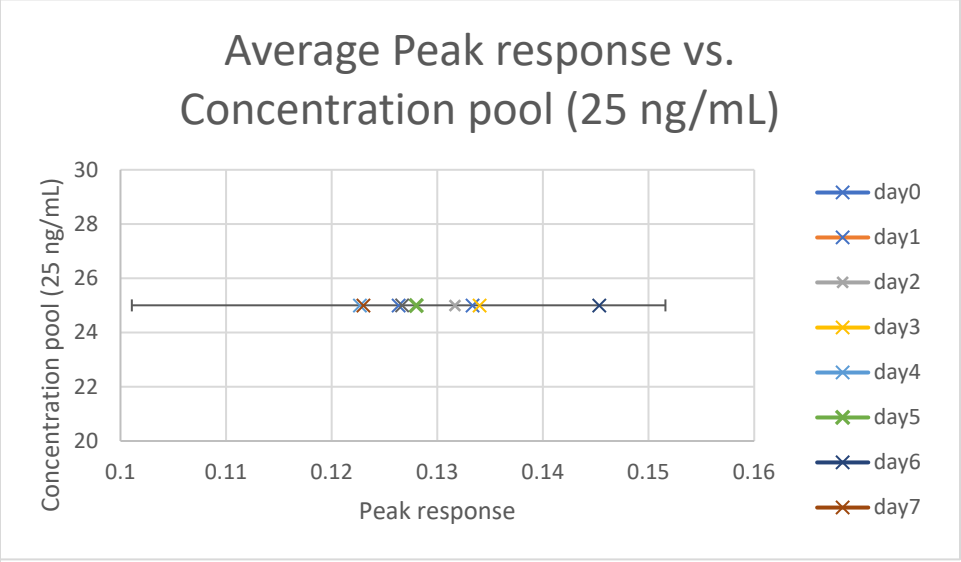


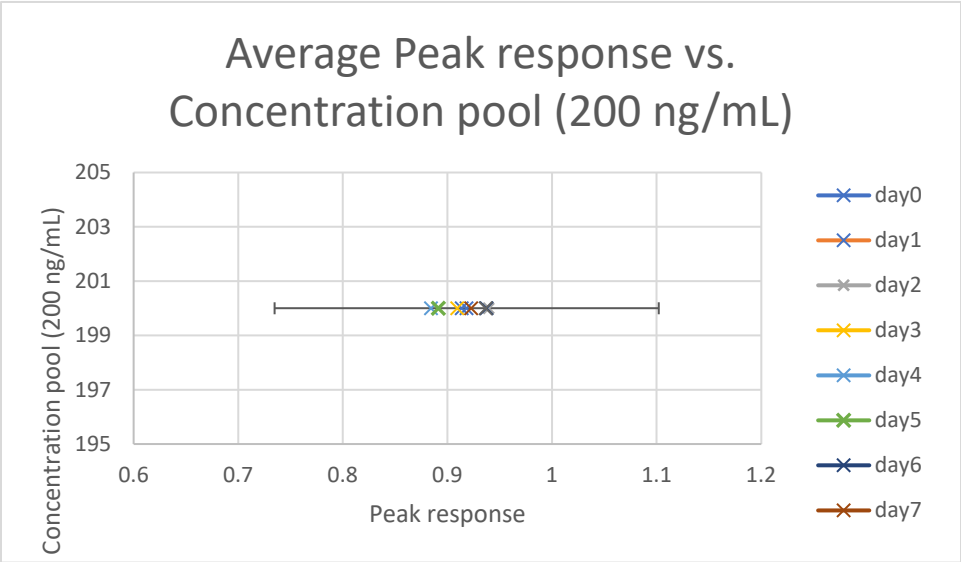




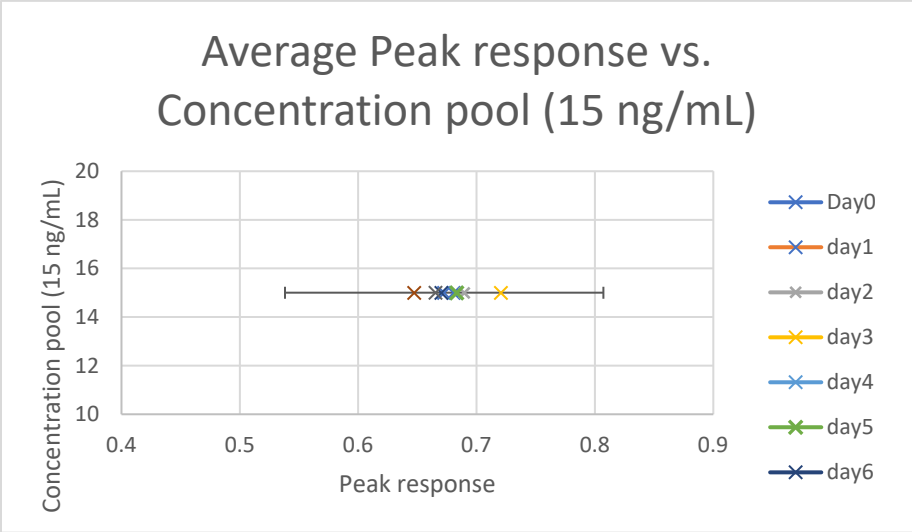
Stability Graphs 45-47 (blood): Alpha-hydroxy triazolam

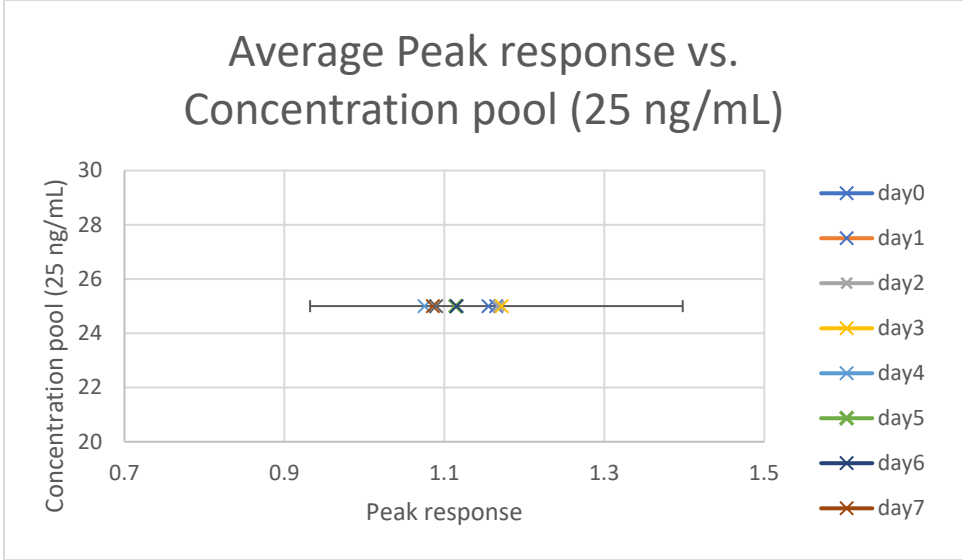


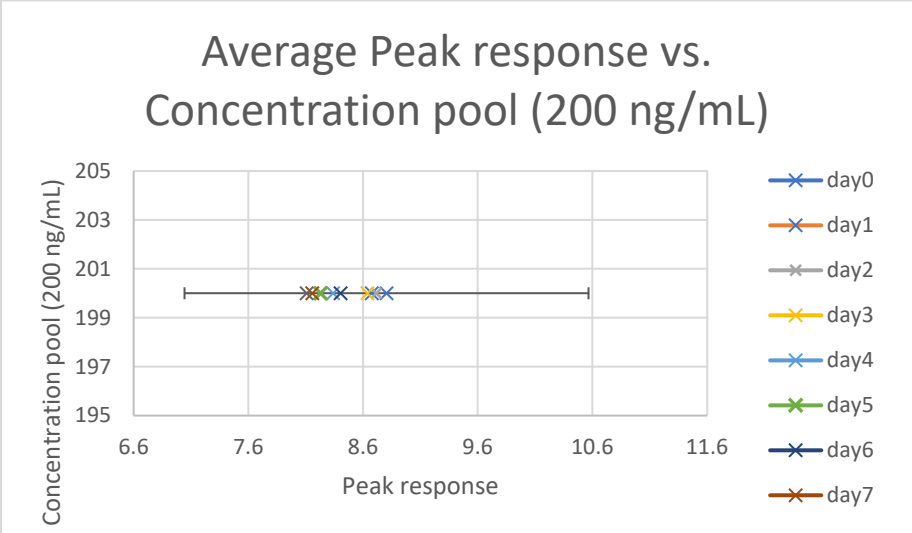




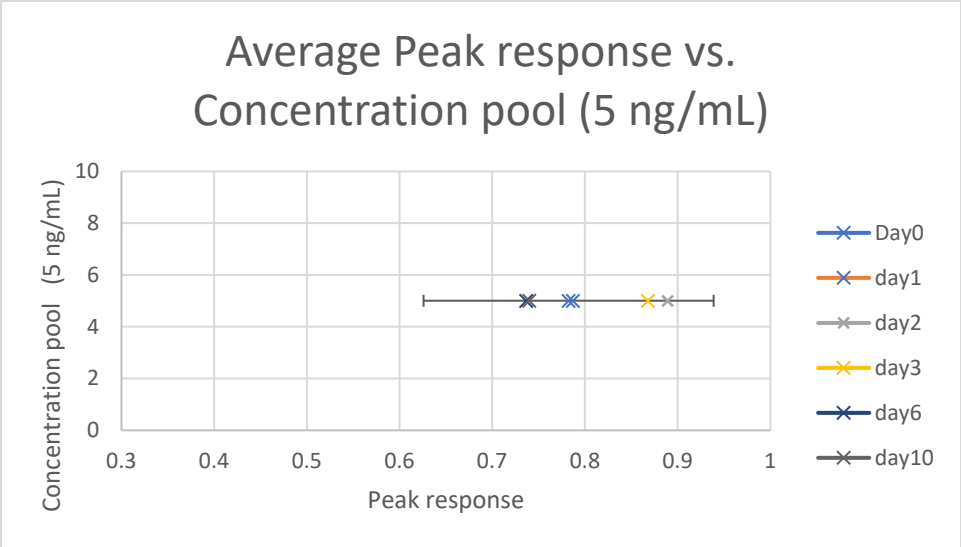
Stability Graphs 48-50 (blood): Alpha-hydroxy alprazolam

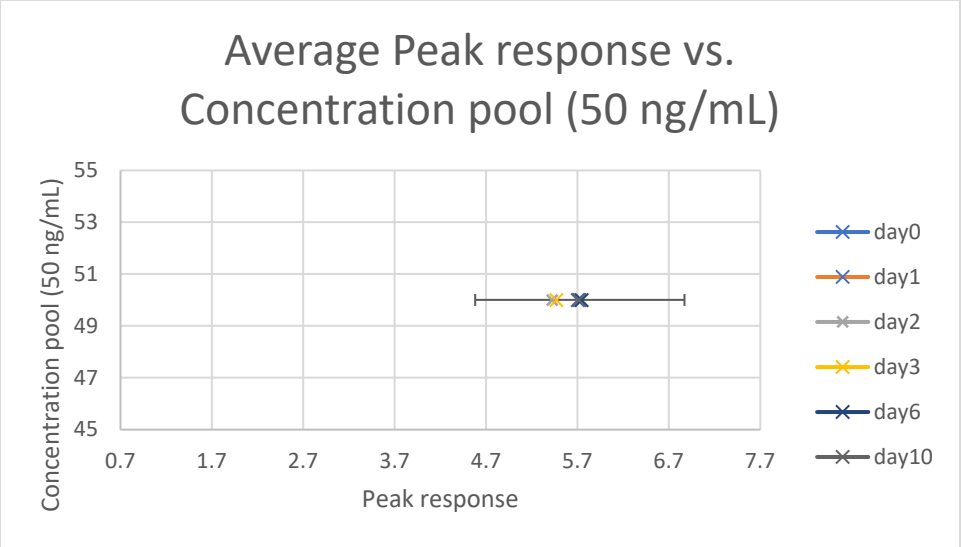


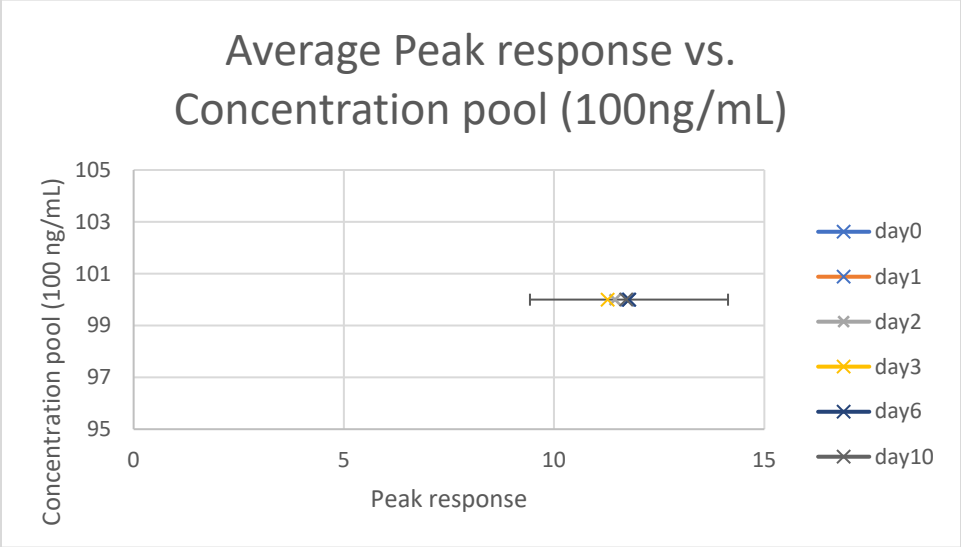




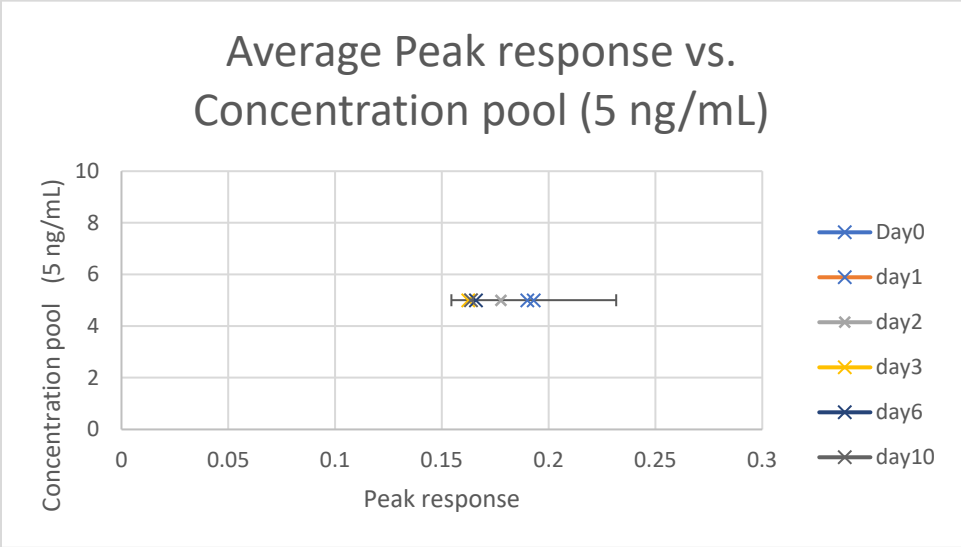
Stability Graphs 51-53 (blood): Hydroxy ethyl flurazepam

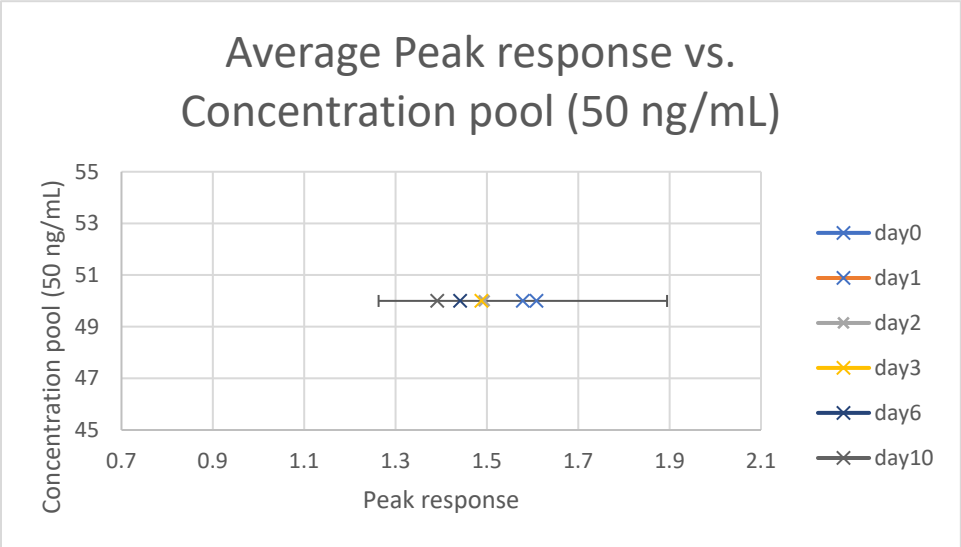


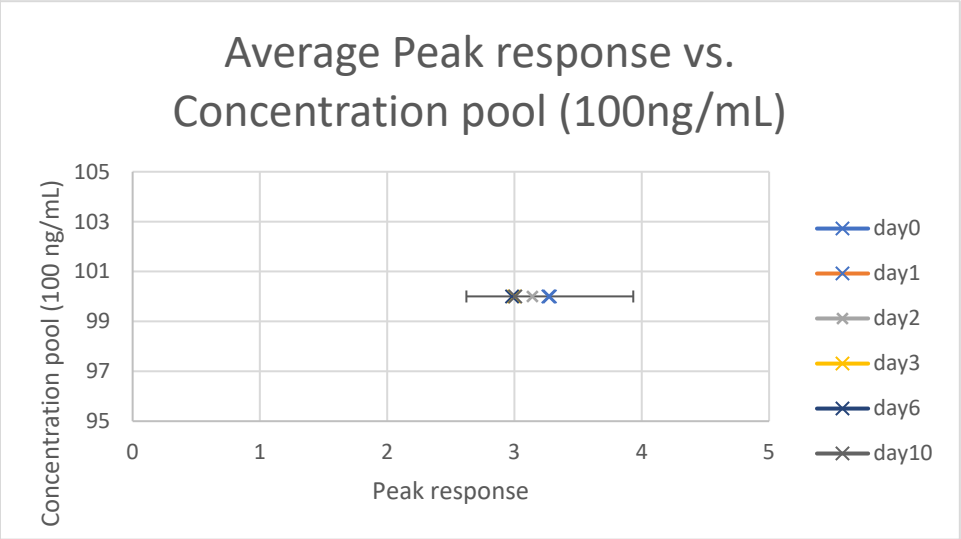




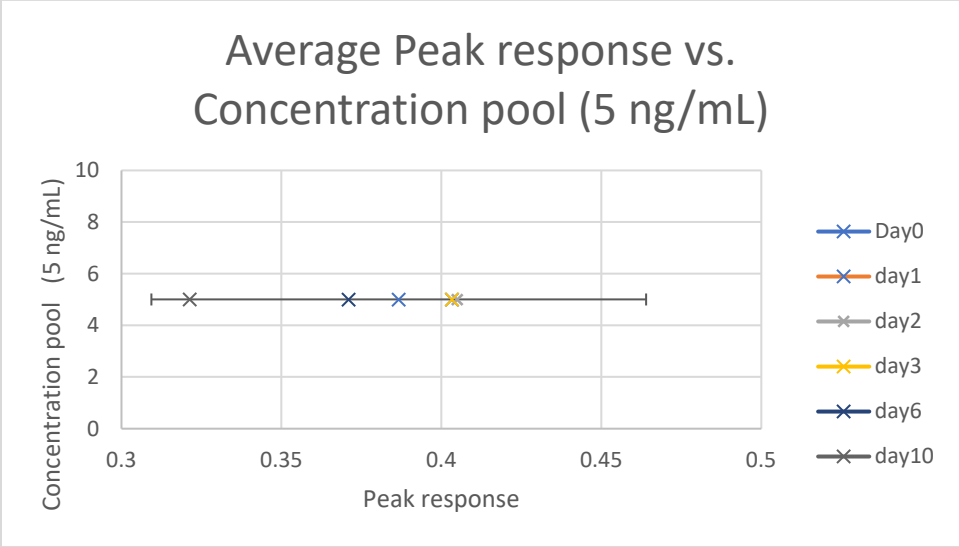
Stability Graphs 54-56 (blood): Bromazepam

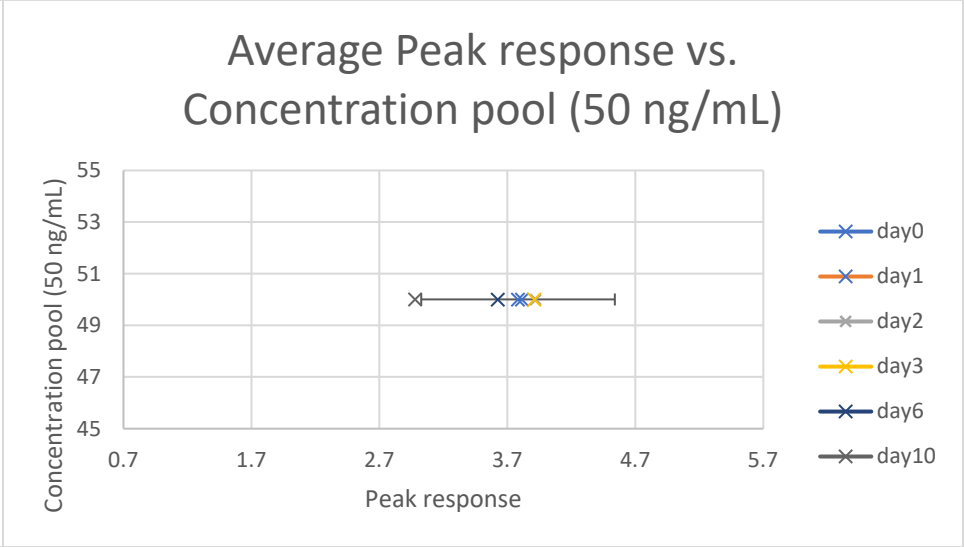


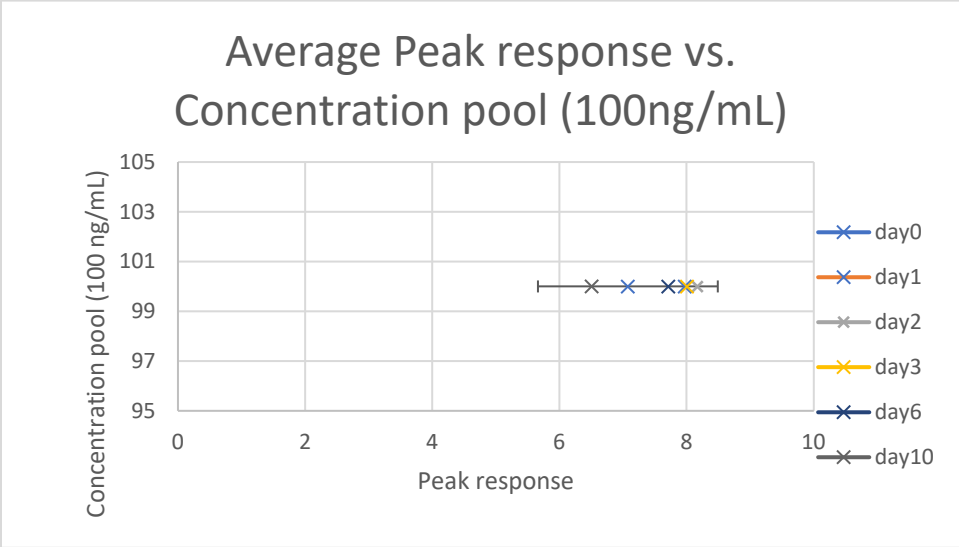




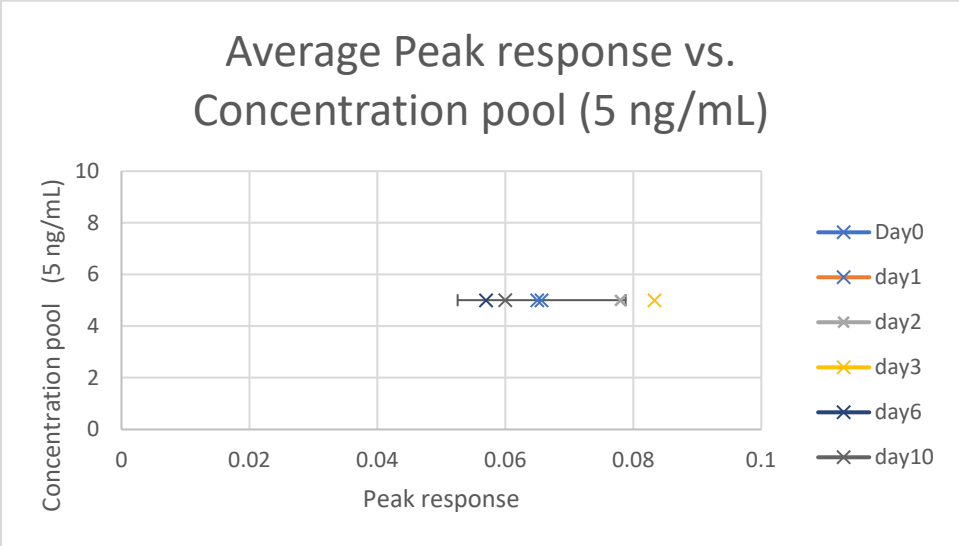
Stability Graphs 57-59 (blood): Chlordiazepoxide

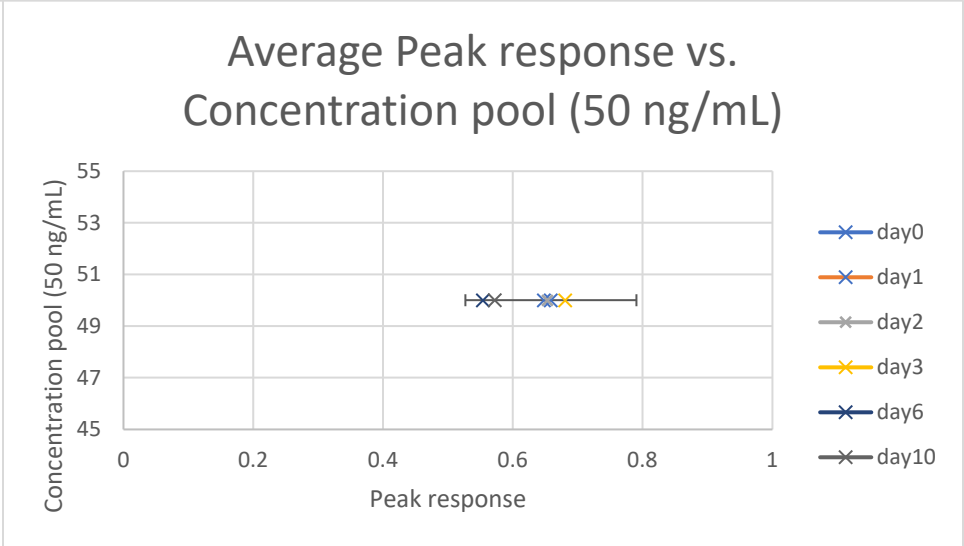


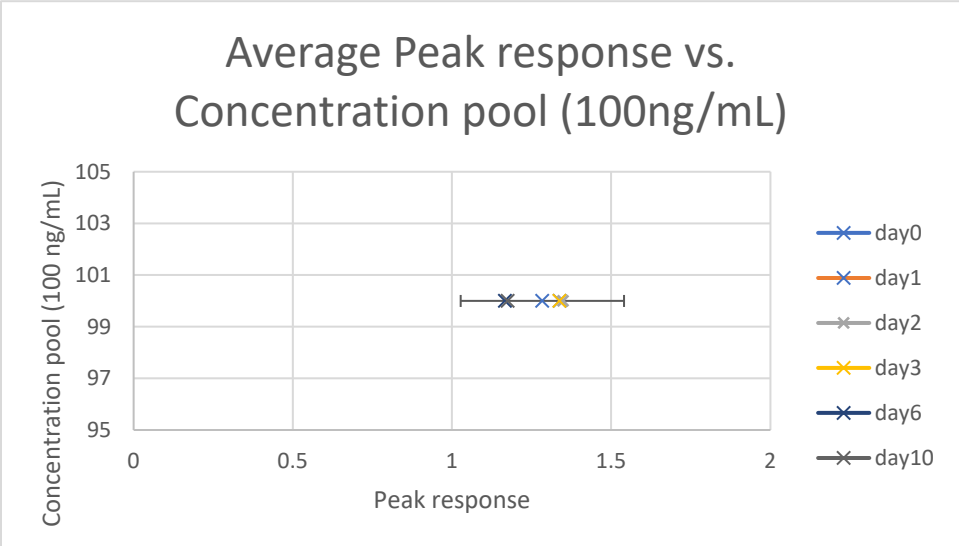




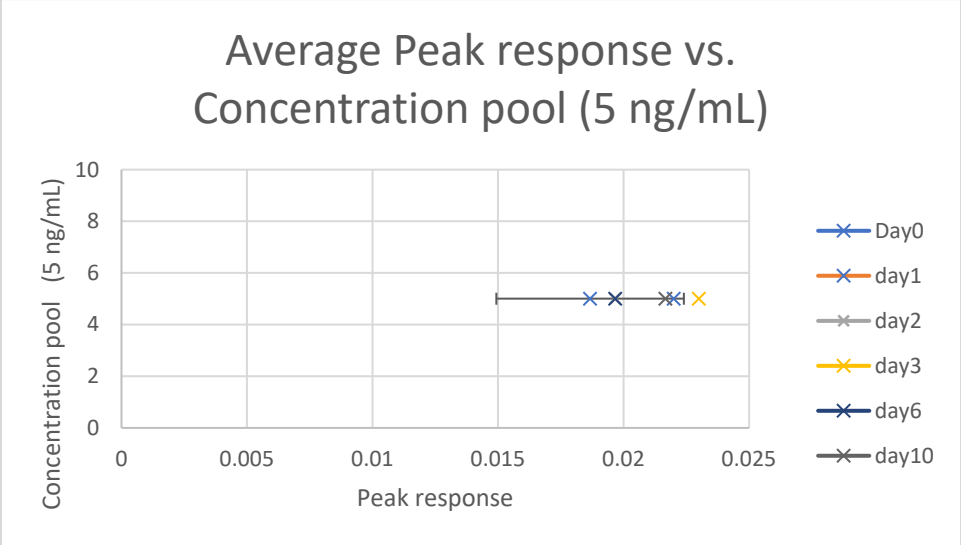
Stability Graphs 60-62 (blood): Clobazam

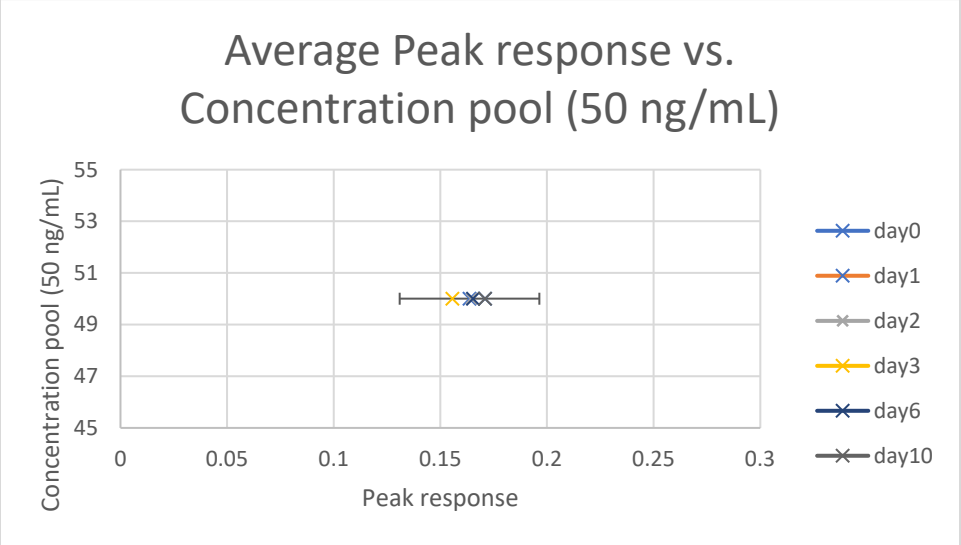


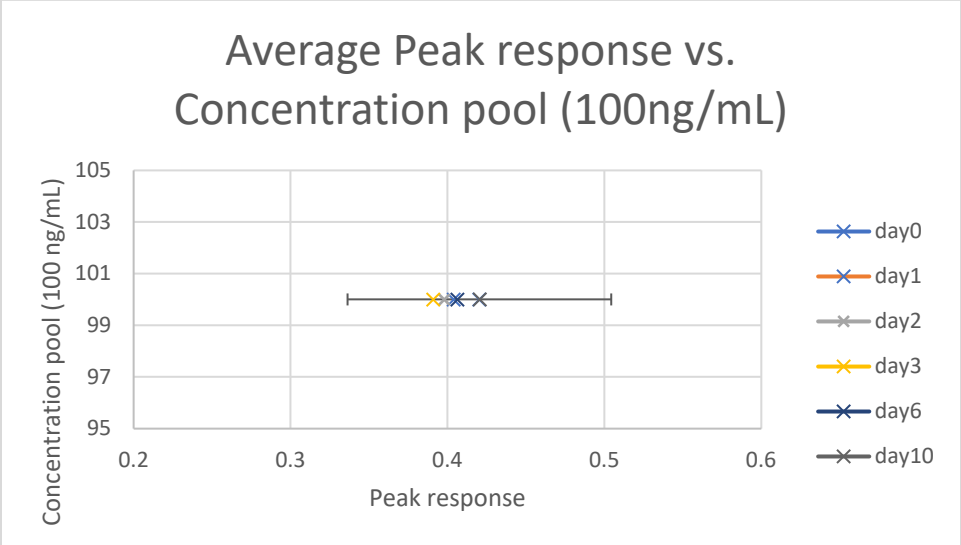




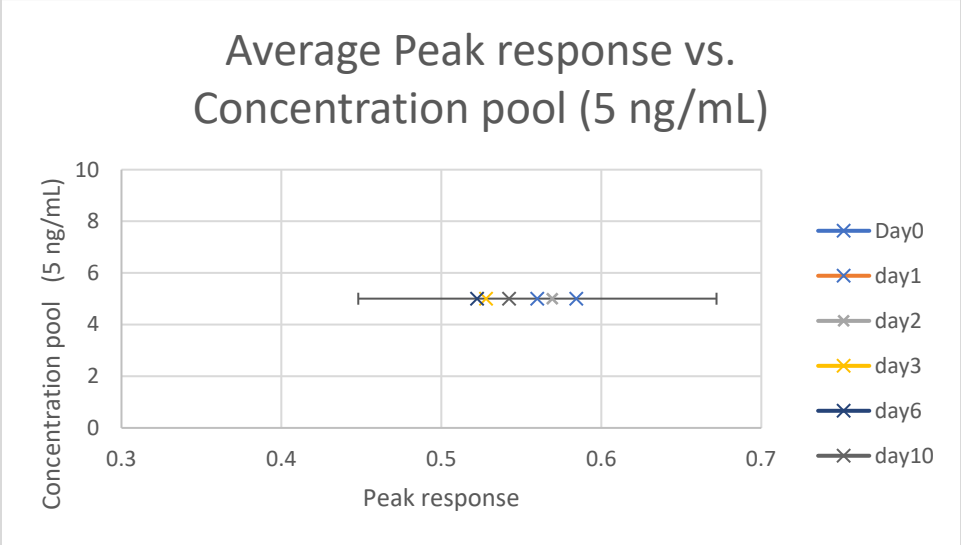
Stability Graphs 63-65 (blood): Clonazolam

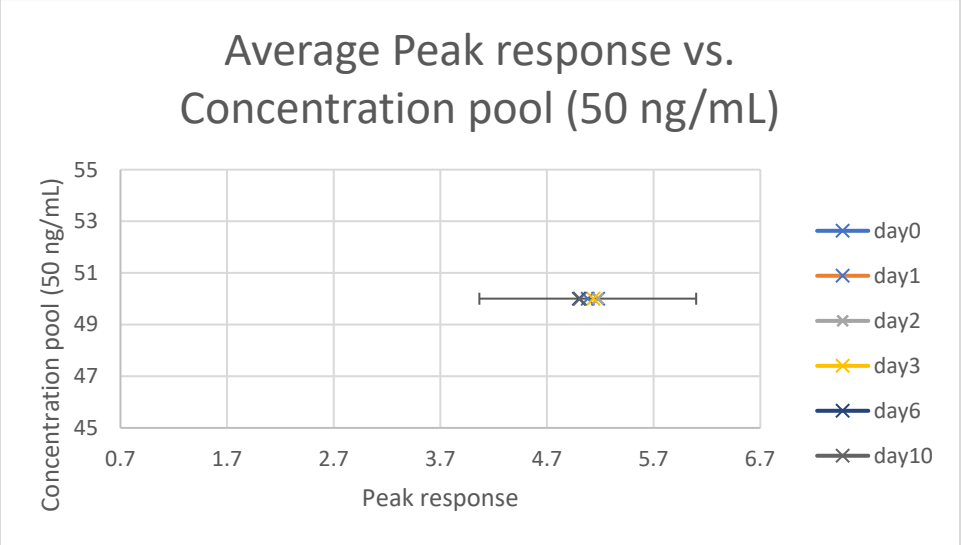


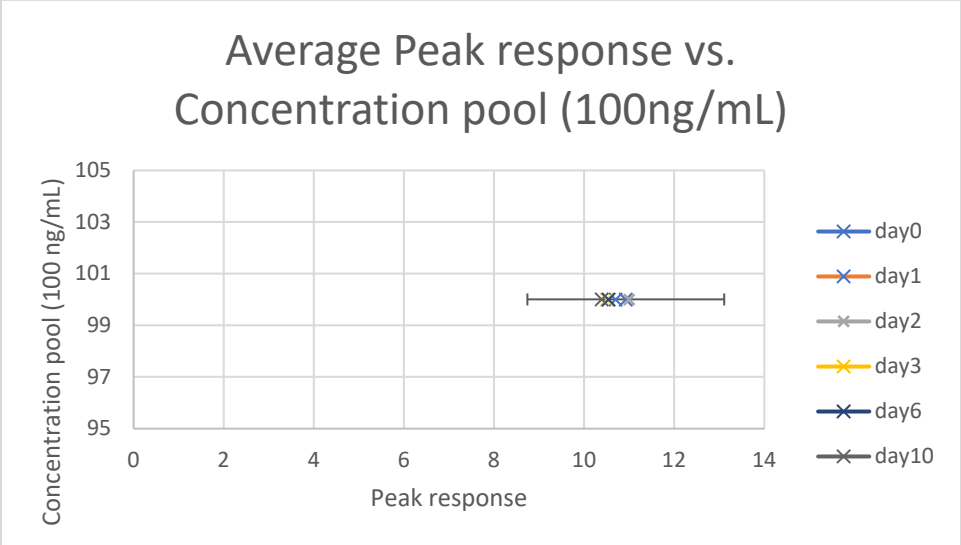




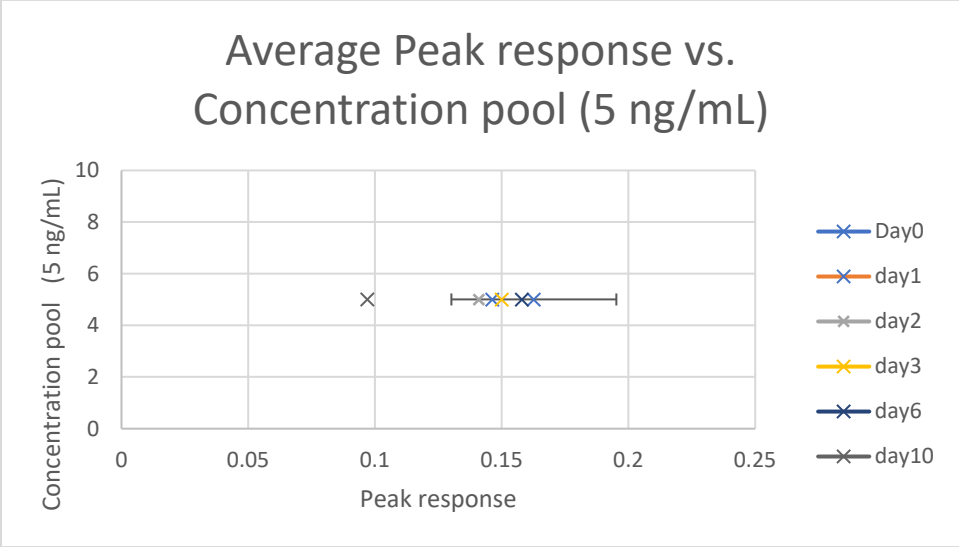
Stability Graphs 66-68 (blood): Delorazepam

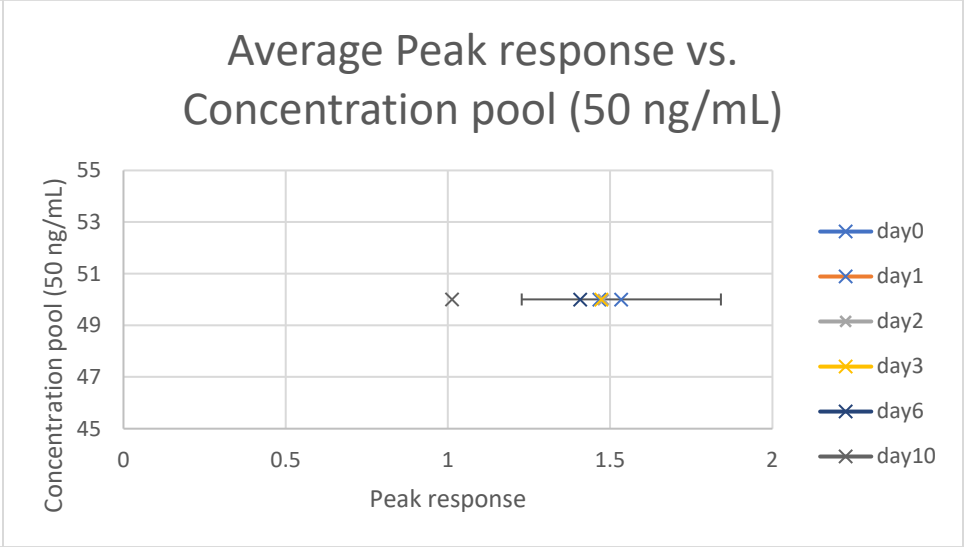


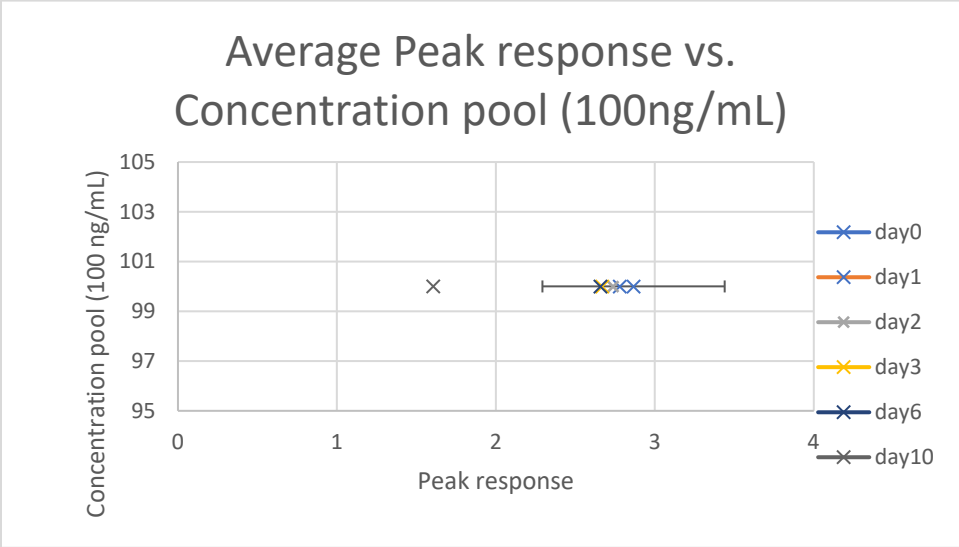




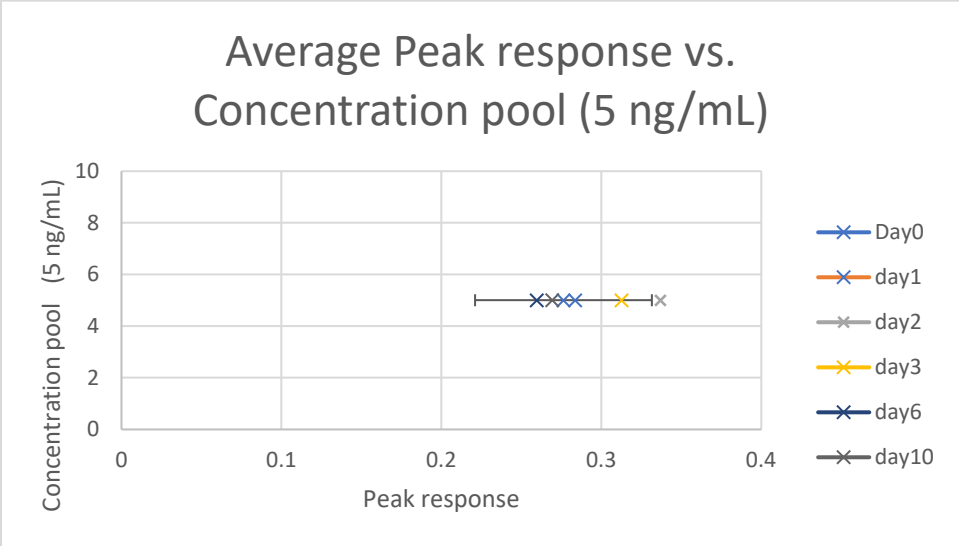
Stability Graphs 69-71 (blood): Demoxepam

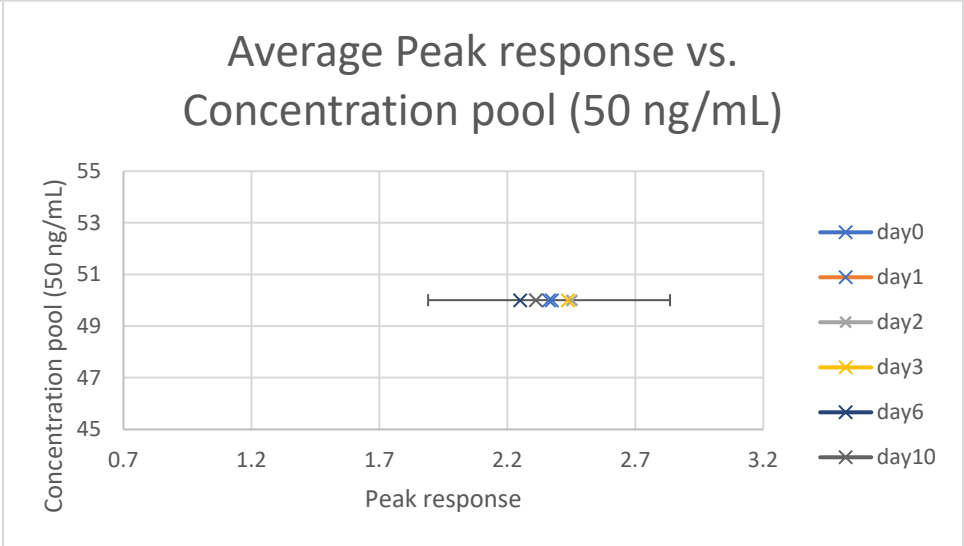


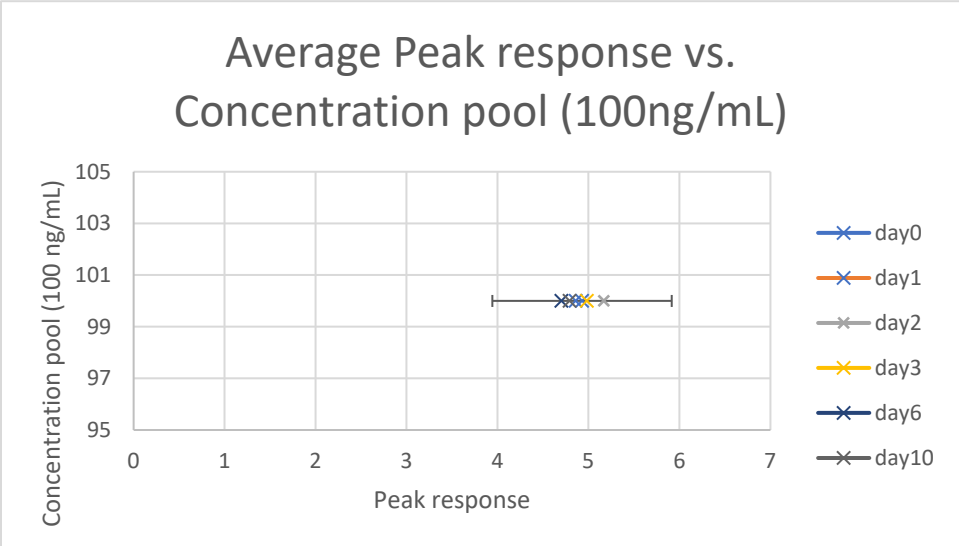




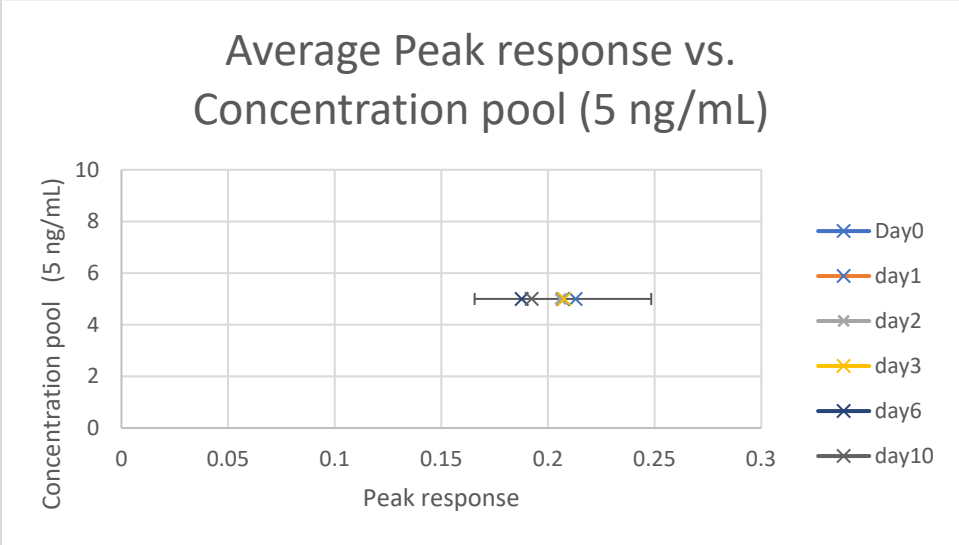
Stability Graphs 72-74 (blood): Diclazepam

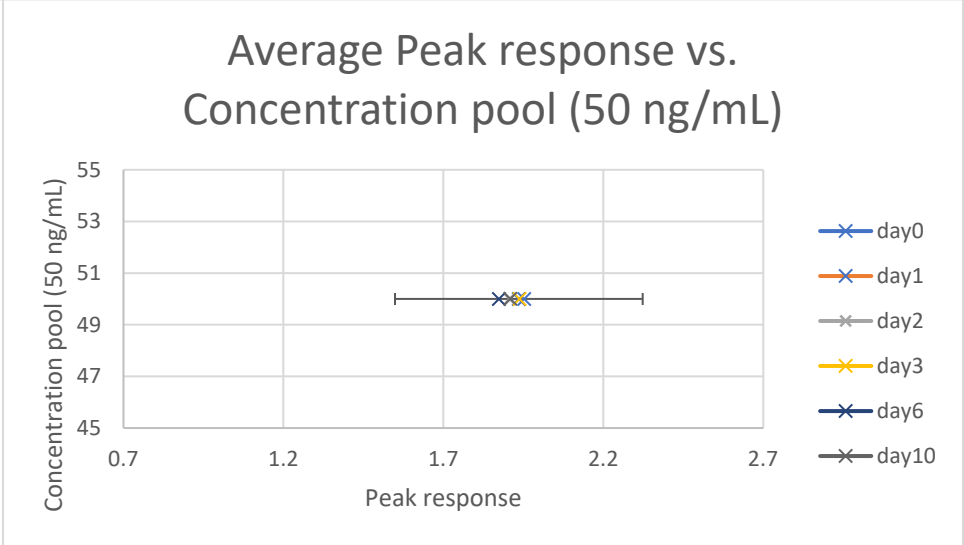


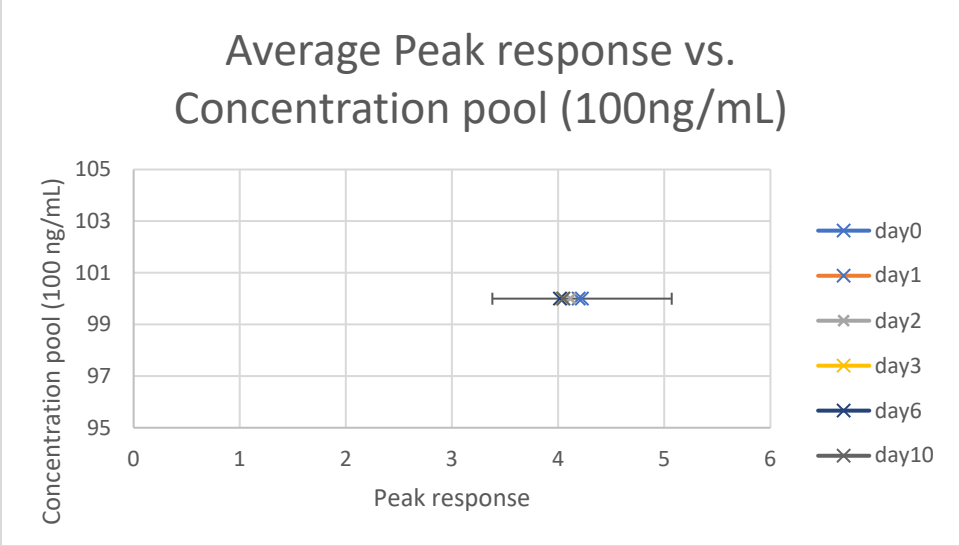




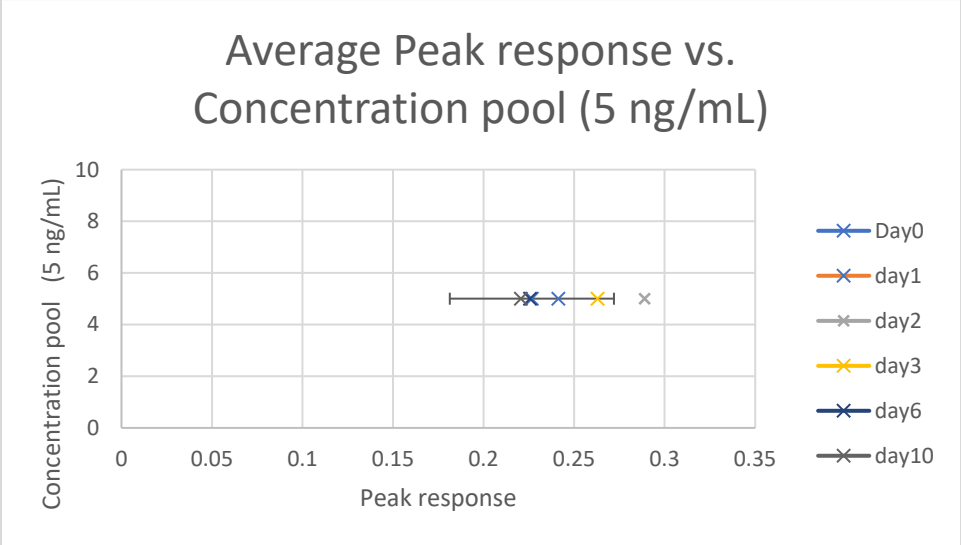
Stability Graphs 75-77 (blood): Estazolam

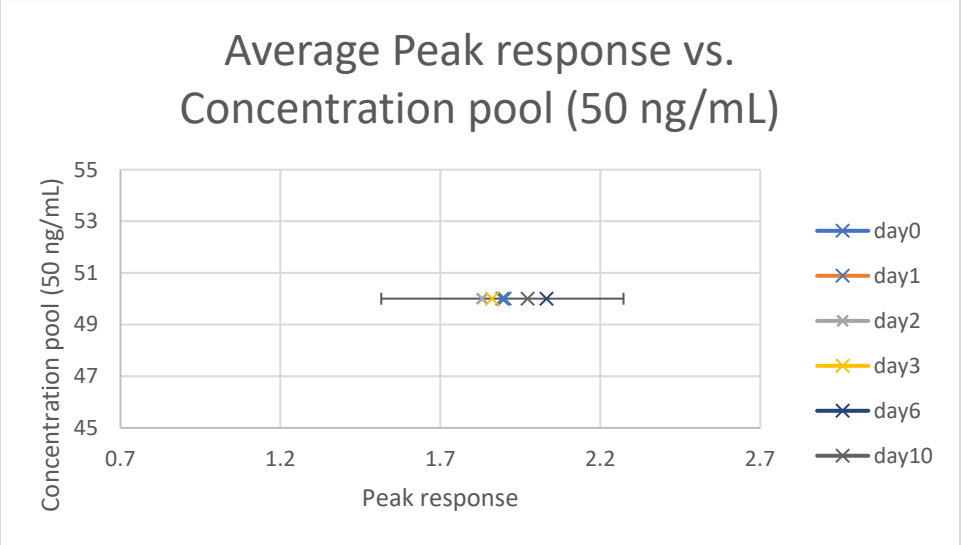


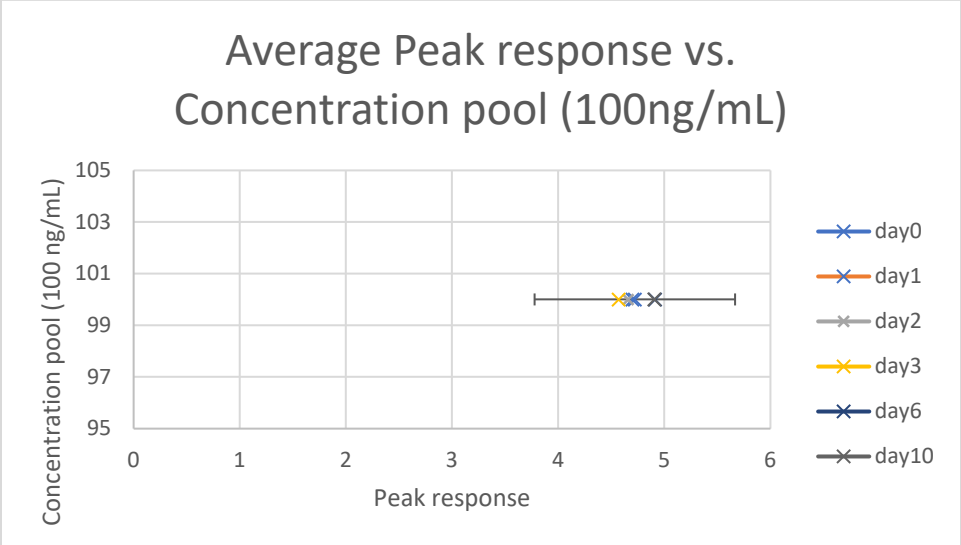




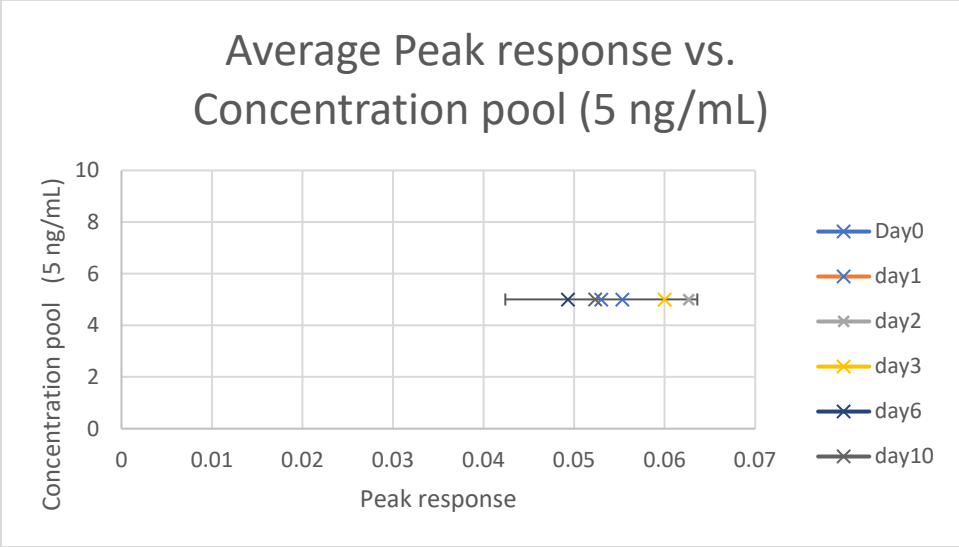
Stability Graphs 78-80 (blood): Flualprazolam

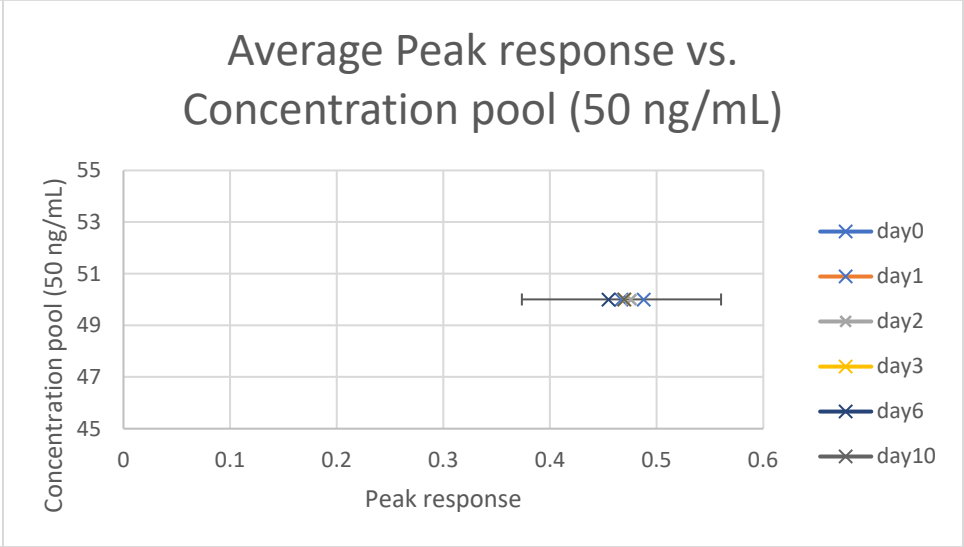


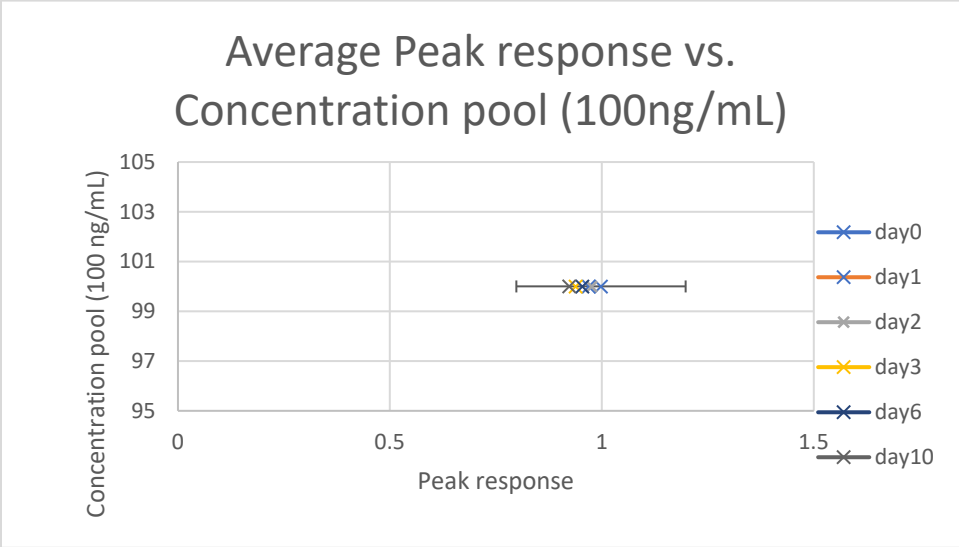




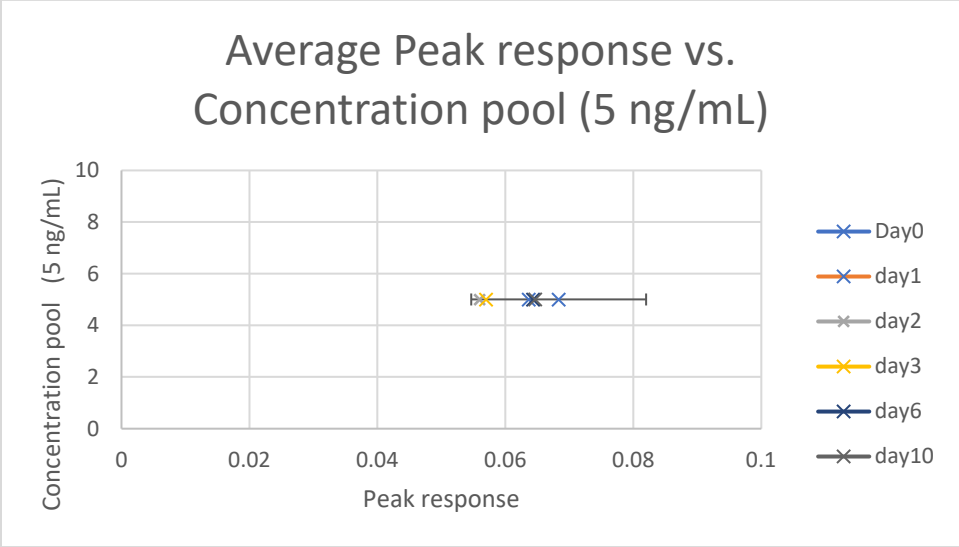
Stability Graphs 81-83 (blood): Flubromazepam

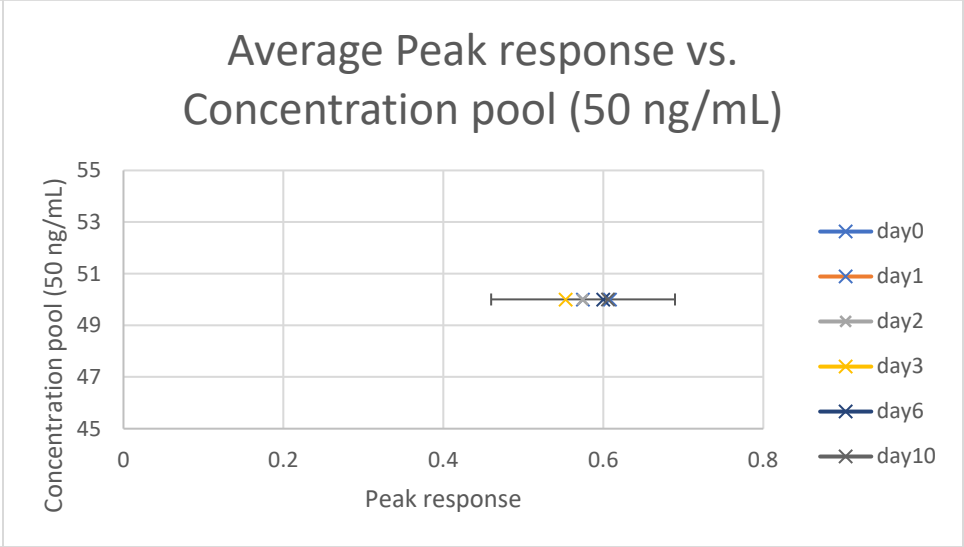


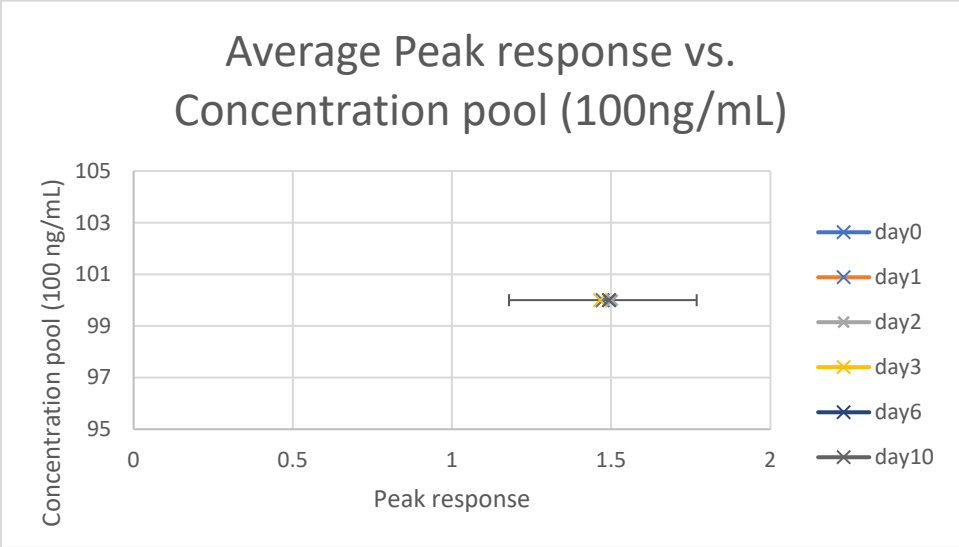




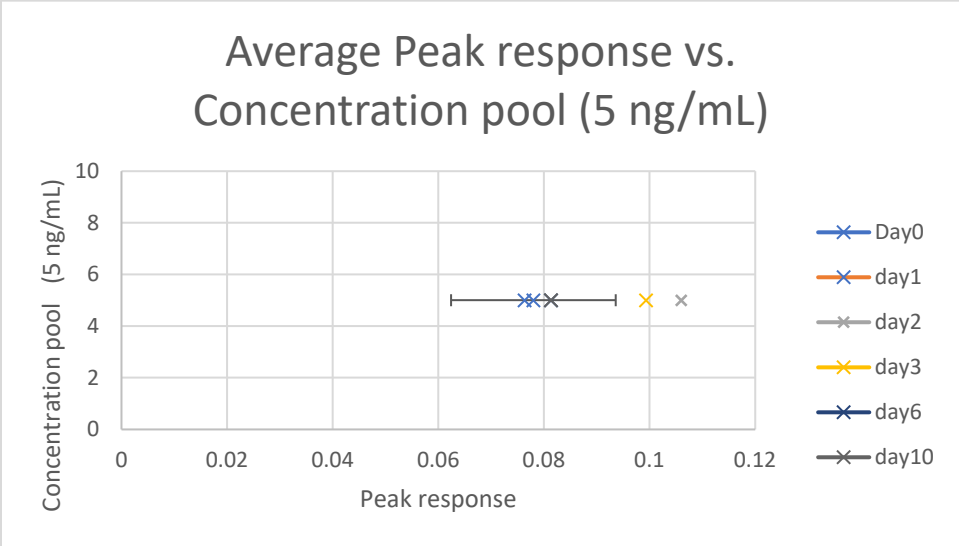
Stability Graphs 84-86 (blood): Flubromazolam

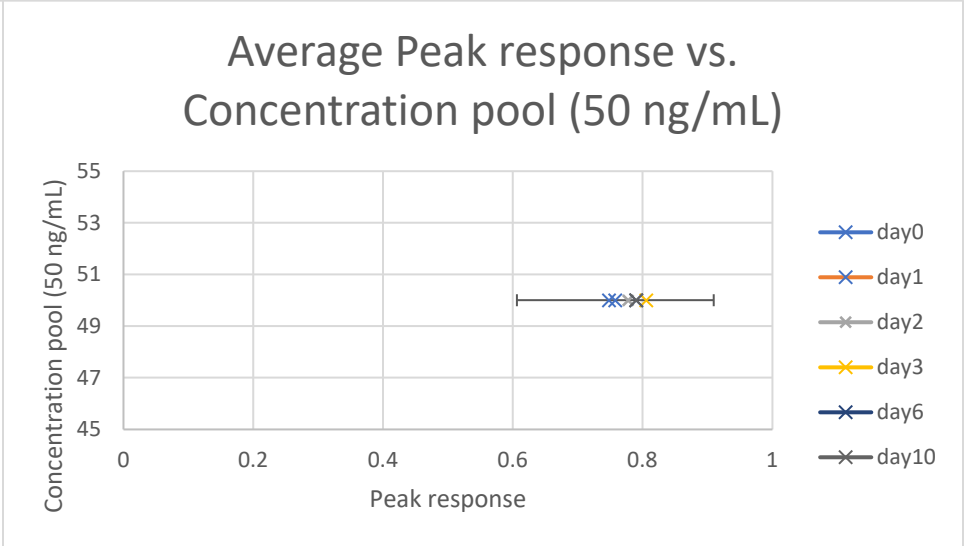


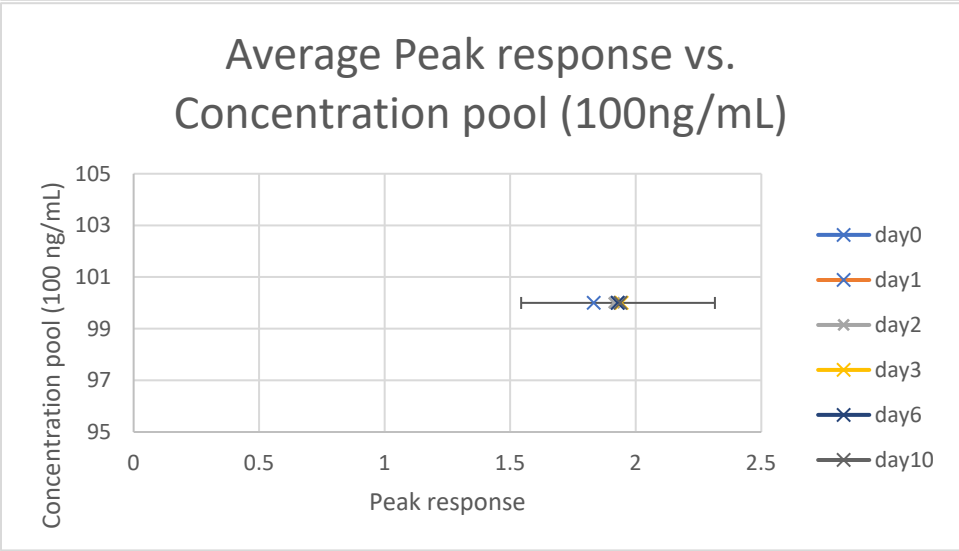




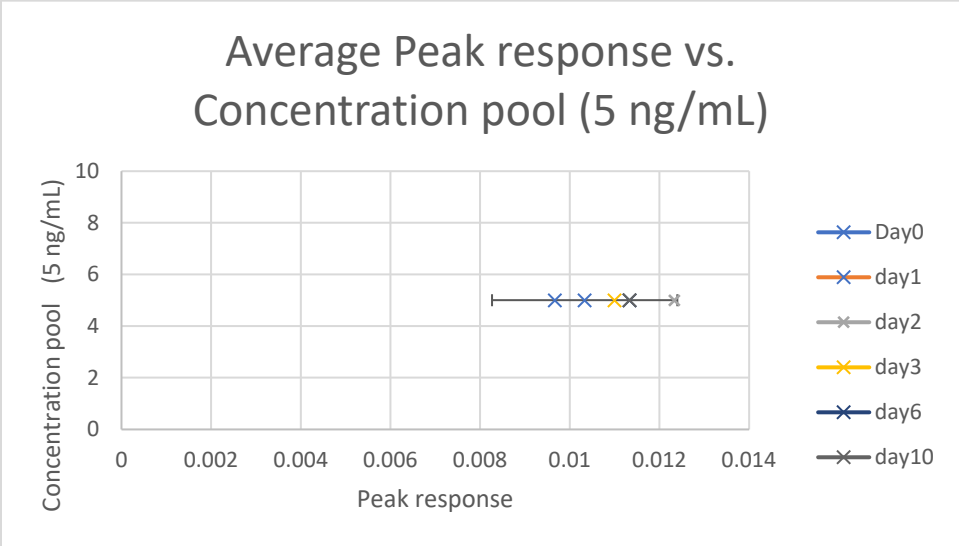
Stability Graphs 87-89 (blood): Flurazepam

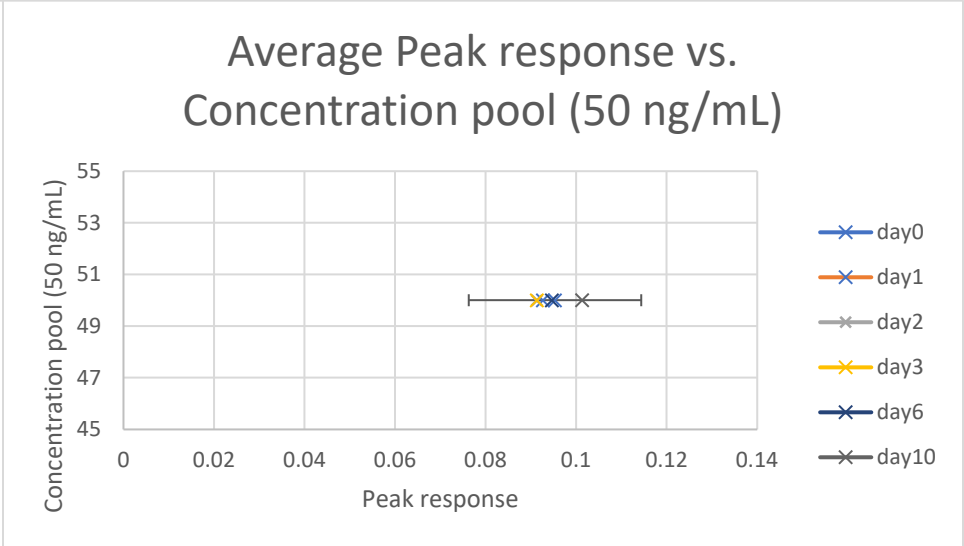


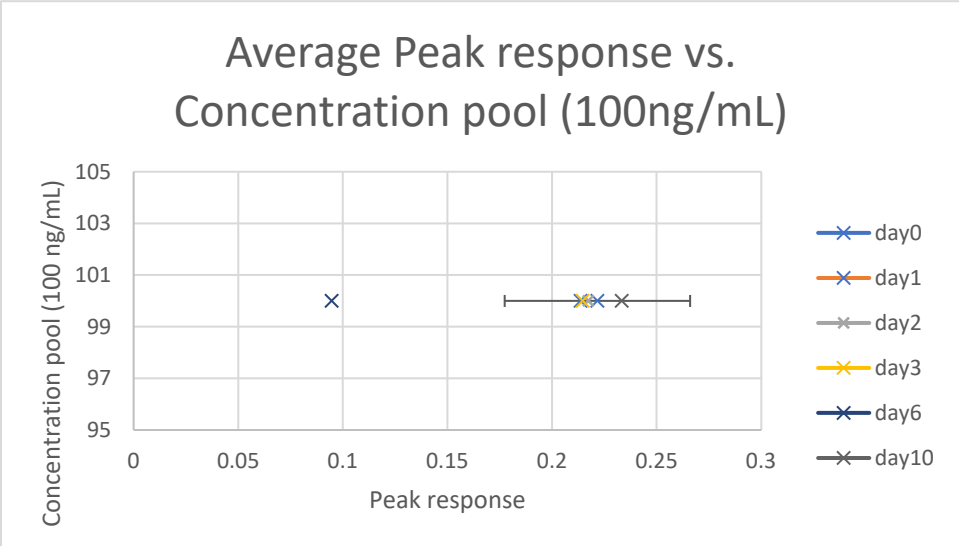




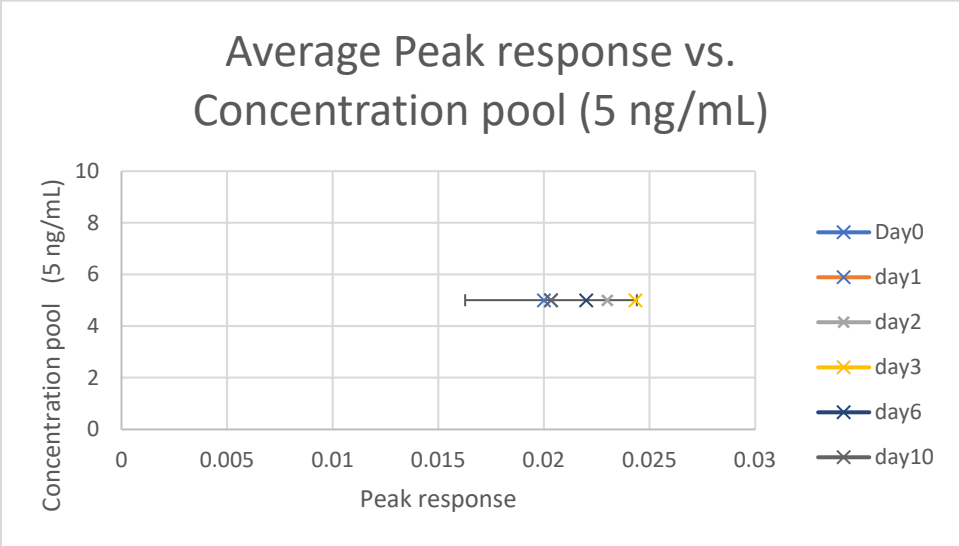
Stability Graphs 90-92 (blood): Lormetazepam

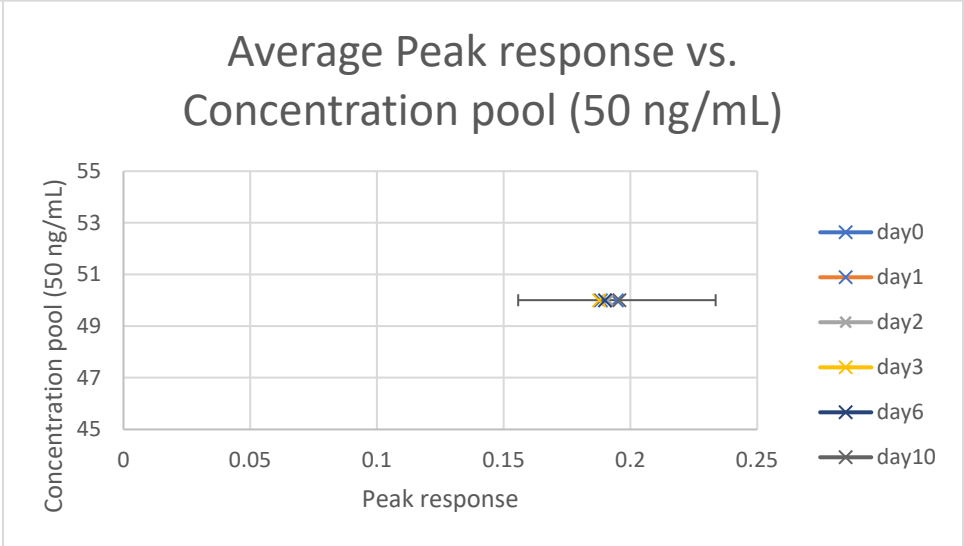


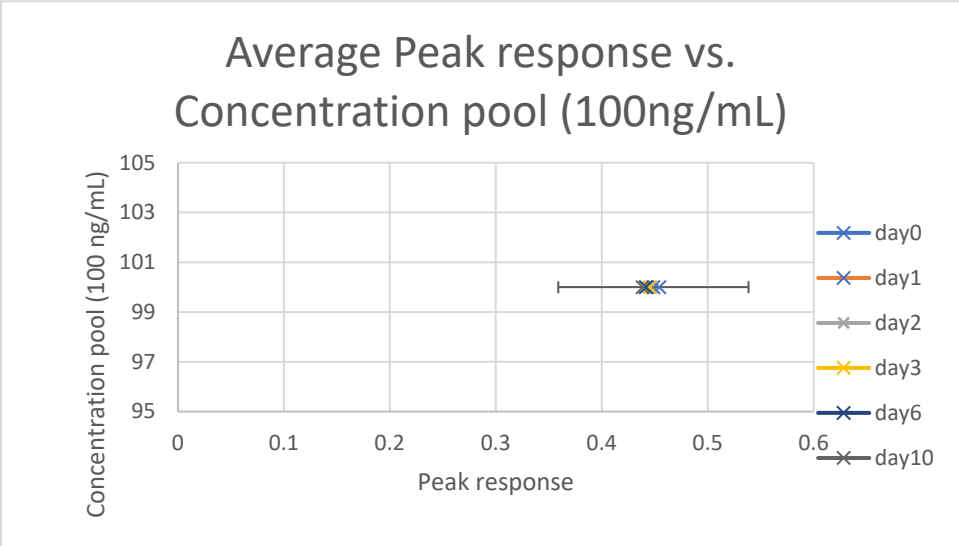




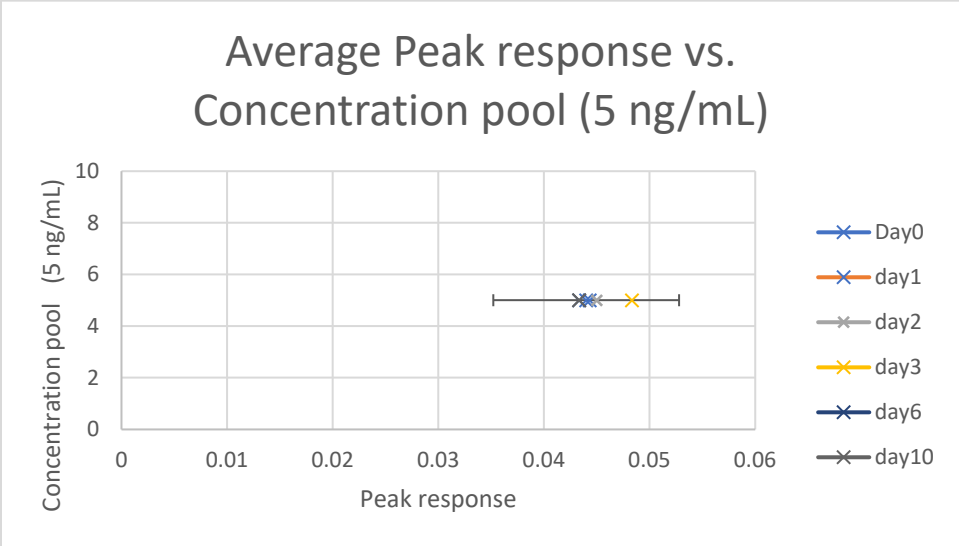
Stability Graphs 93-95 (blood): Nimetazepam

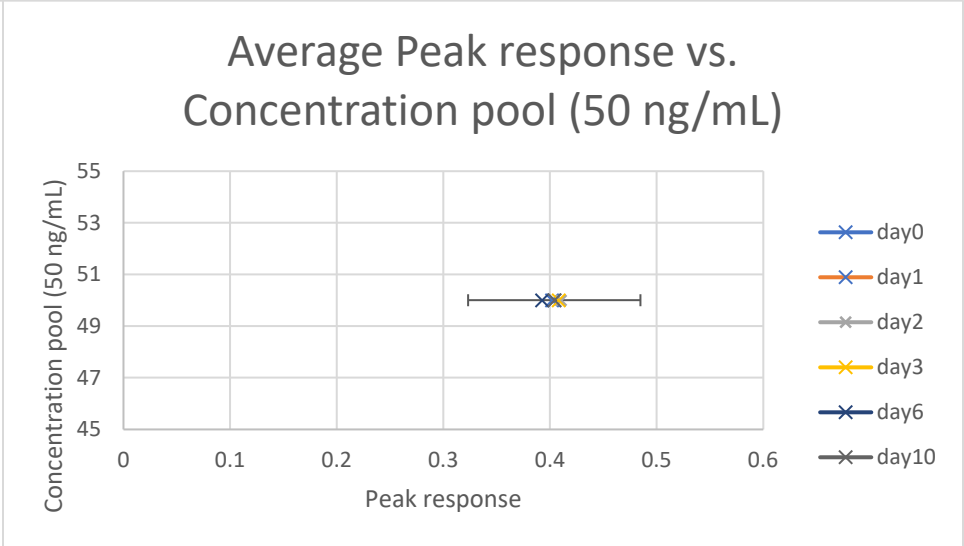


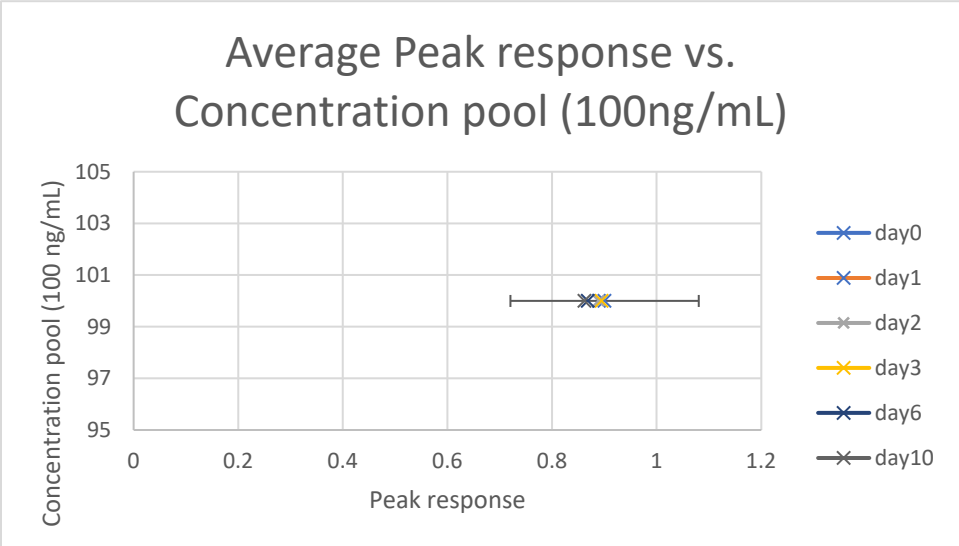




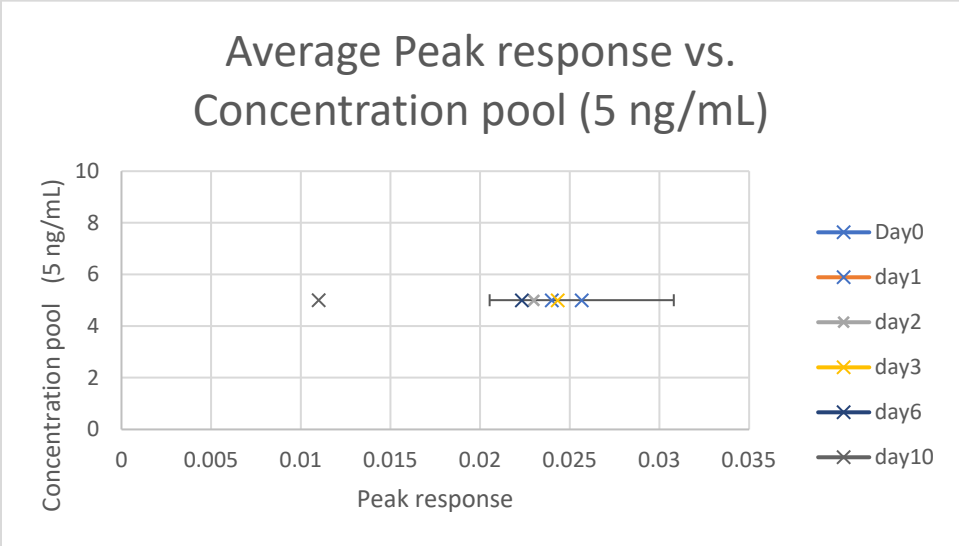
Stability Graphs 96-98 (blood): Nitrazepam

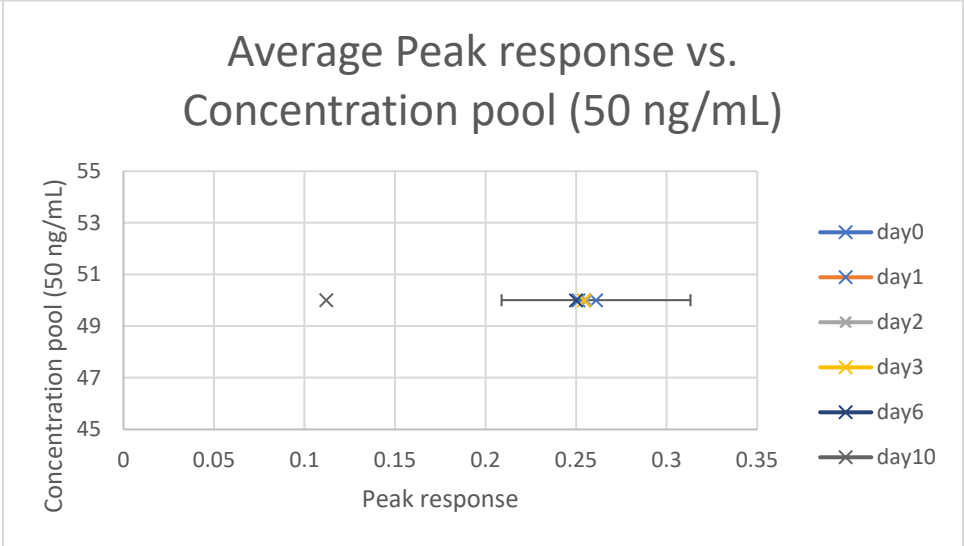


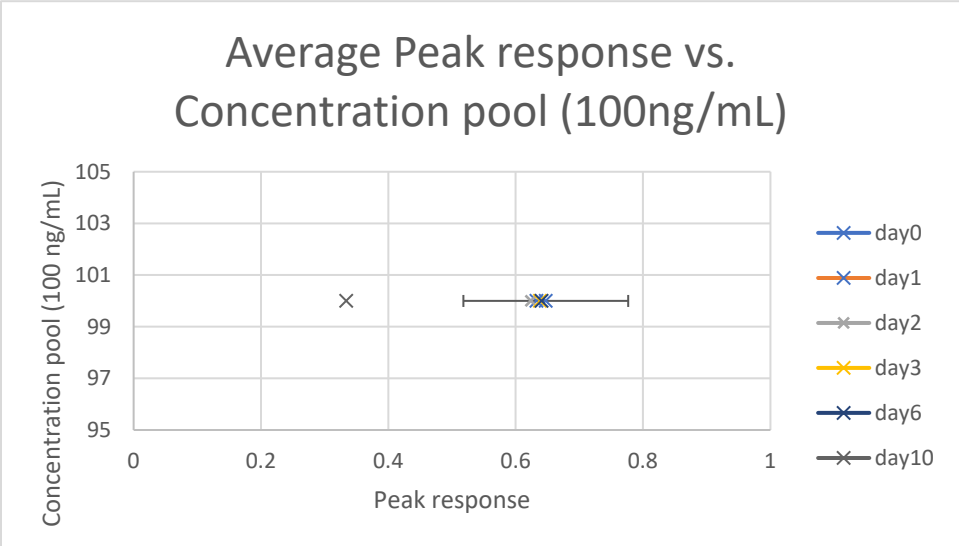




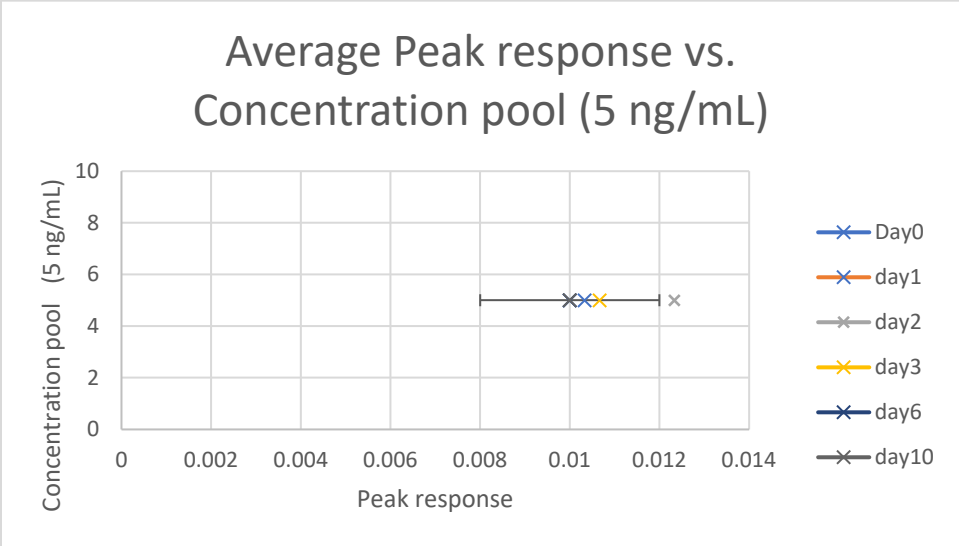
Stability Graphs 99-101 (blood): Norchlordiazepoxide

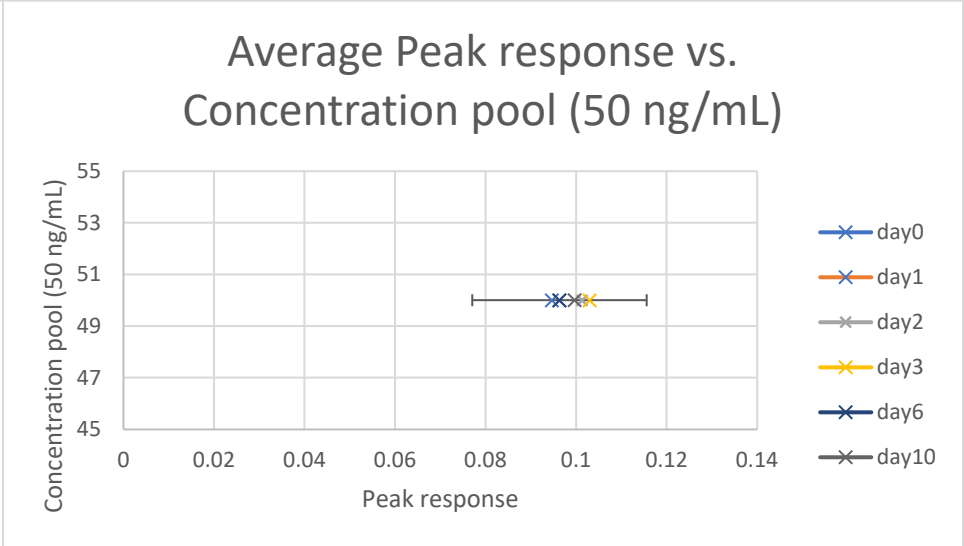


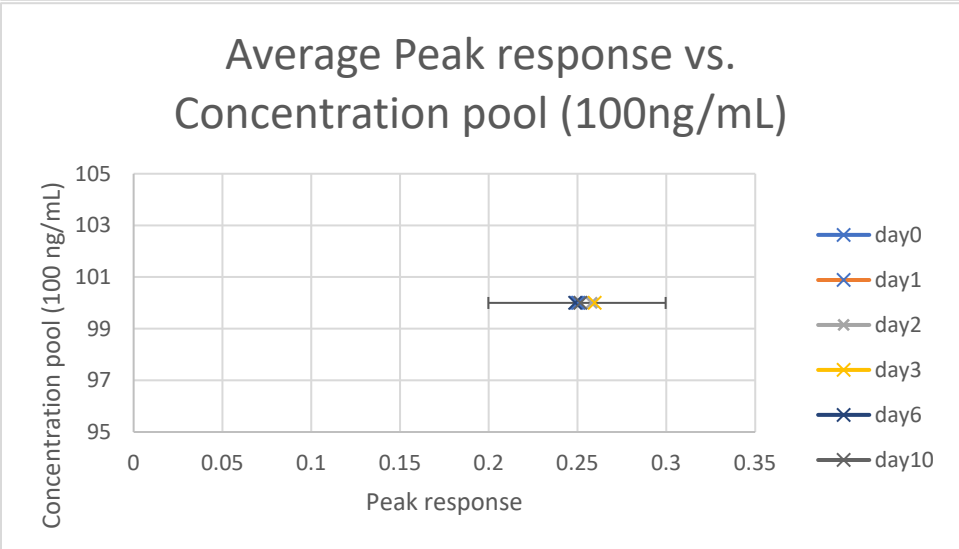




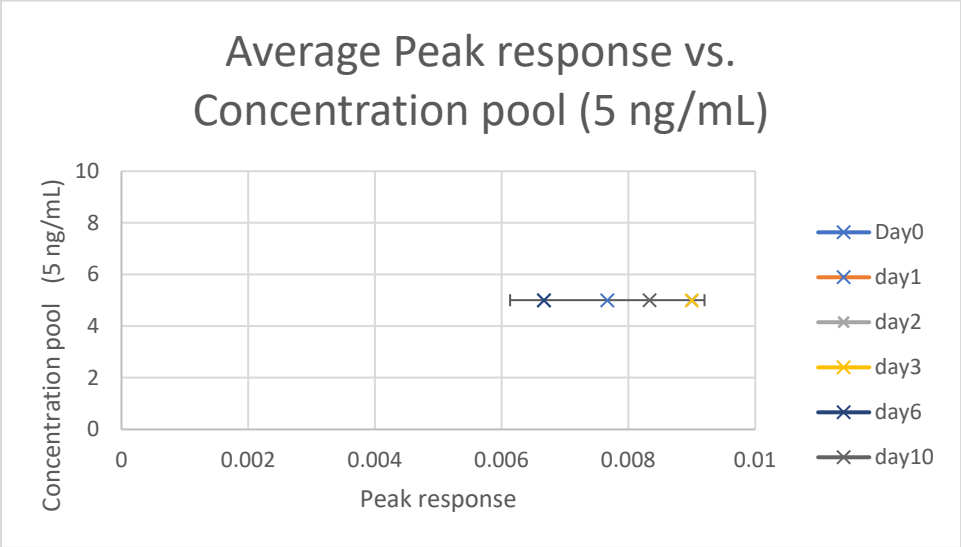
Stability Graphs 102-104 (blood): Phenazepam

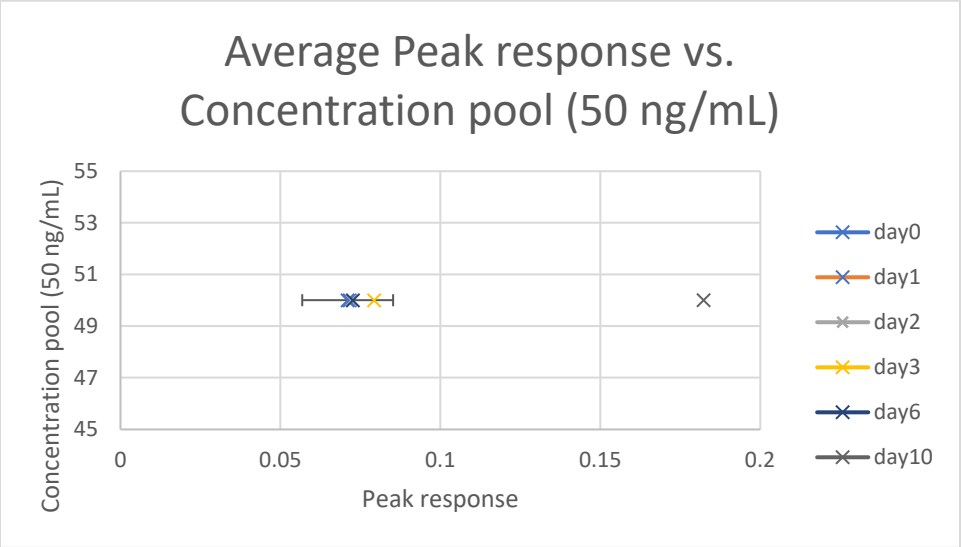


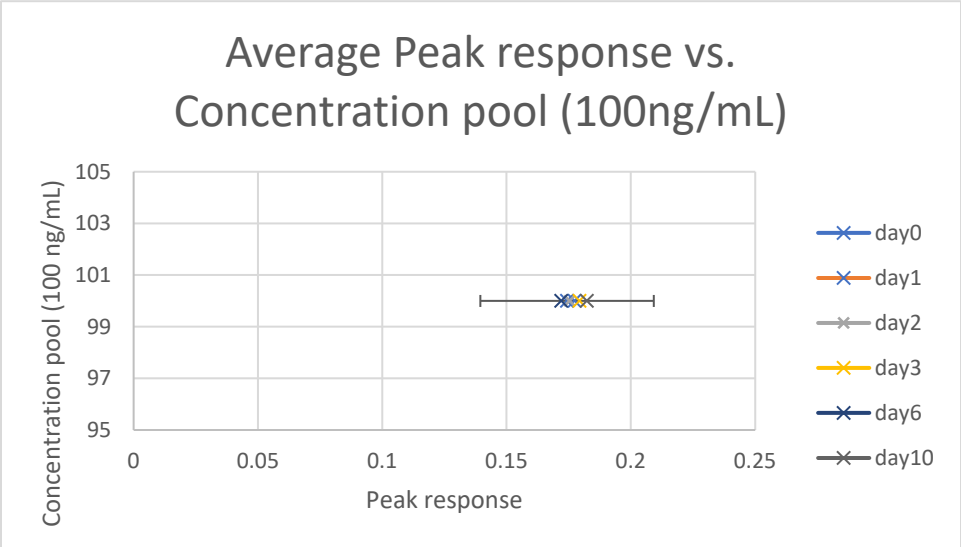




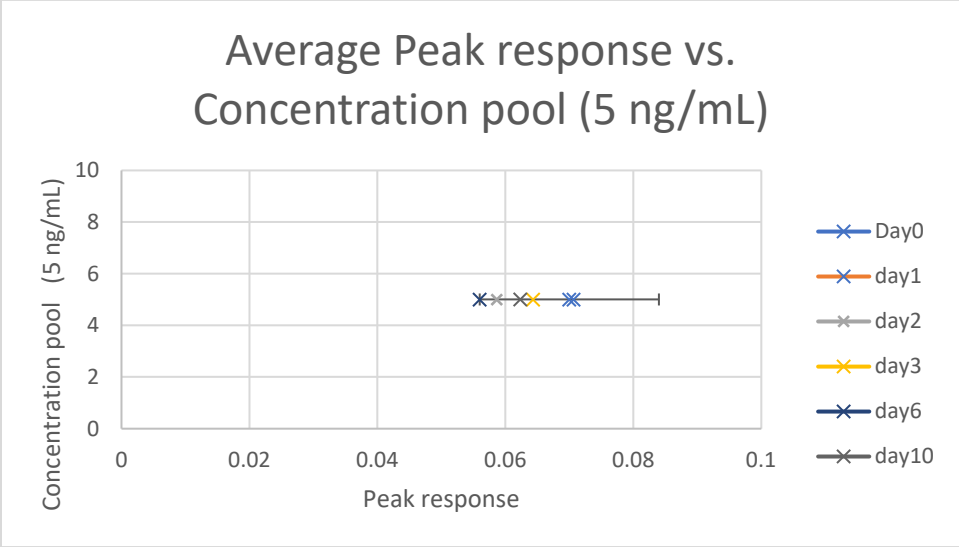
Stability Graphs 105-107 (blood): Pyrazolam

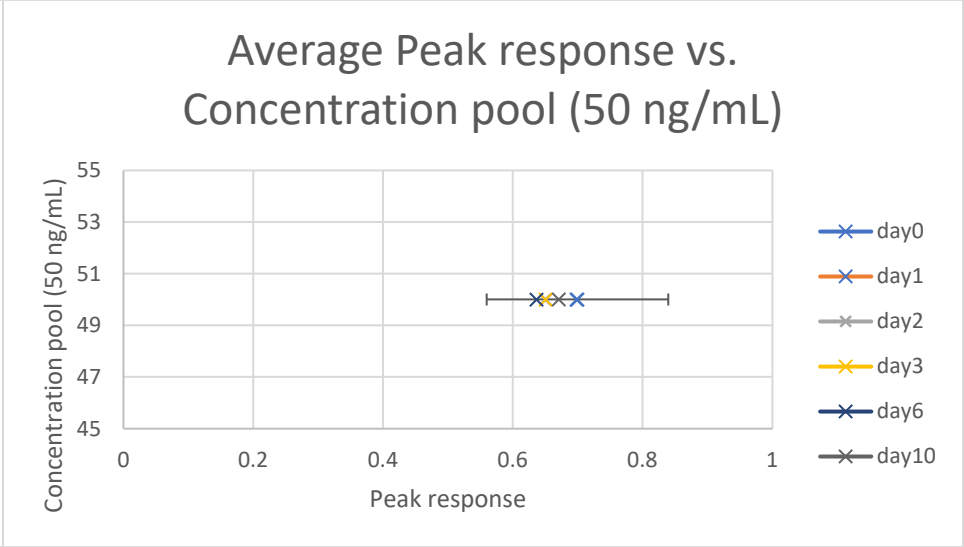


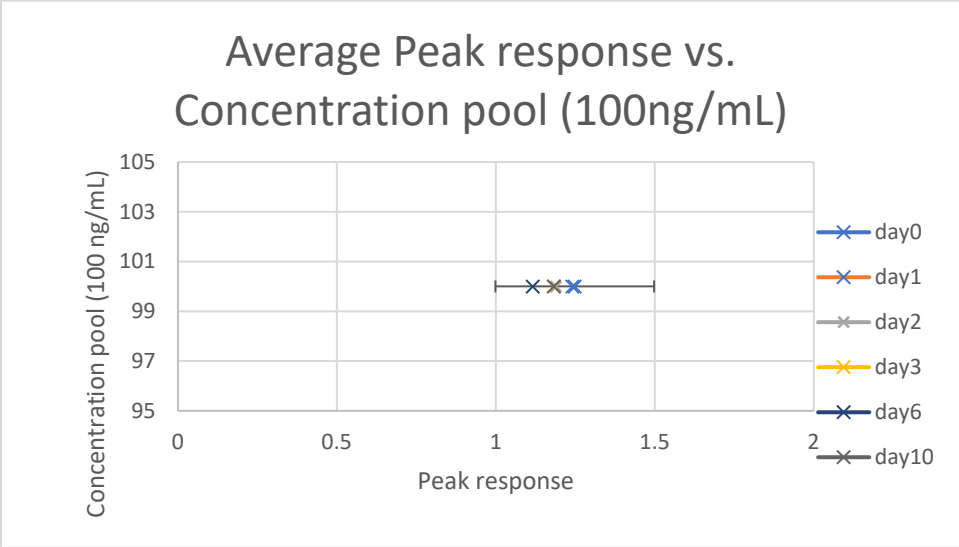




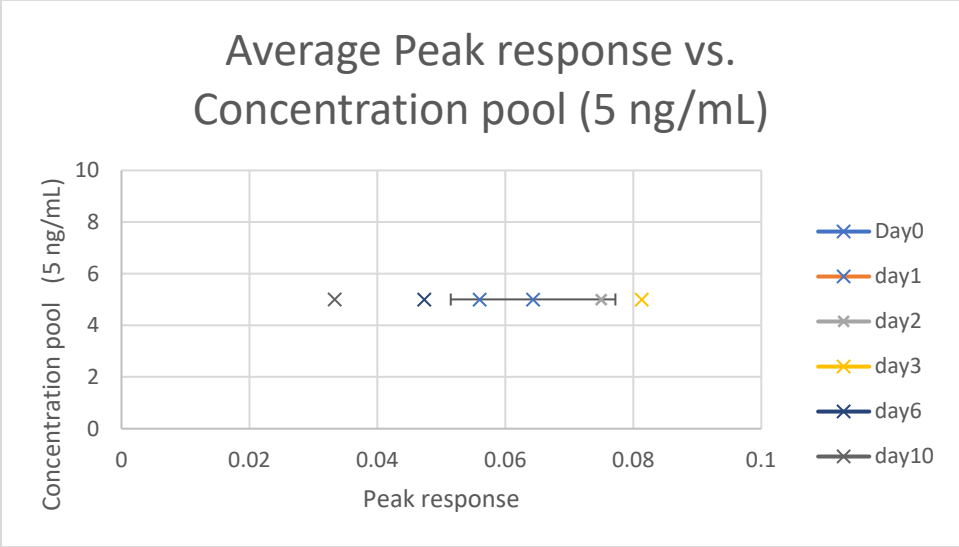
Stability Graphs 108-110 (blood): Zaleplon

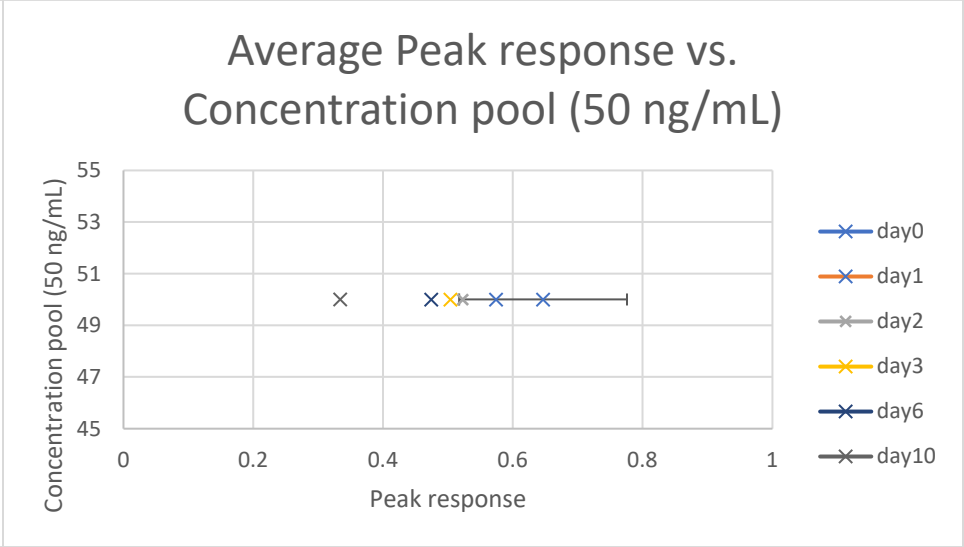


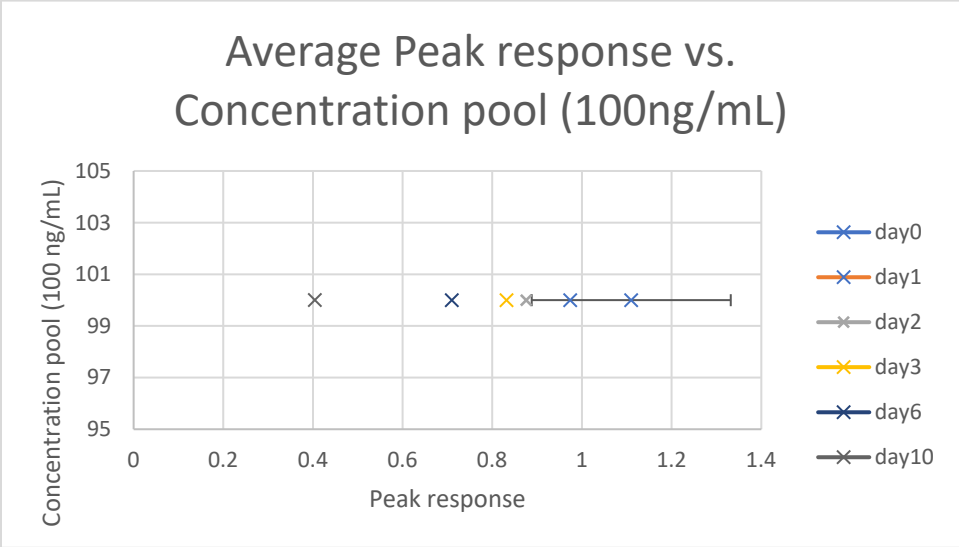




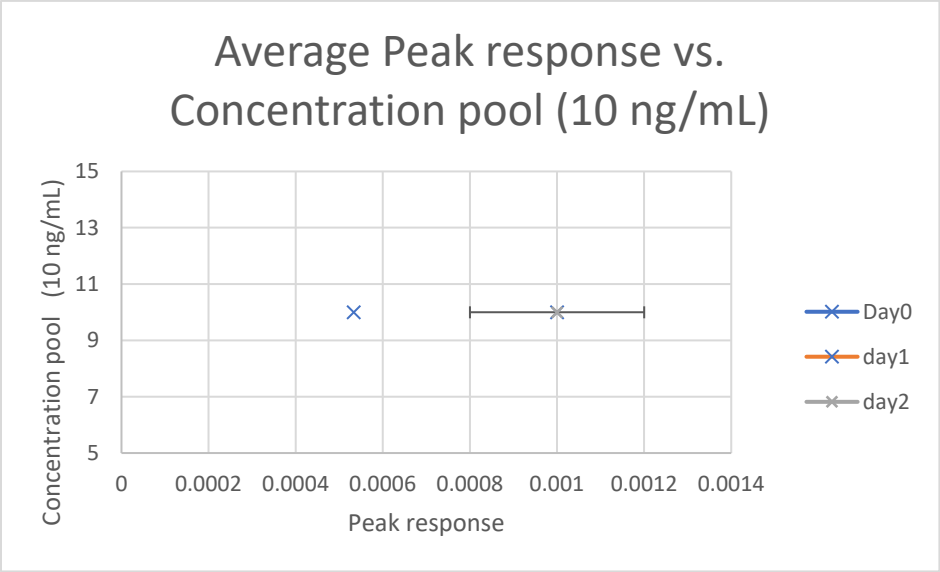
Stability Graphs 111-113 (blood): Zopiclone

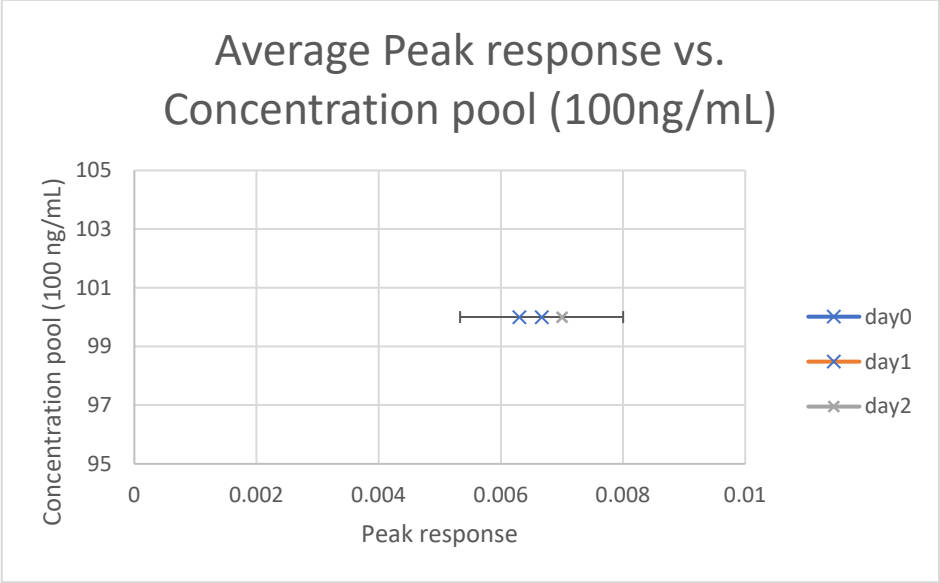


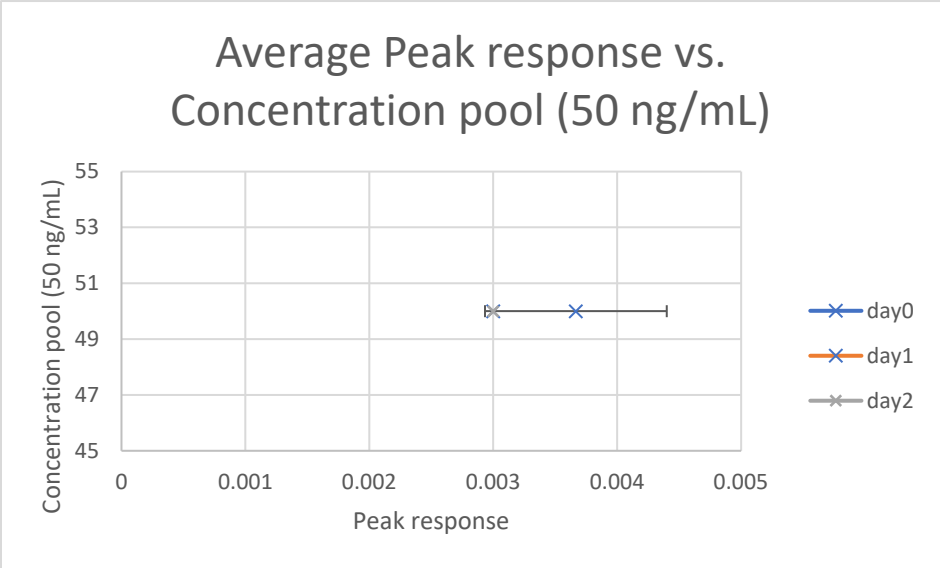




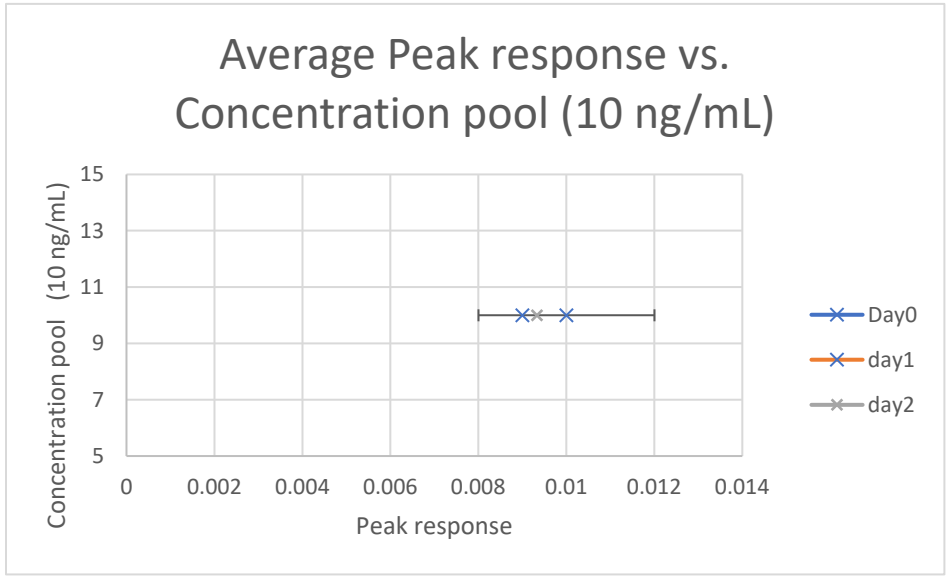
Stability Graphs 114-116 (blood): Adinazolam

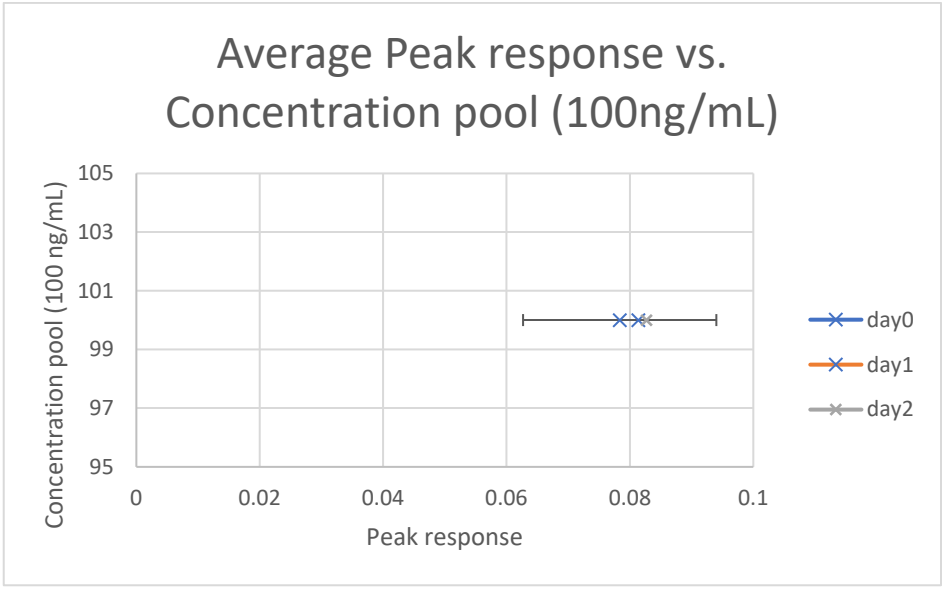


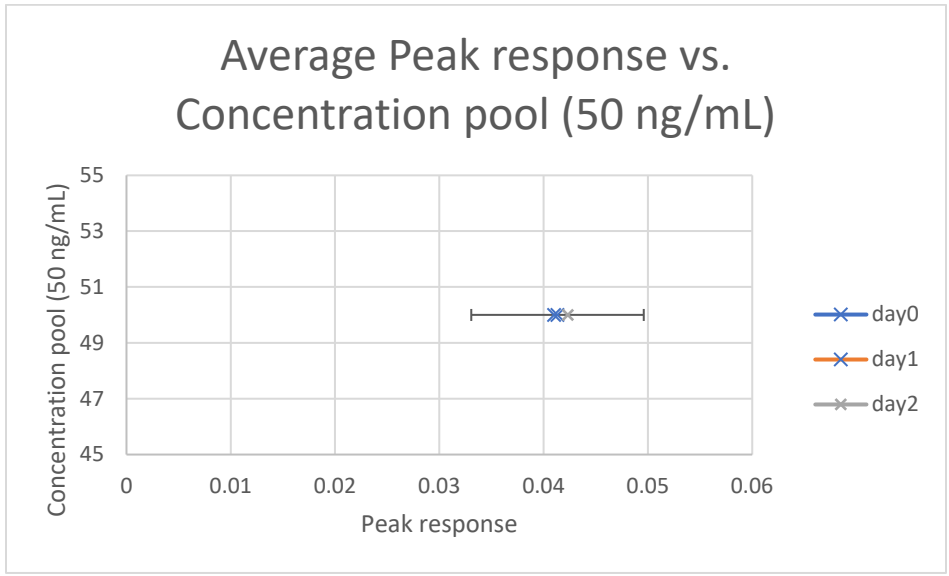




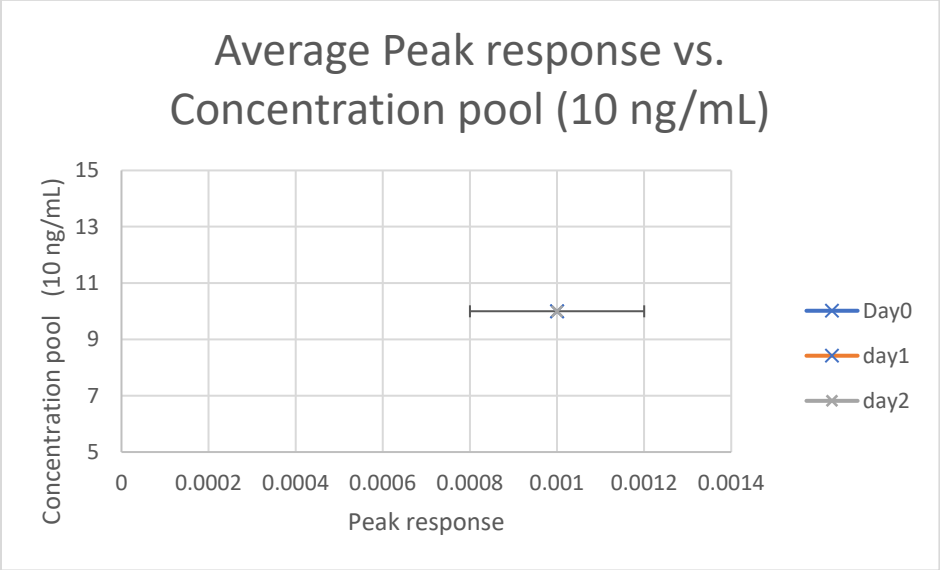
Stability Graphs 117-119 (blood): 8-amino clonazolam

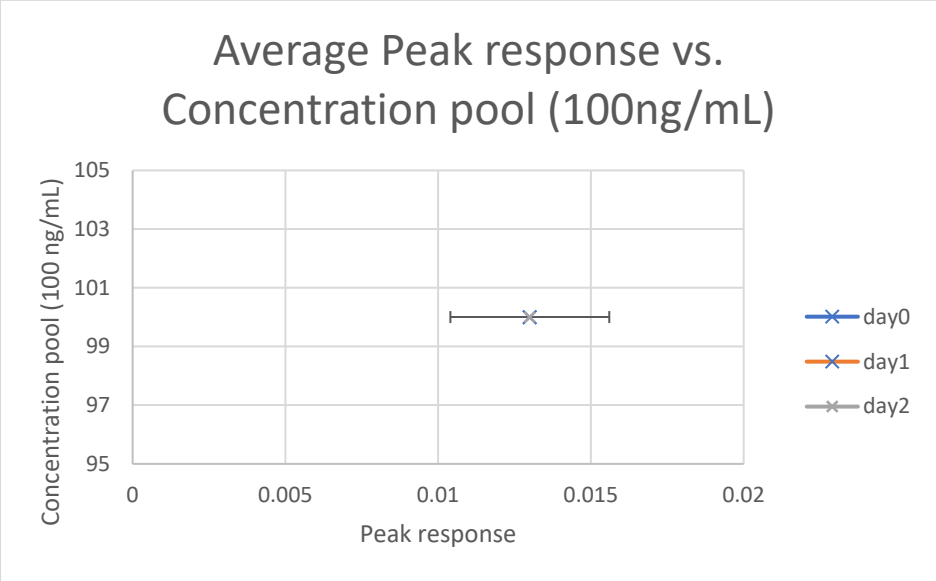


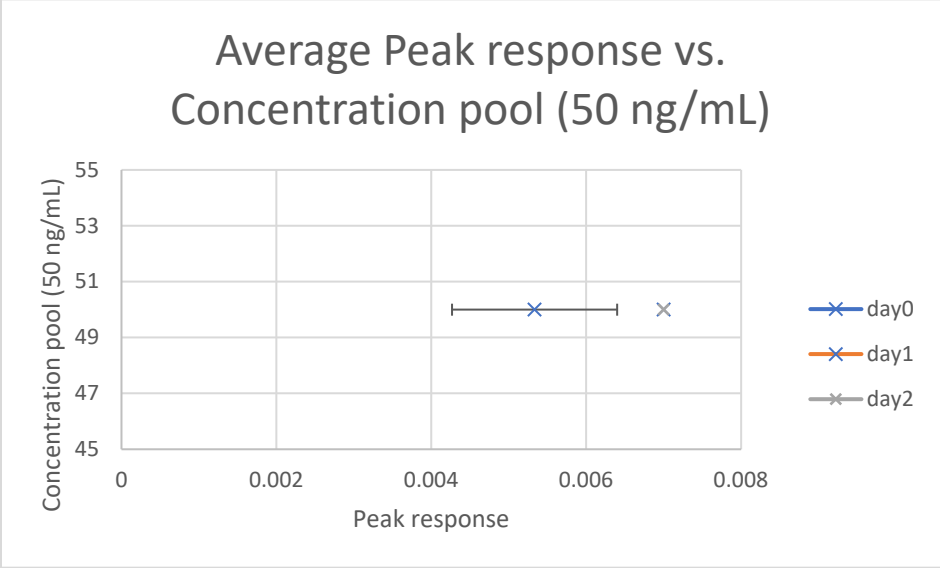




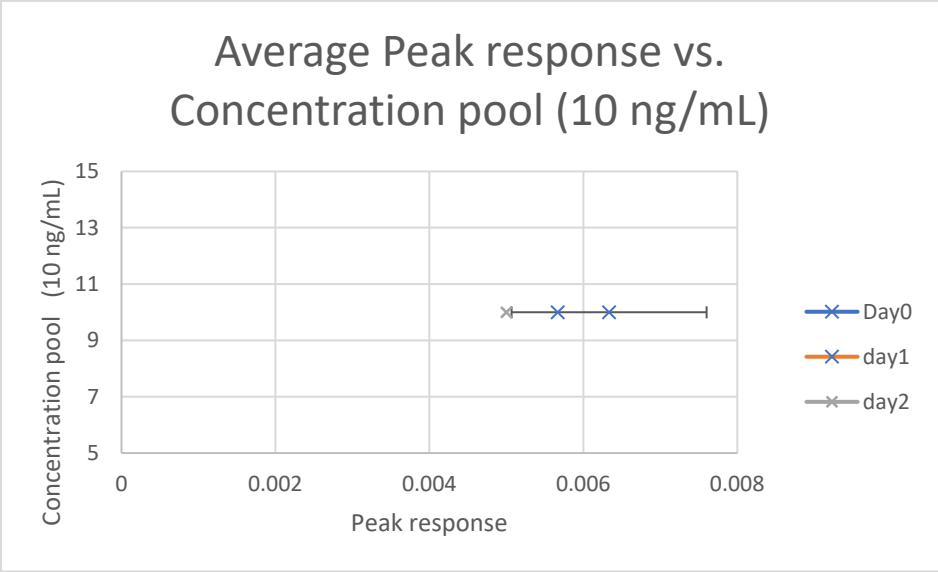
Stability Graphs 120-122 (blood): alpha hydroxy clonazolam

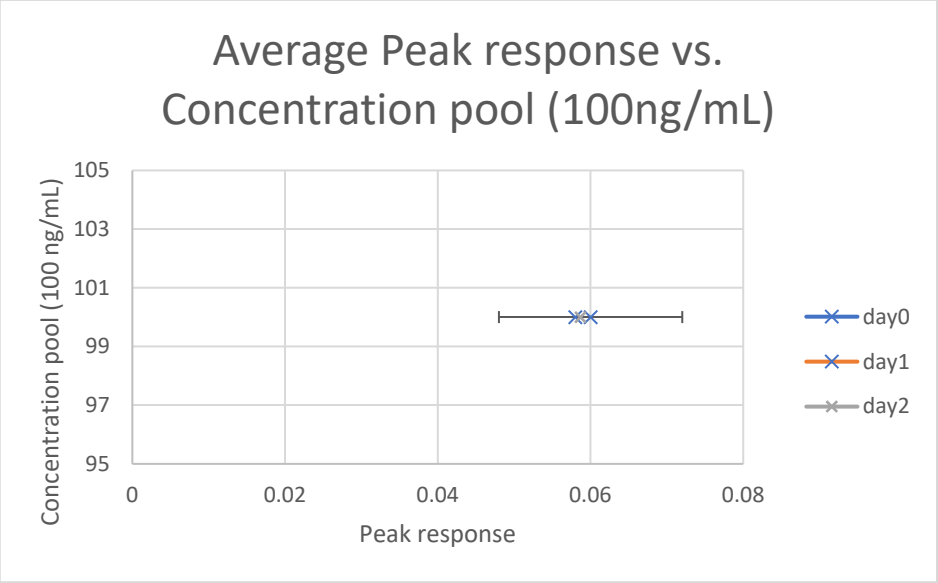


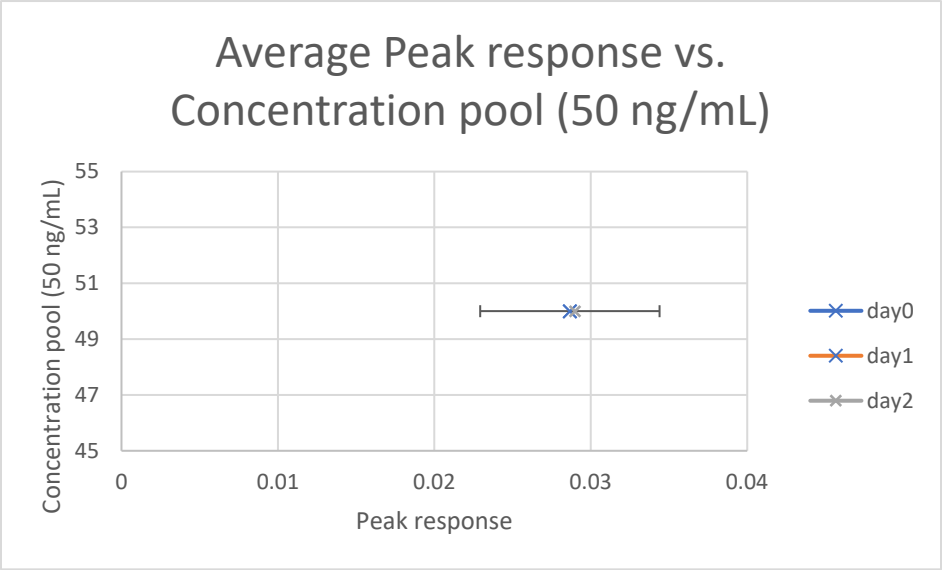




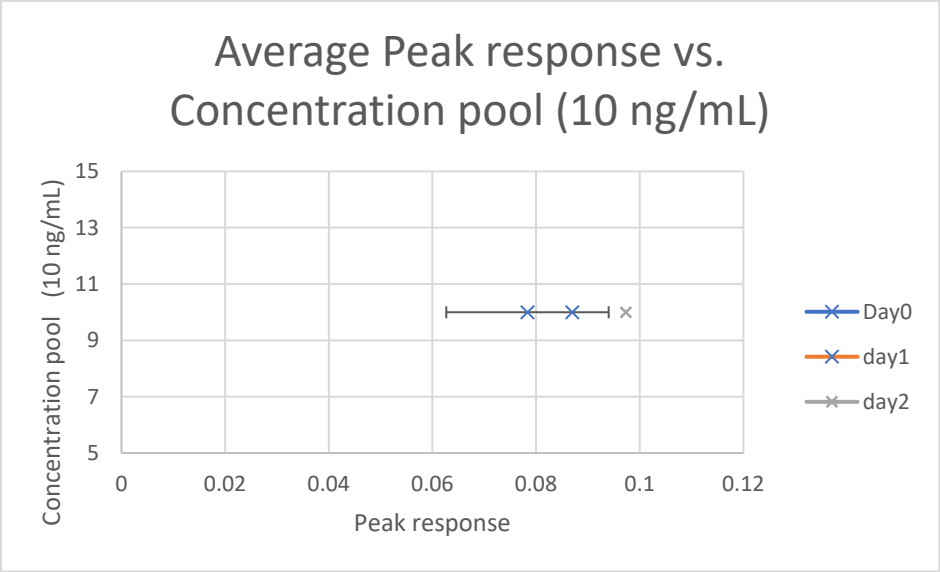
Stability Graphs 123-125 (blood): alpha hydroxy Flubromazolam

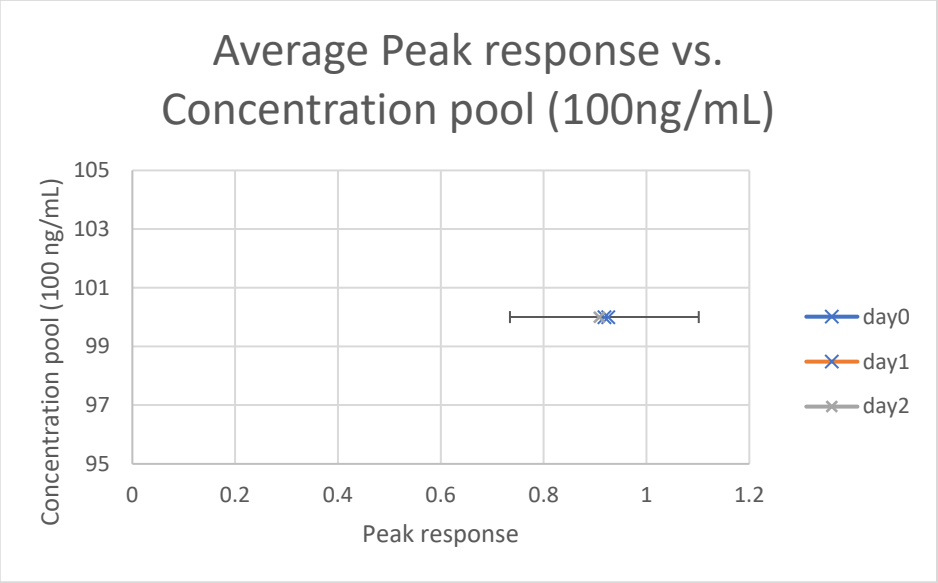


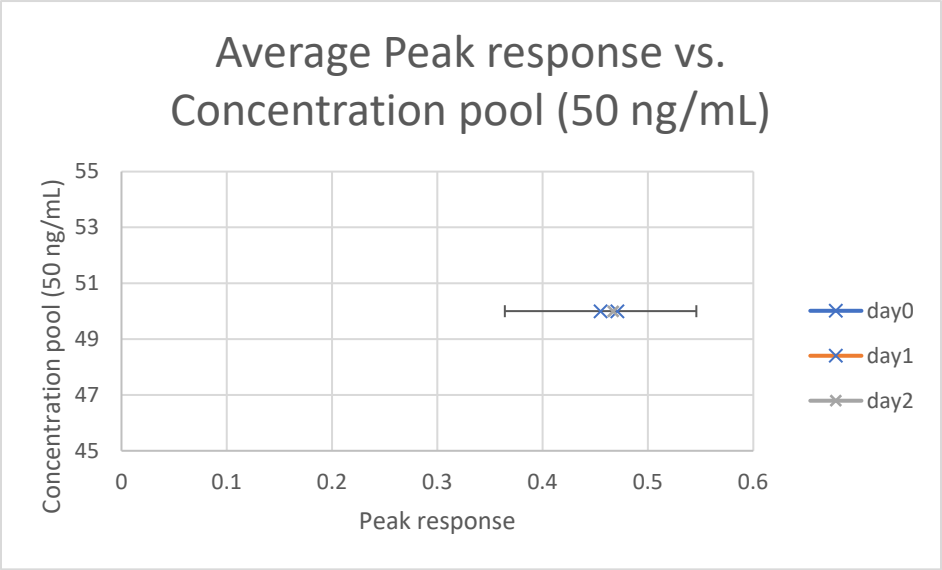




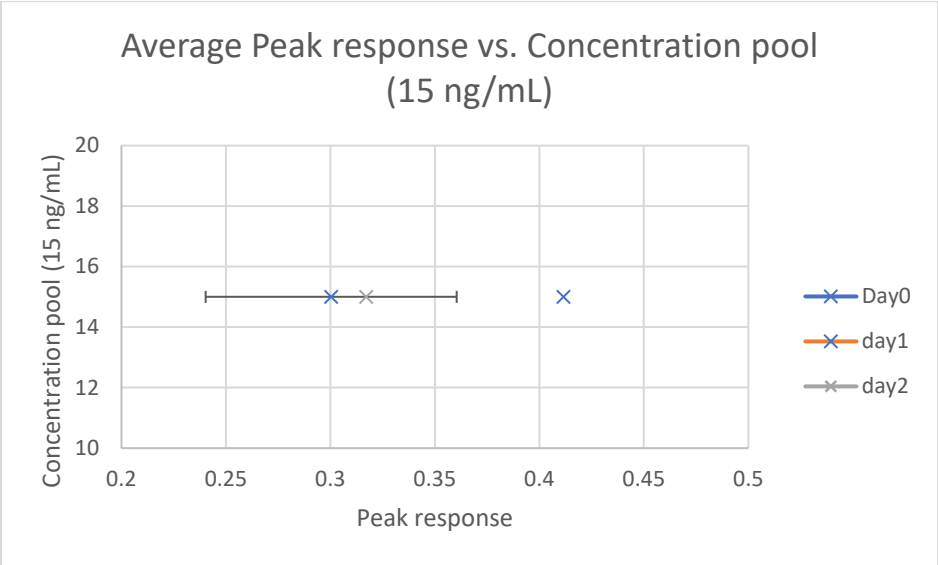
Stability Graphs 126-128 (blood): Bromazolam

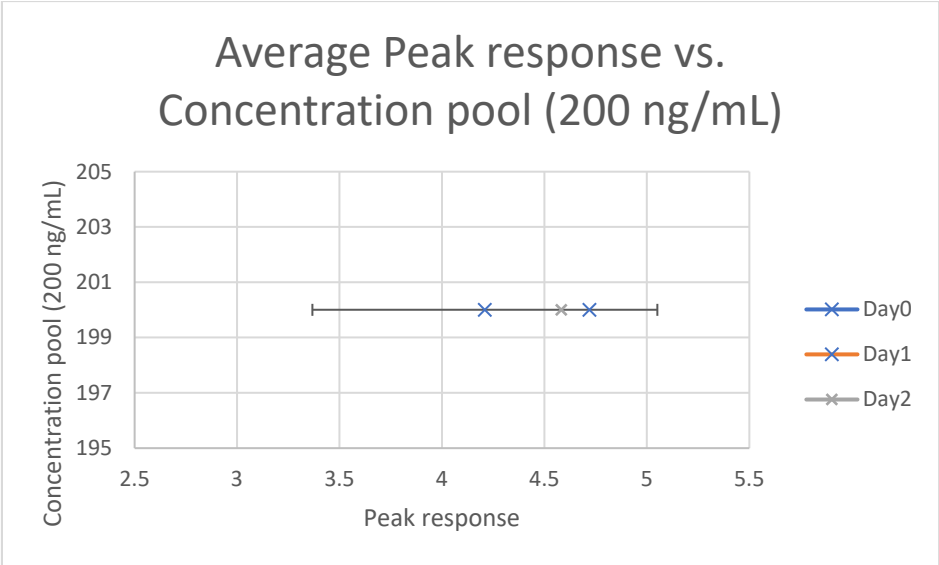


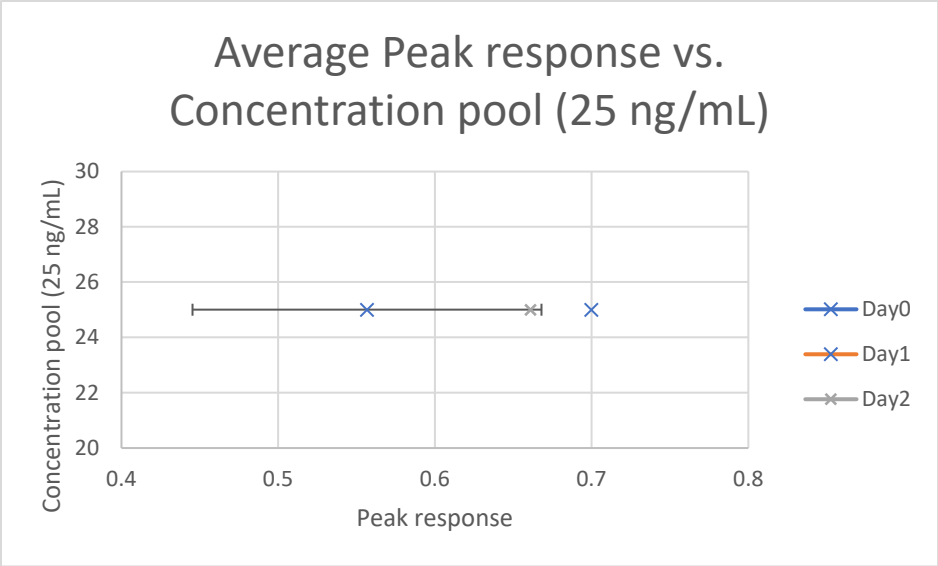




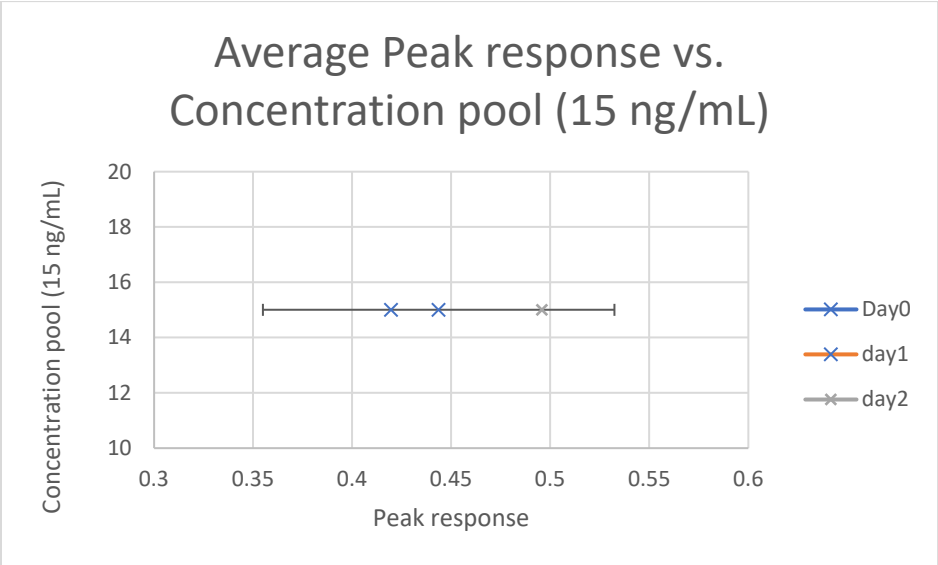
Stability Graphs 129-131 (urine): 7-amino clonazepam

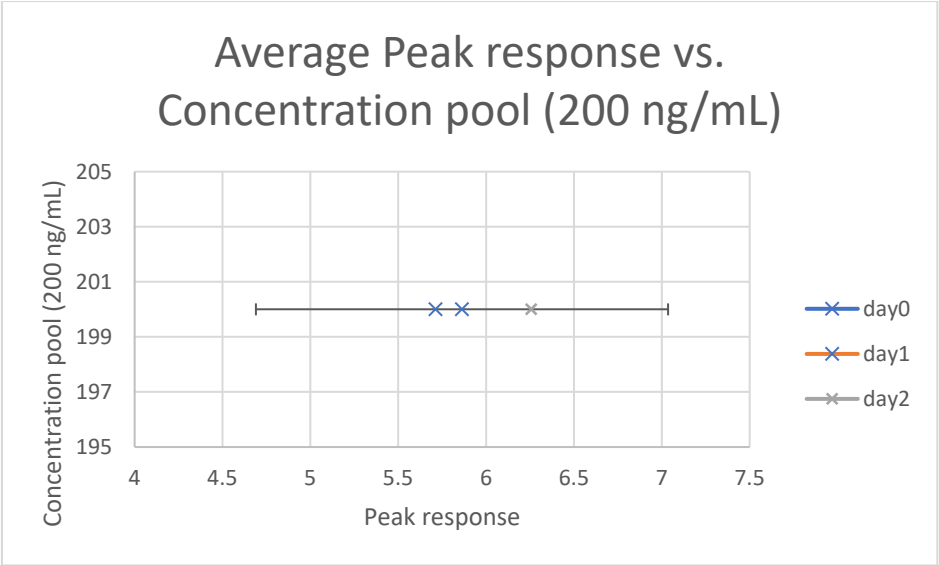


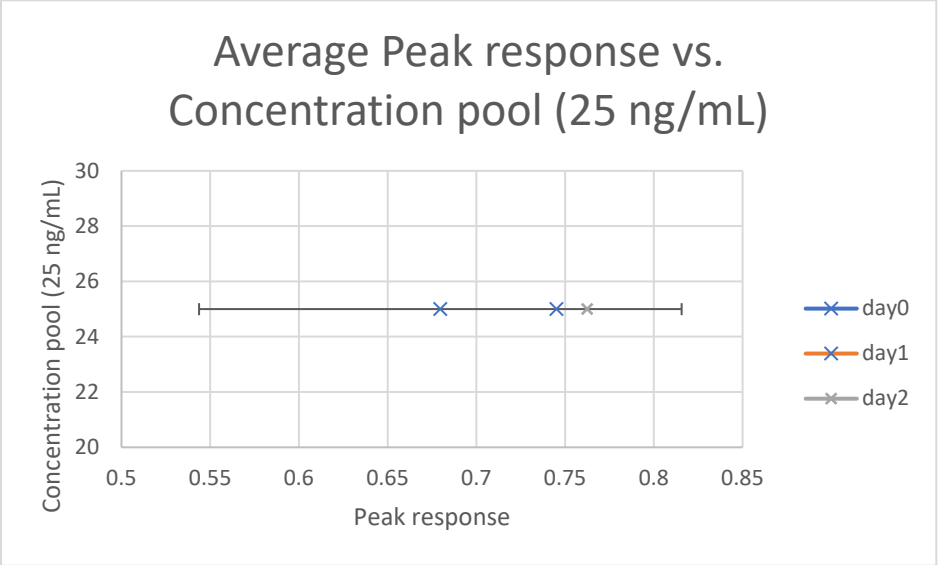




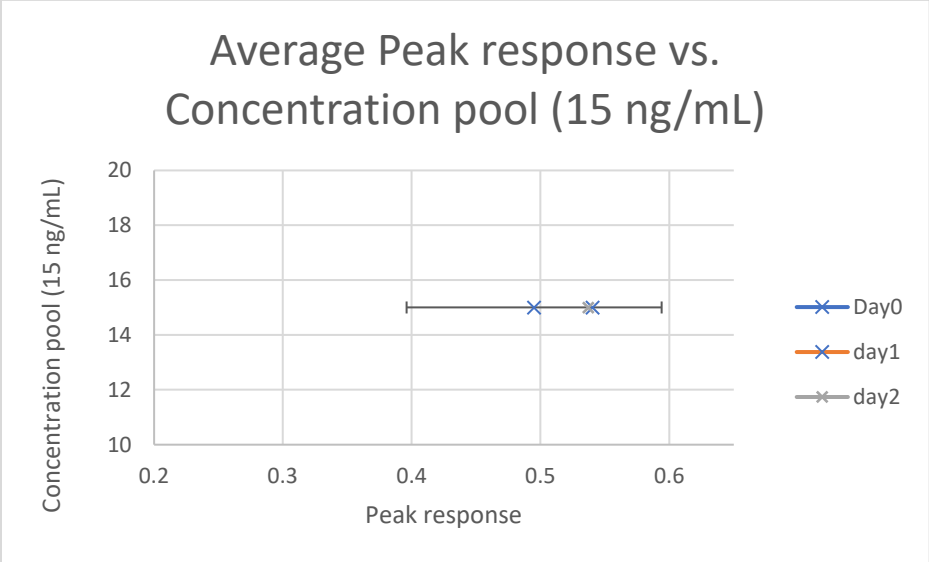
Stability Graphs 132-134(urine): Alprazolam

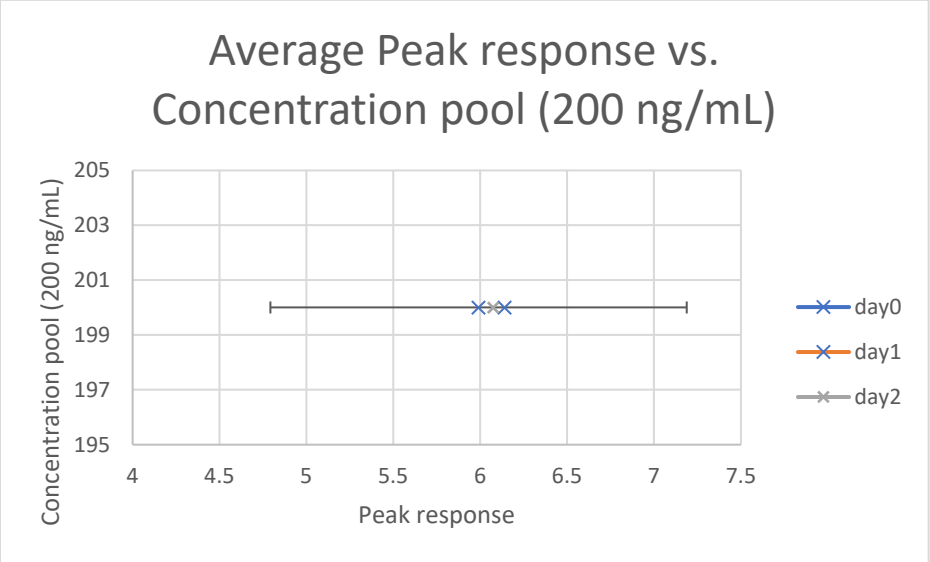


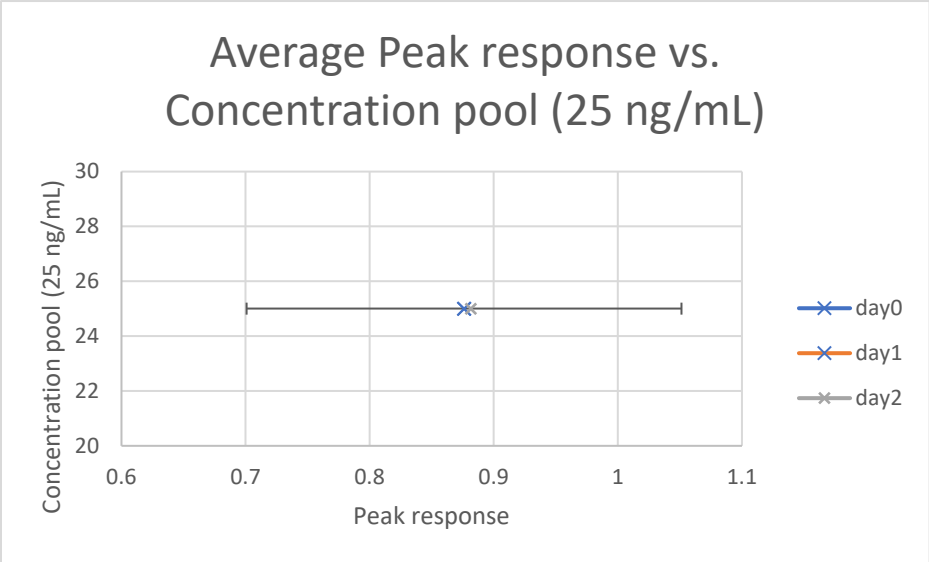




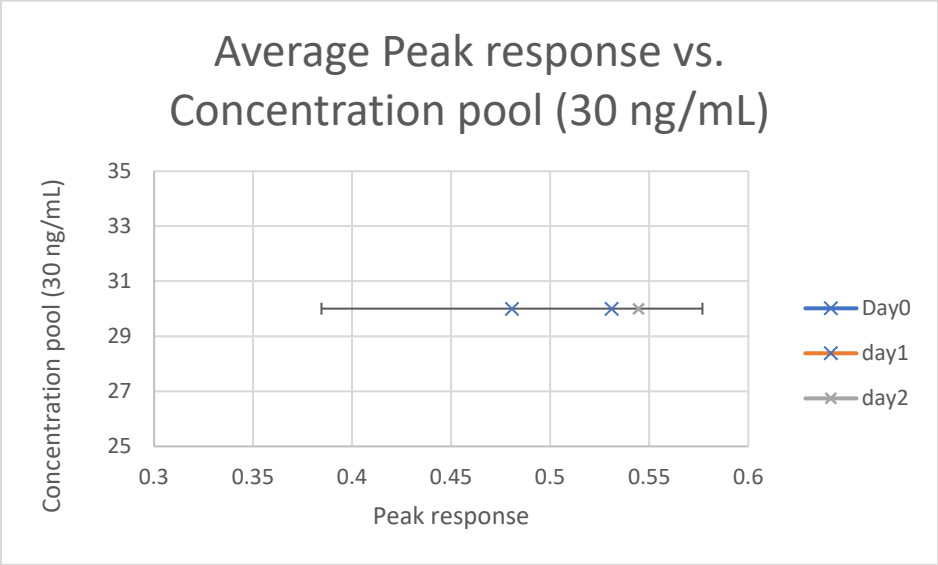
Stability Graphs 135-137 (urine): Clonazepam

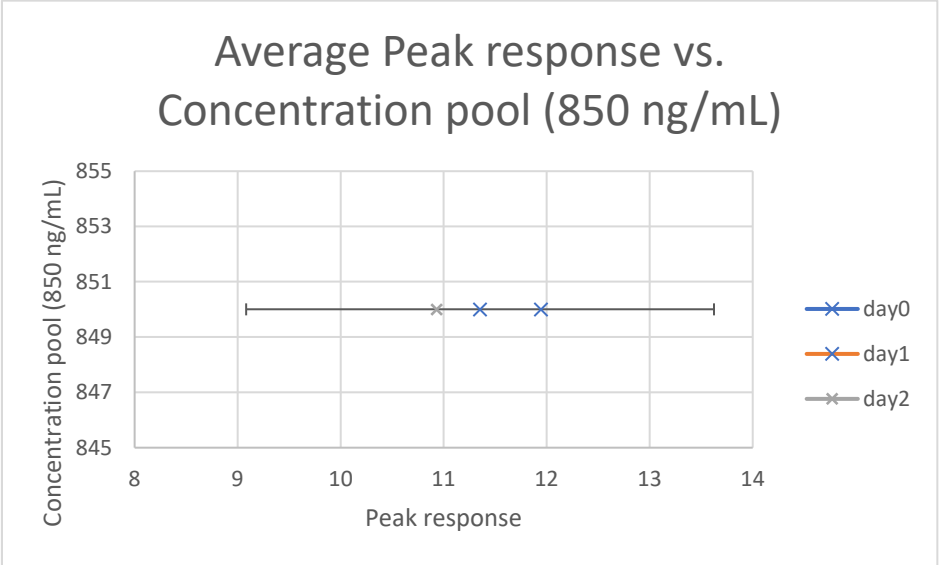


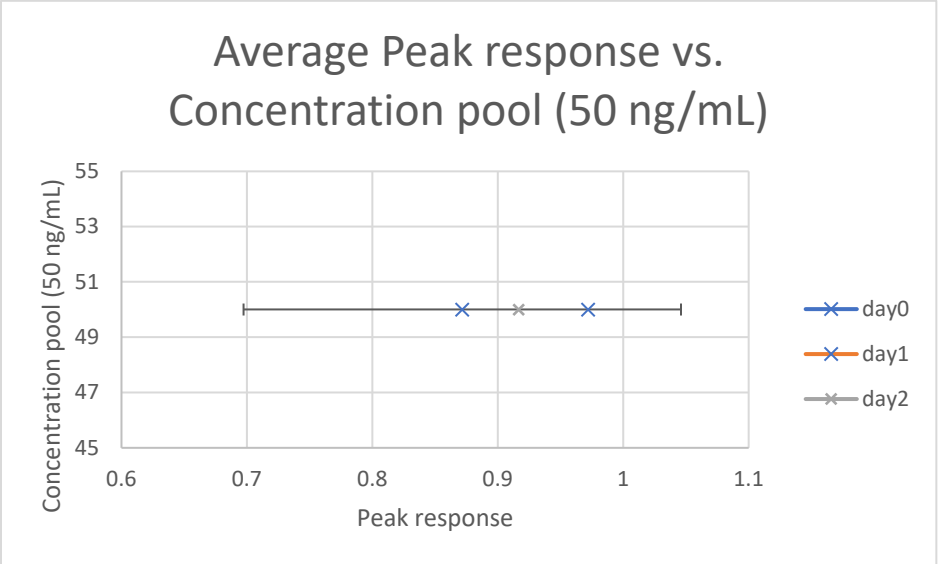




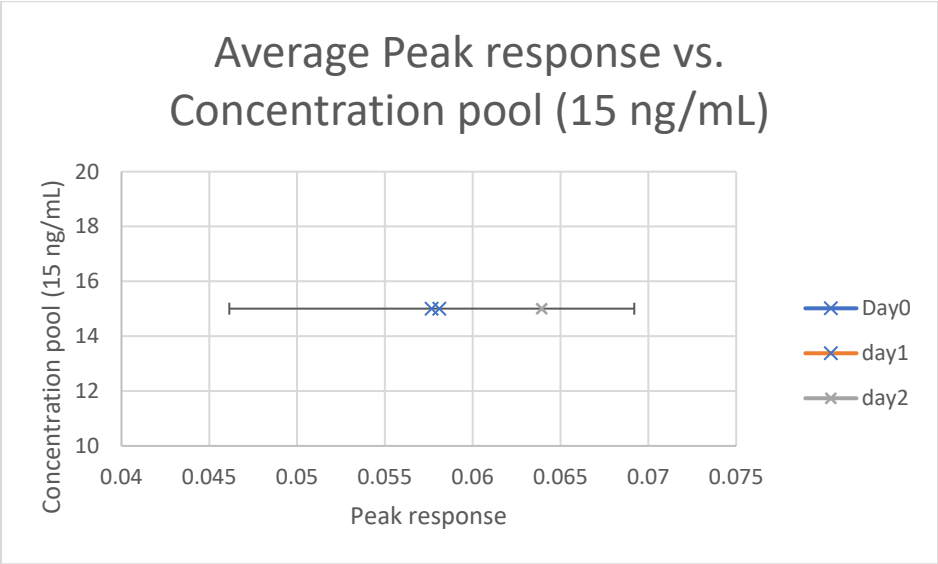
Stability Graphs 138-140 (urine): Diazepam

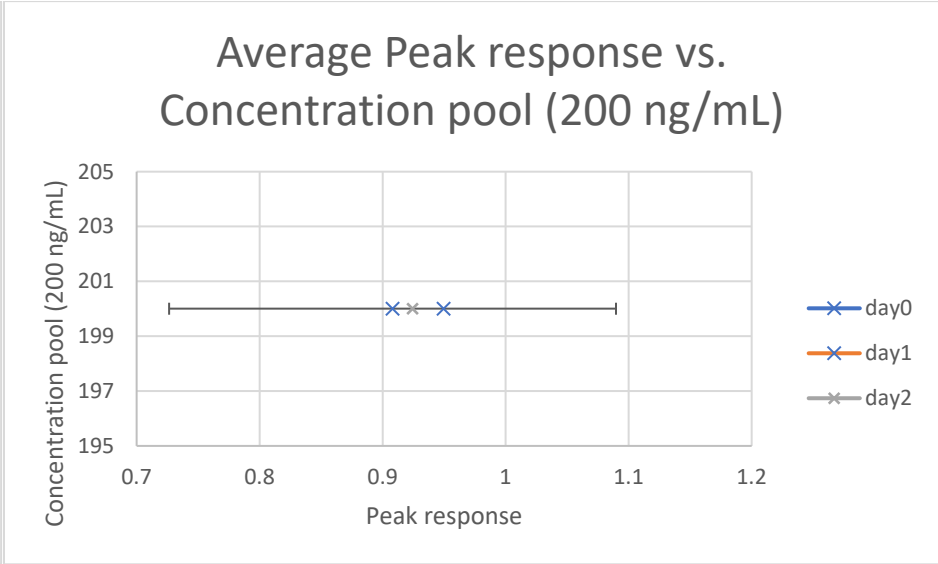


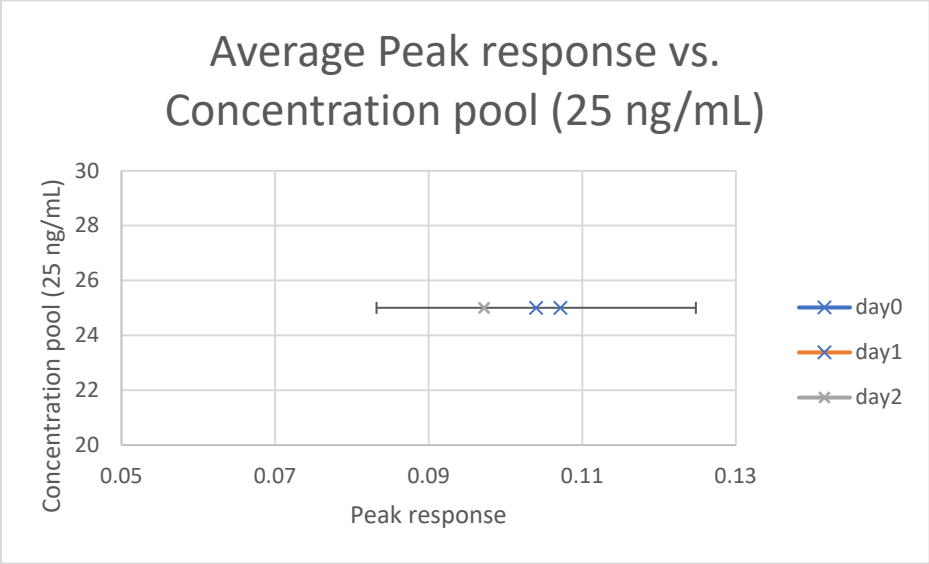




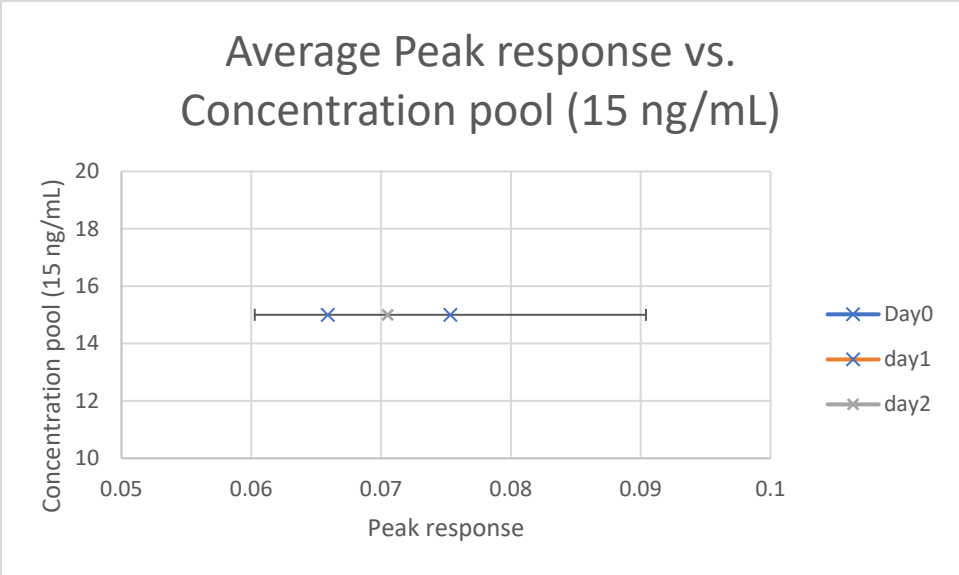
Stability Graphs 141-143(urine): Etizolam

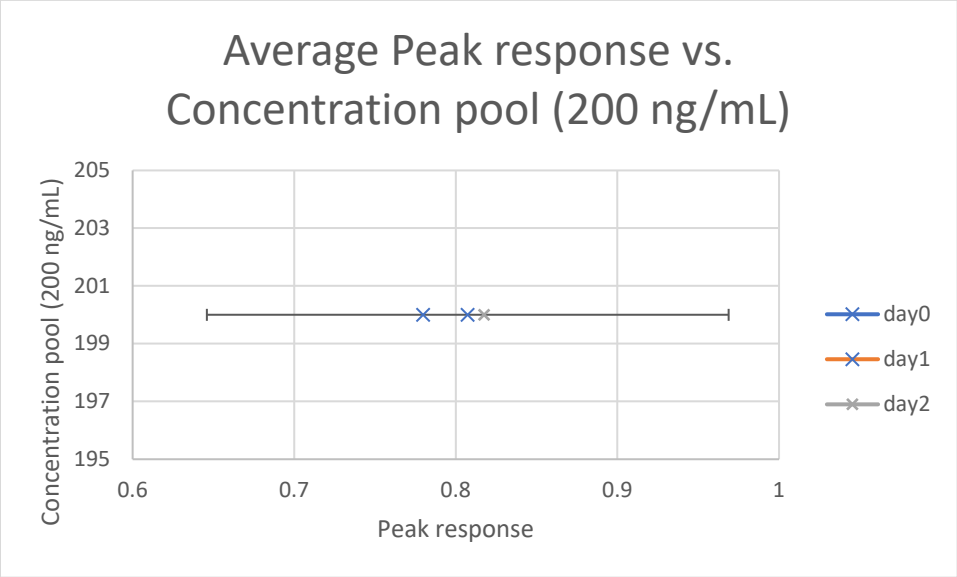


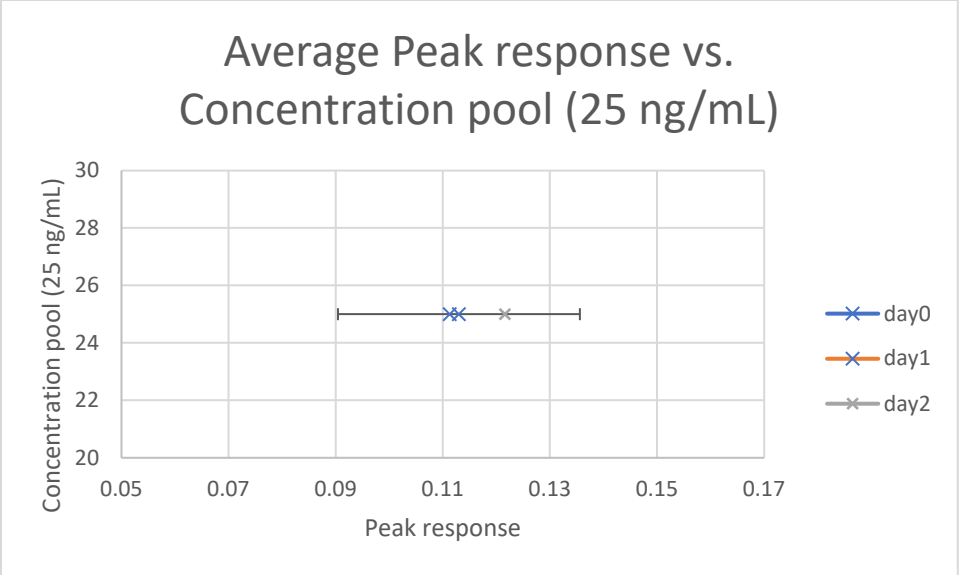




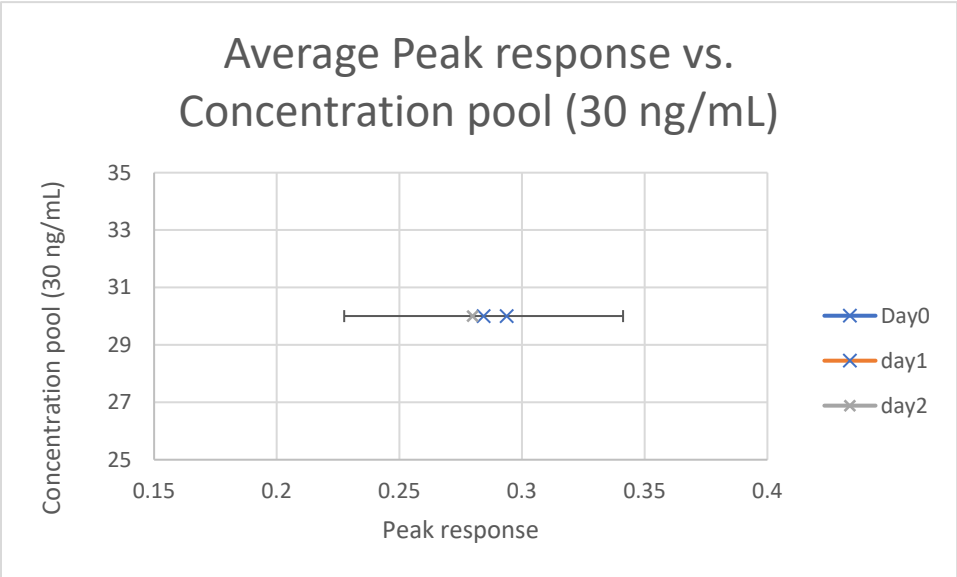
Stability Graphs 144-146 (urine): Lorazepam

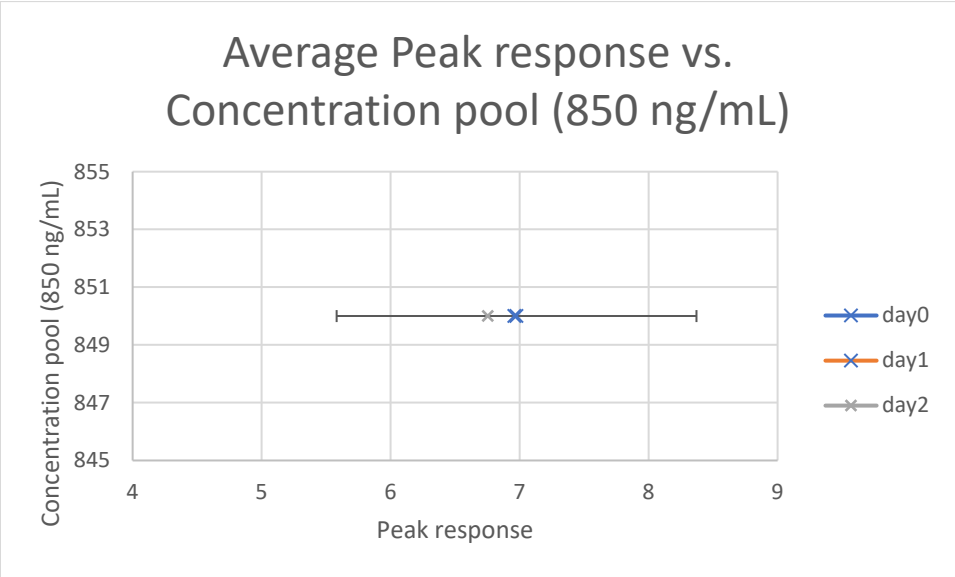


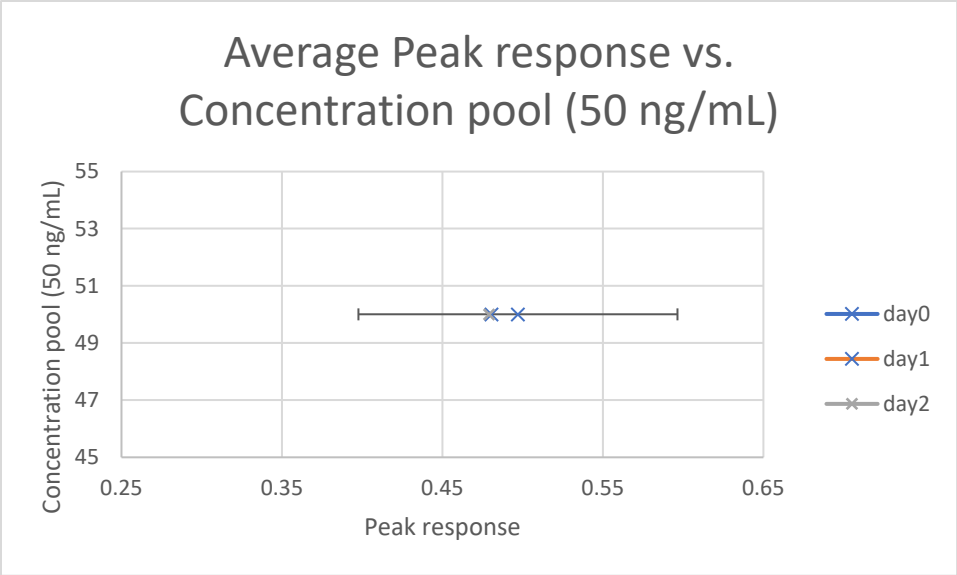




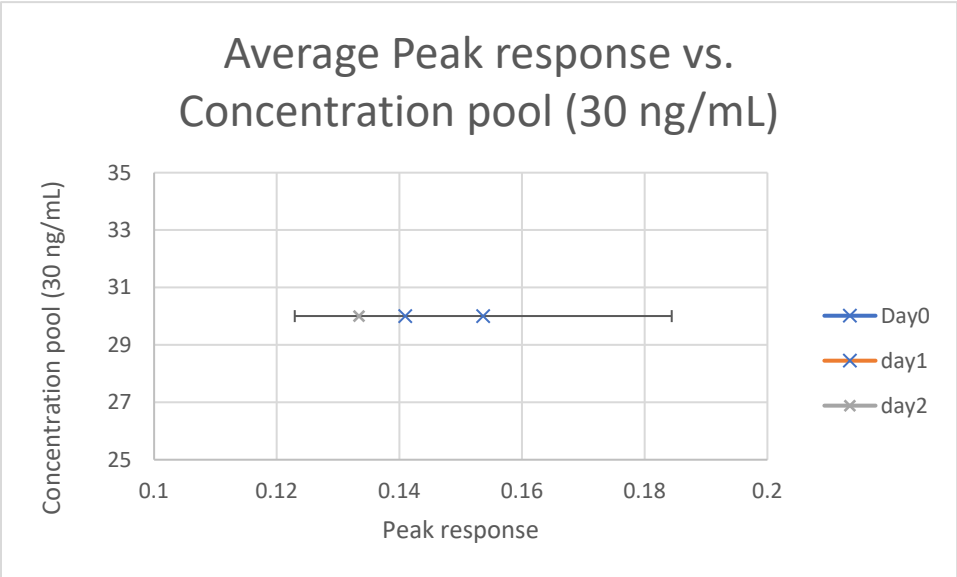
Stability Graphs 147-149 (urine): Nordiazepam

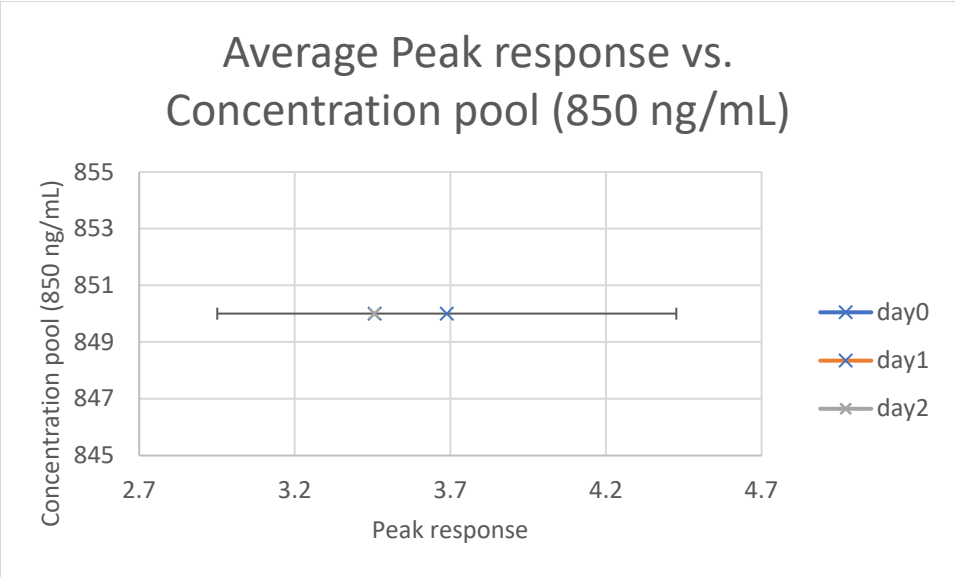


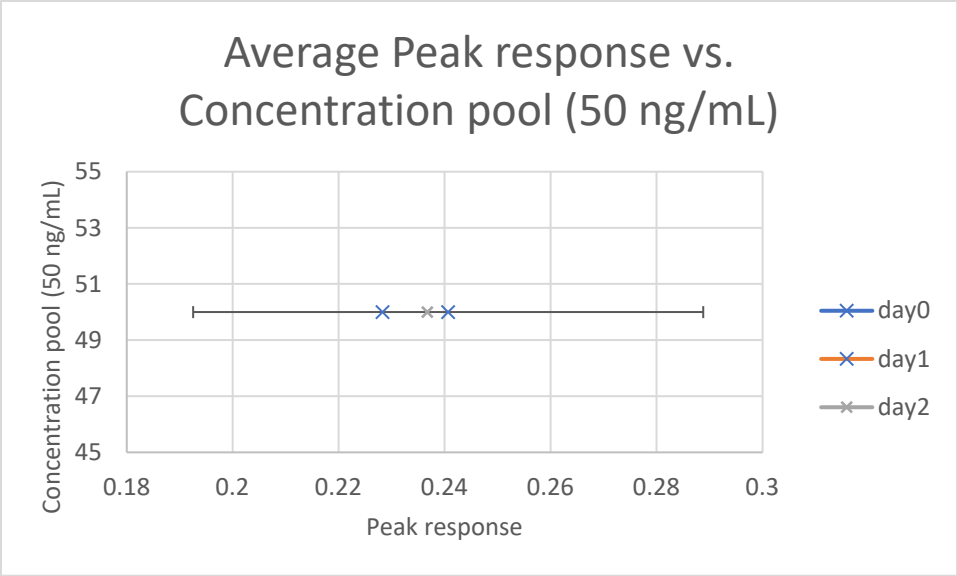




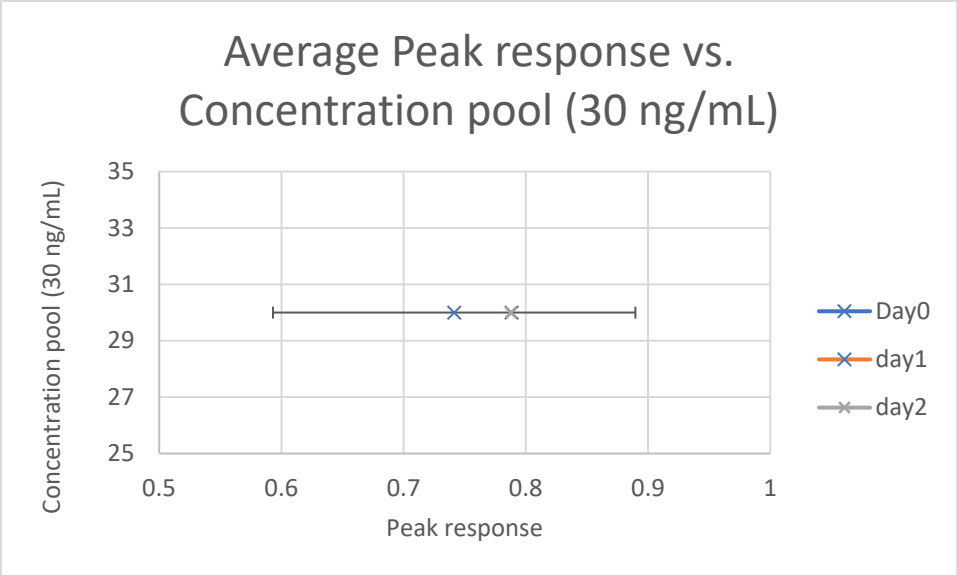
Stability Graphs 150-152 (urine): Oxazepam

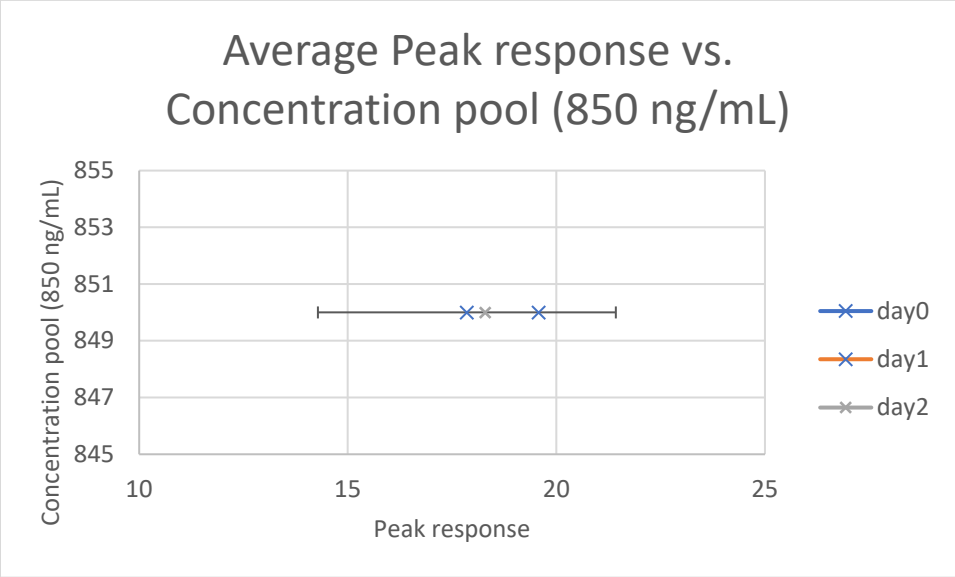


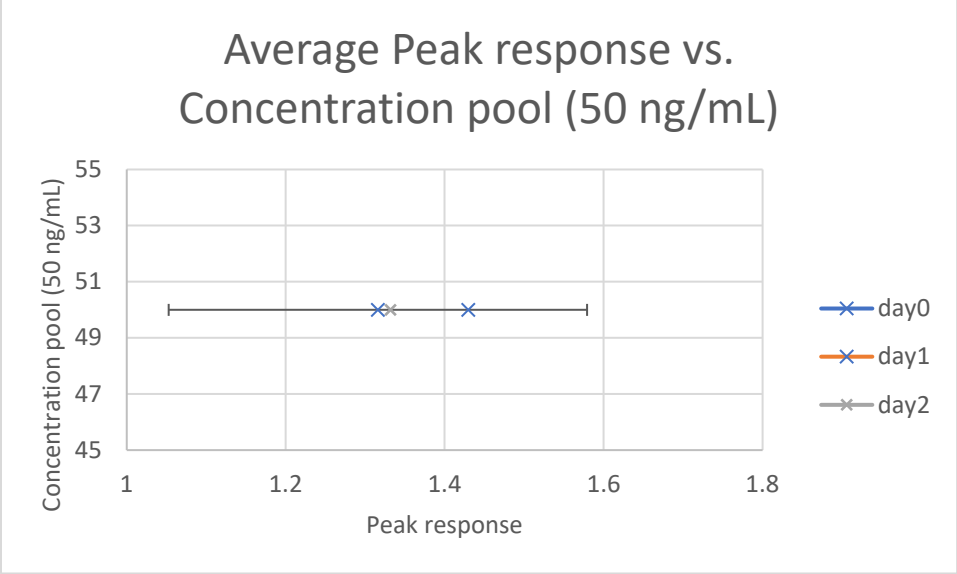




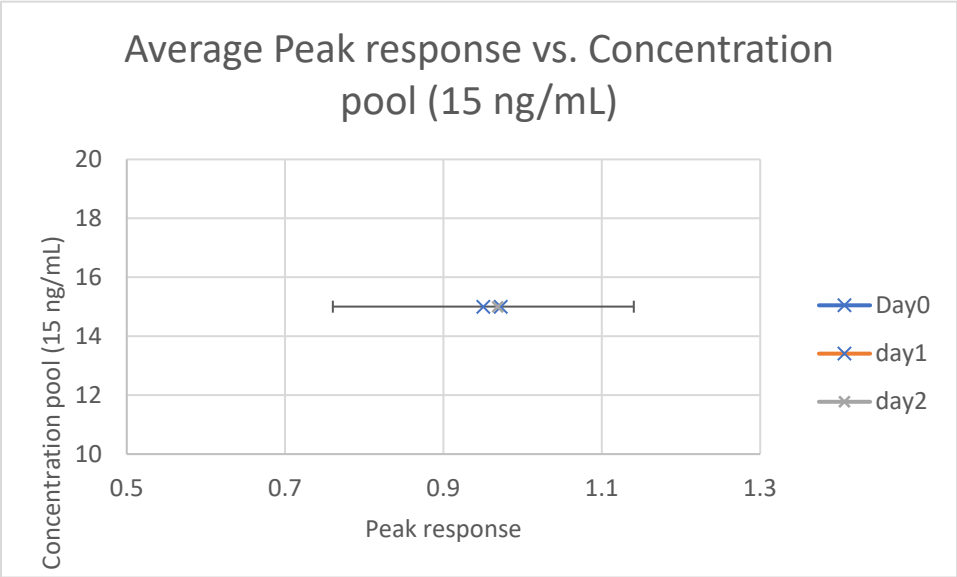
Stability Graphs 153-155 (urine): Temazepam

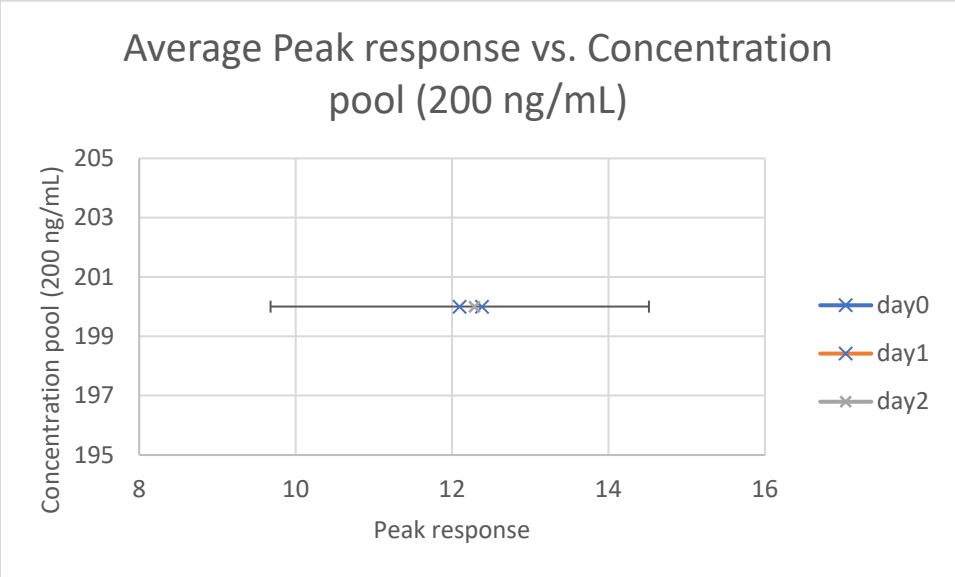


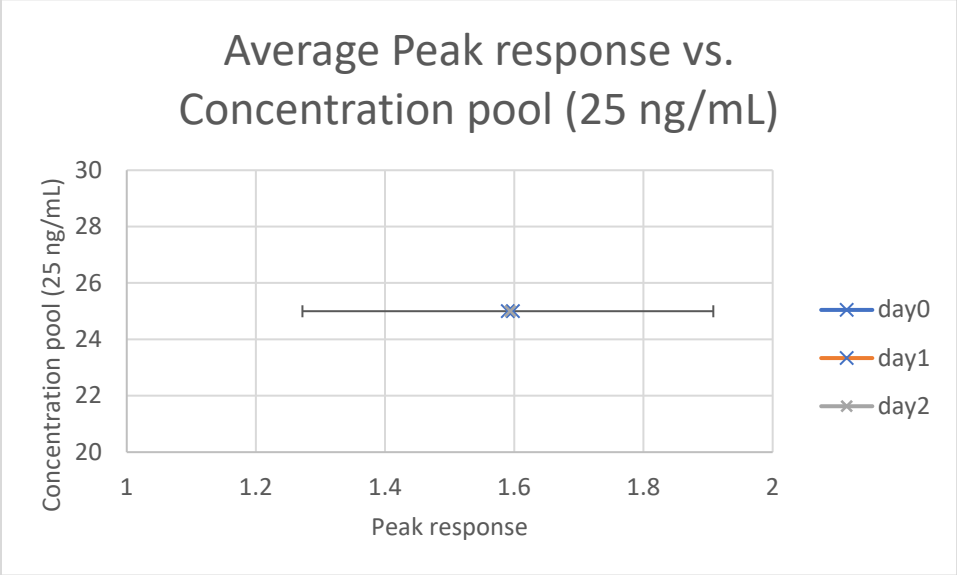




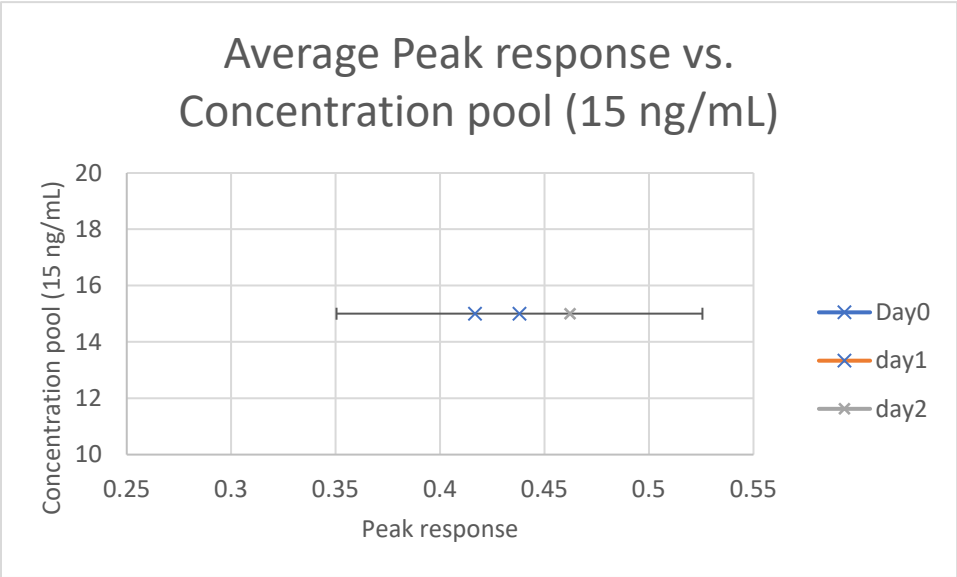
Stability Graphs 156-158 (urine): Zolpidem

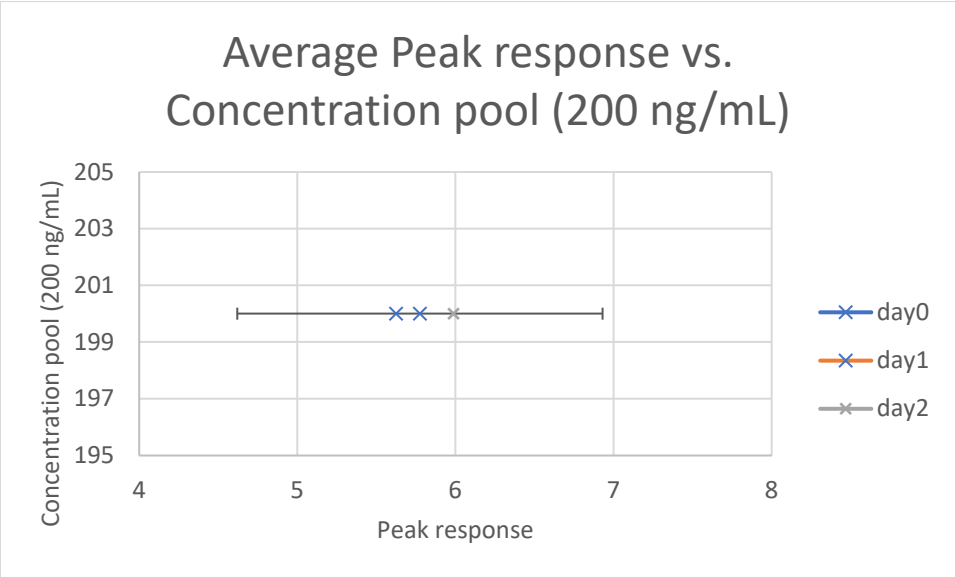


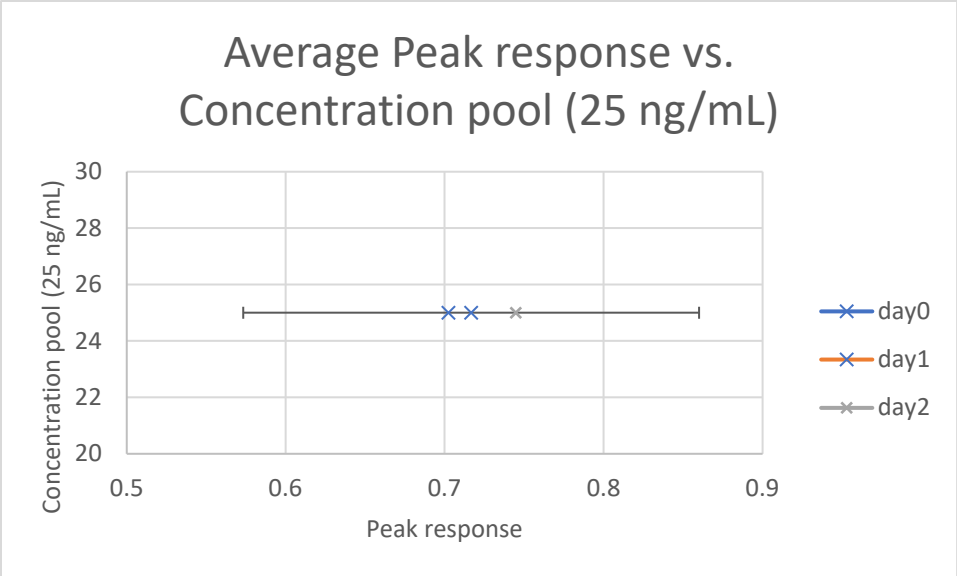




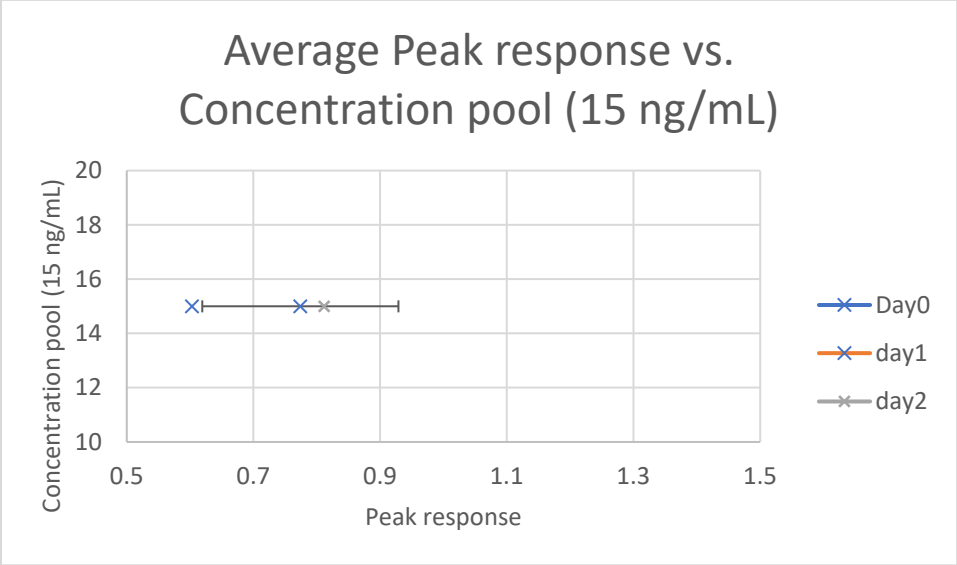
Stability Graphs 159-161 (urine): Alpha-hydroxy alprazolam

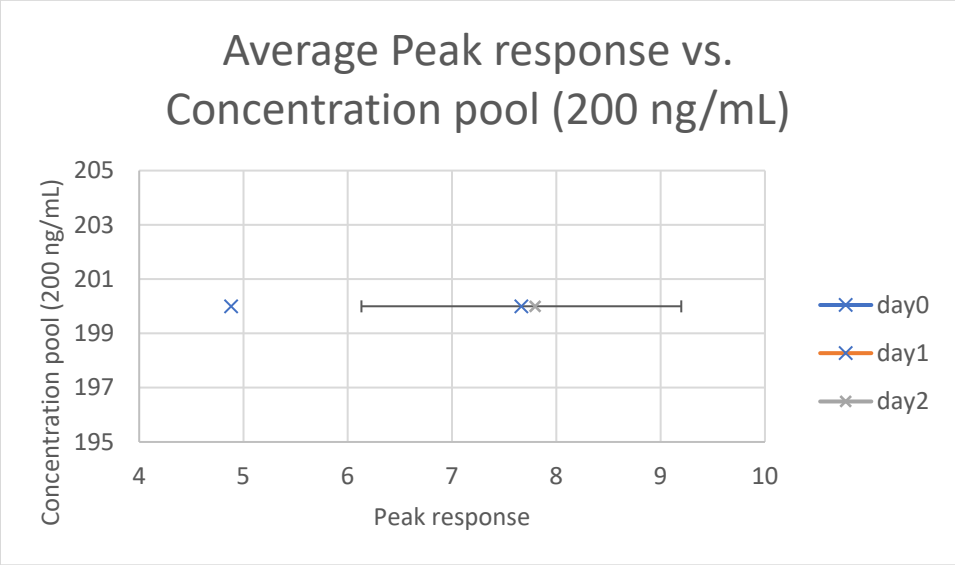


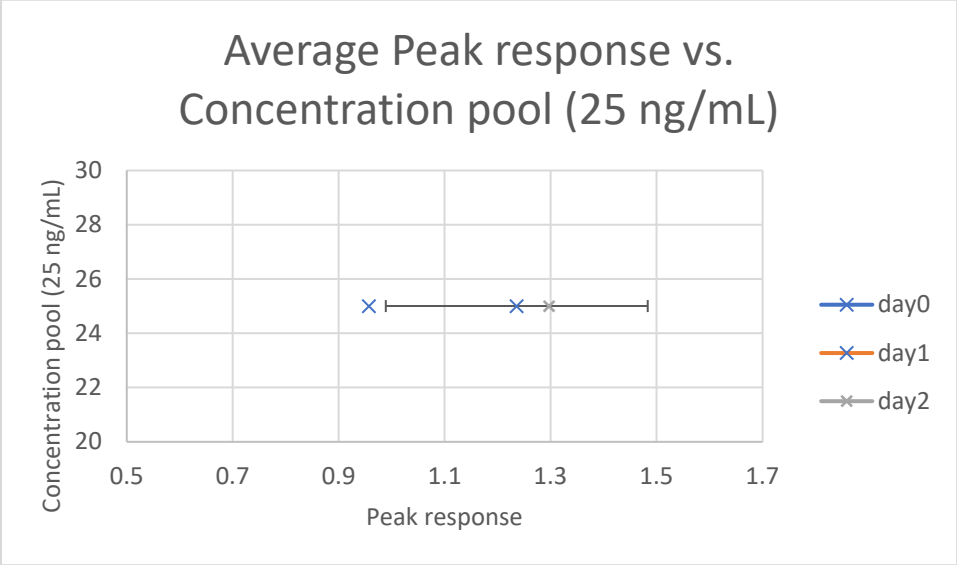




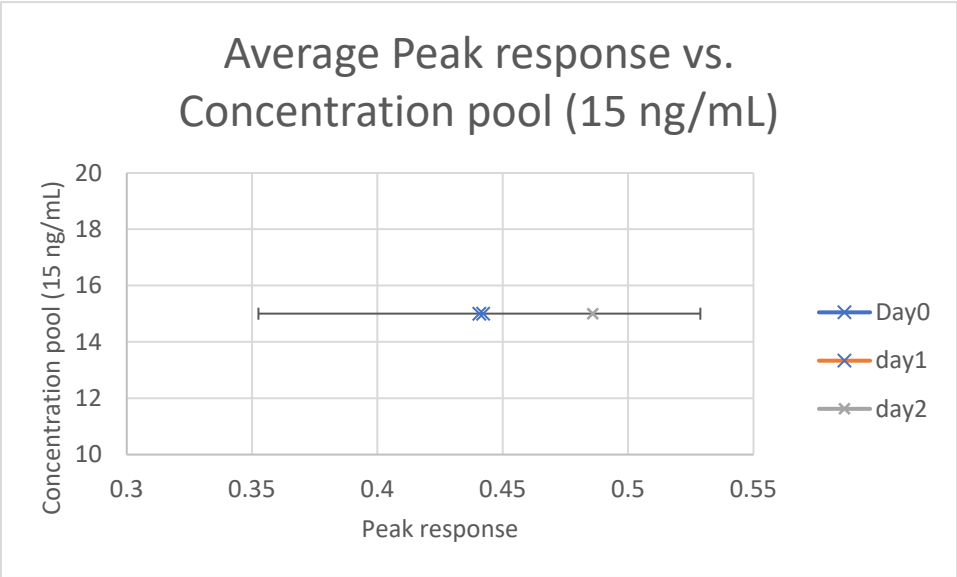
Stability Graphs 162-164 (urine): 7-amino flunitrazepam

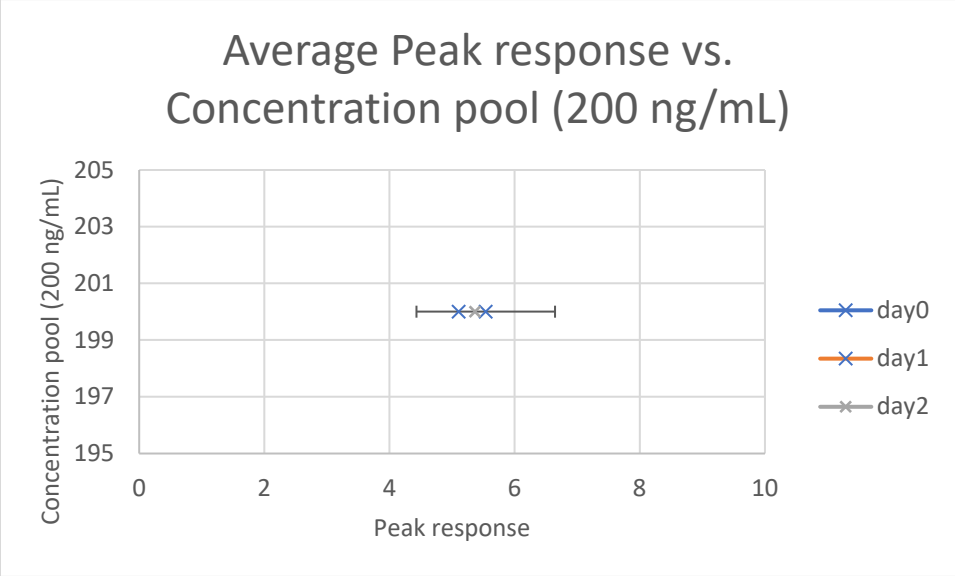


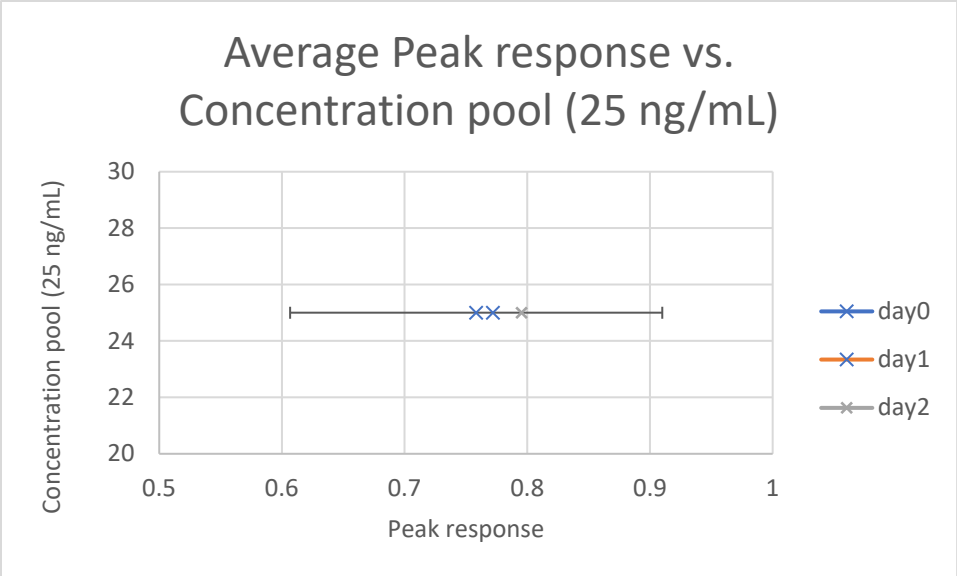




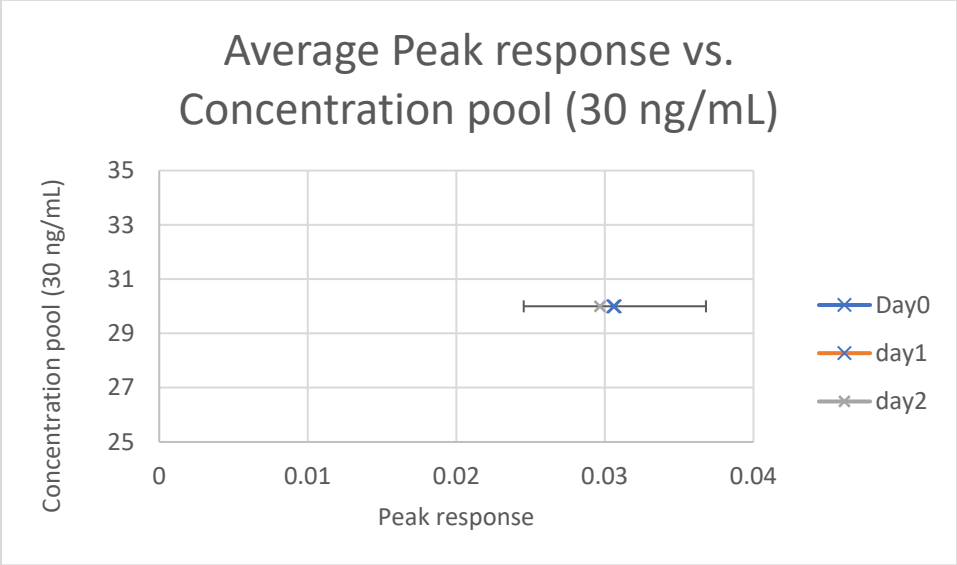
Stability Graphs 165-167 (urine): Flunitrazepam

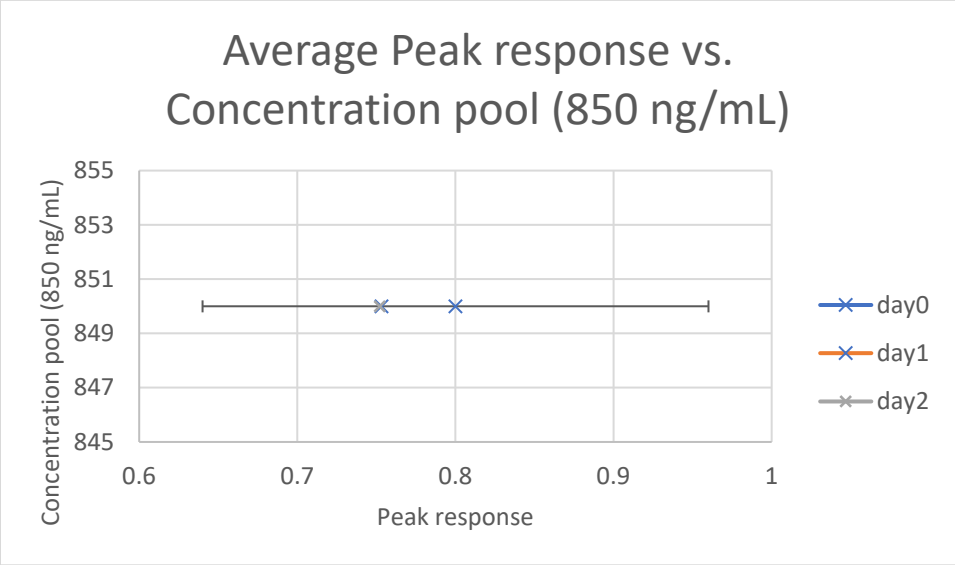


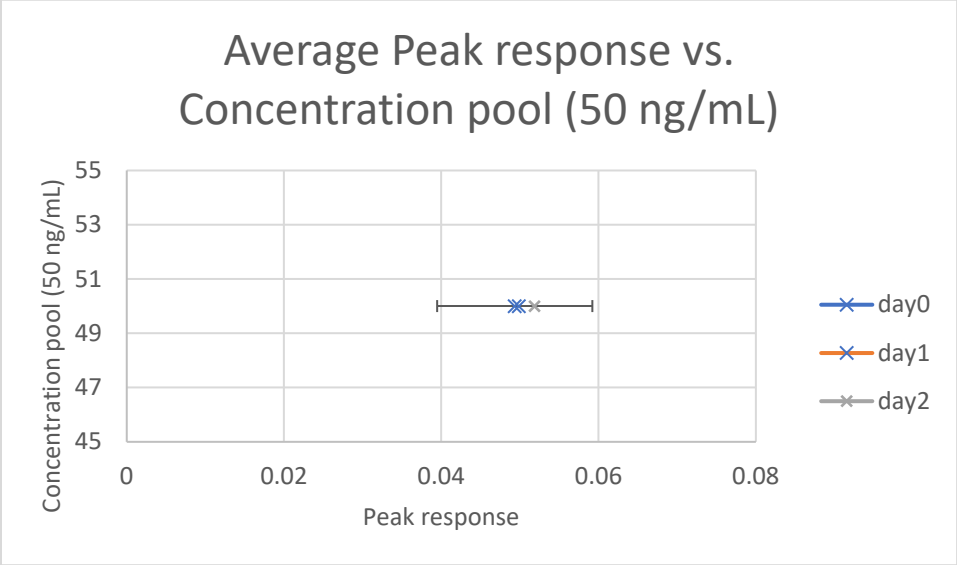




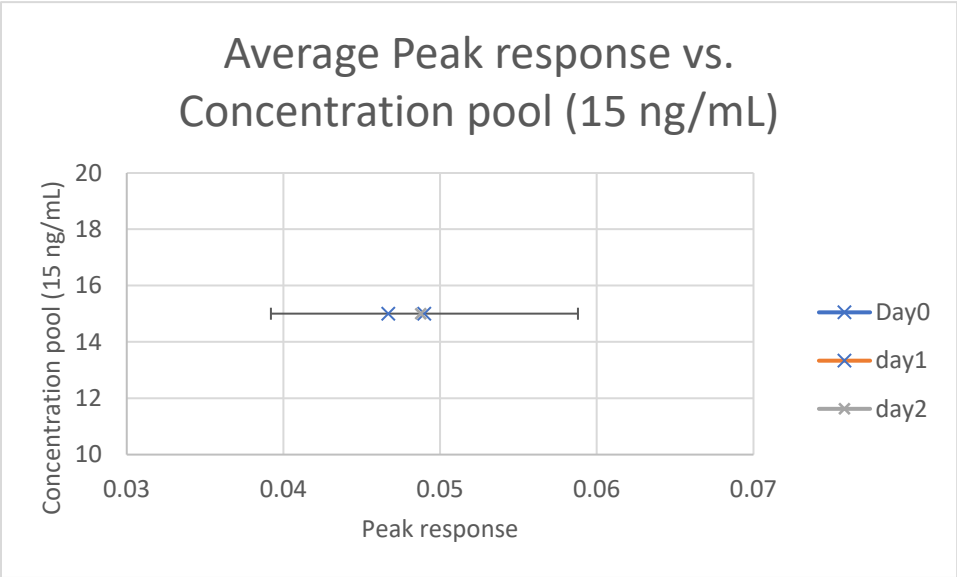
Stability Graphs 168-170 (urine): Midazolam

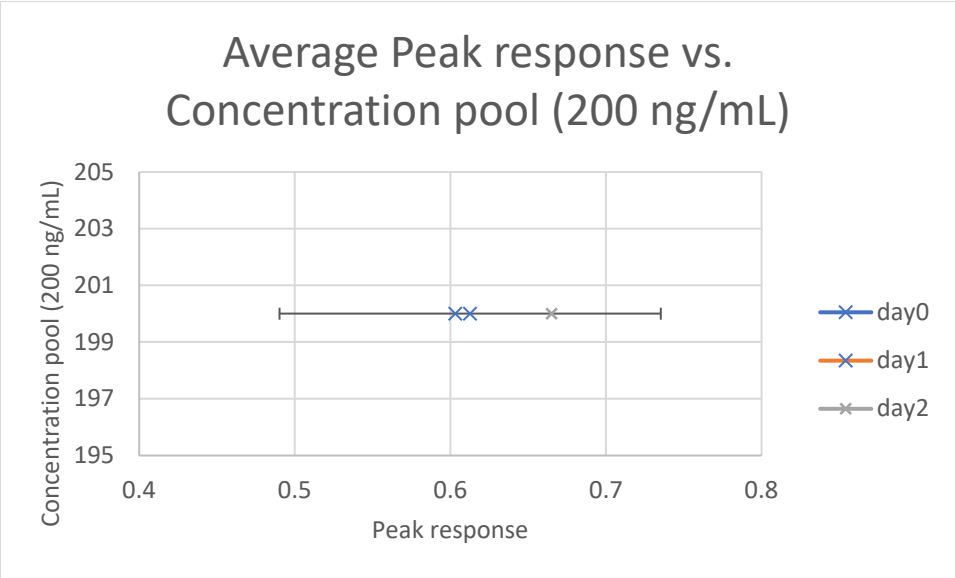


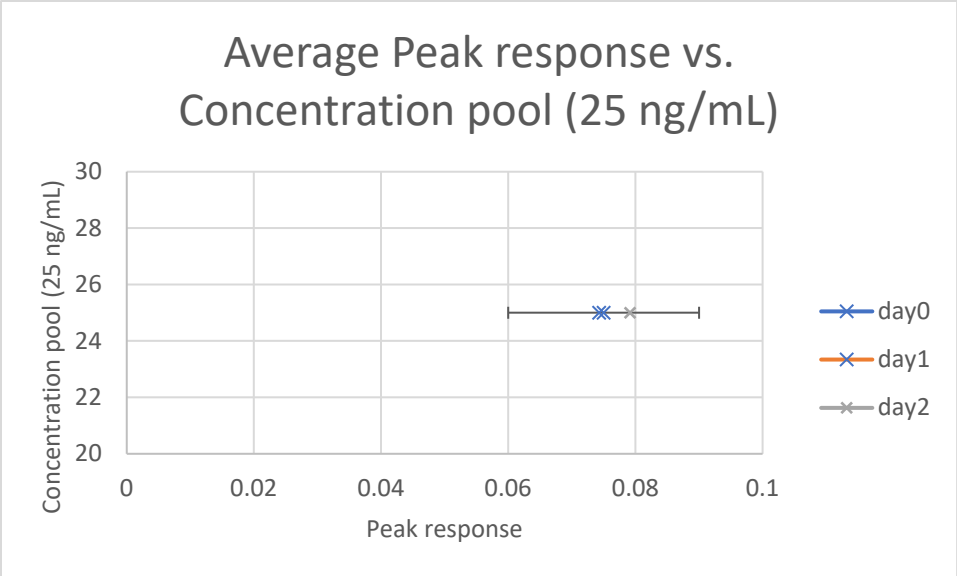




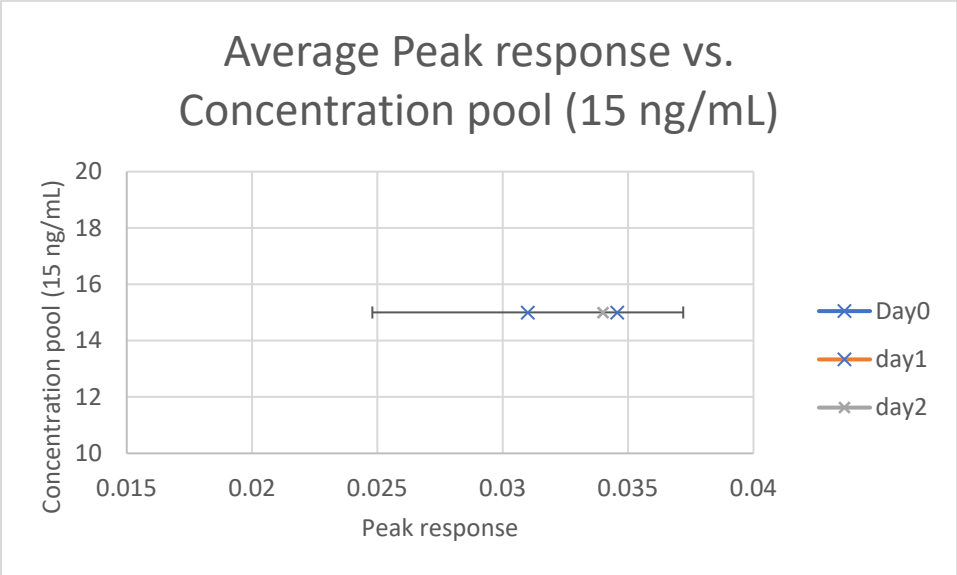
Stability Graphs 171-173 (urine): Triazolam

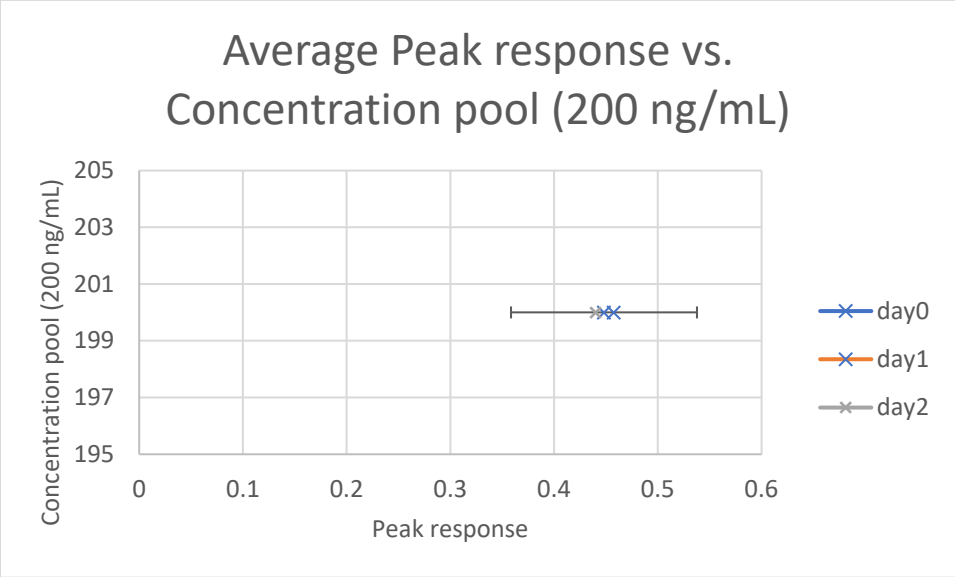


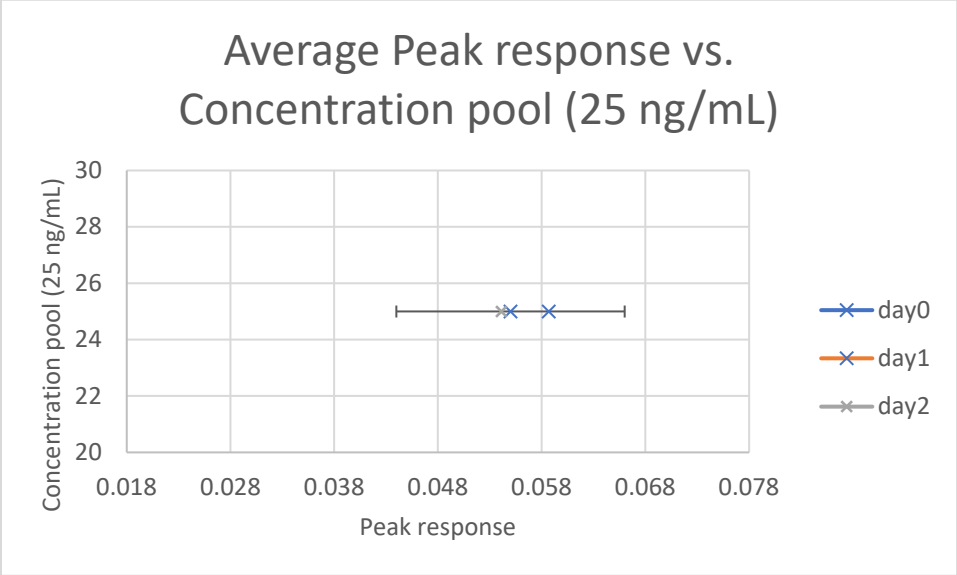




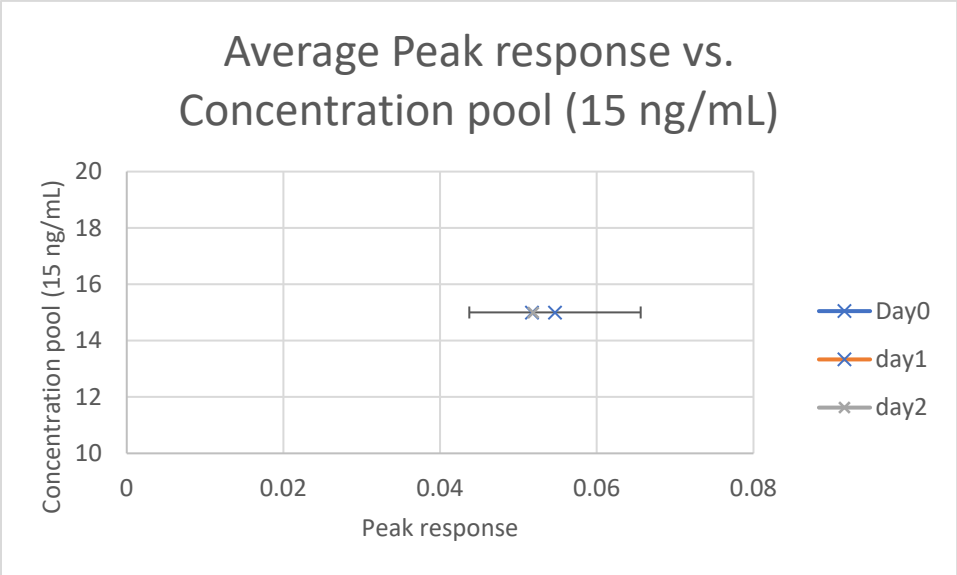
Stability Graphs 174-176 (urine): Alpha hydroxy etizolam

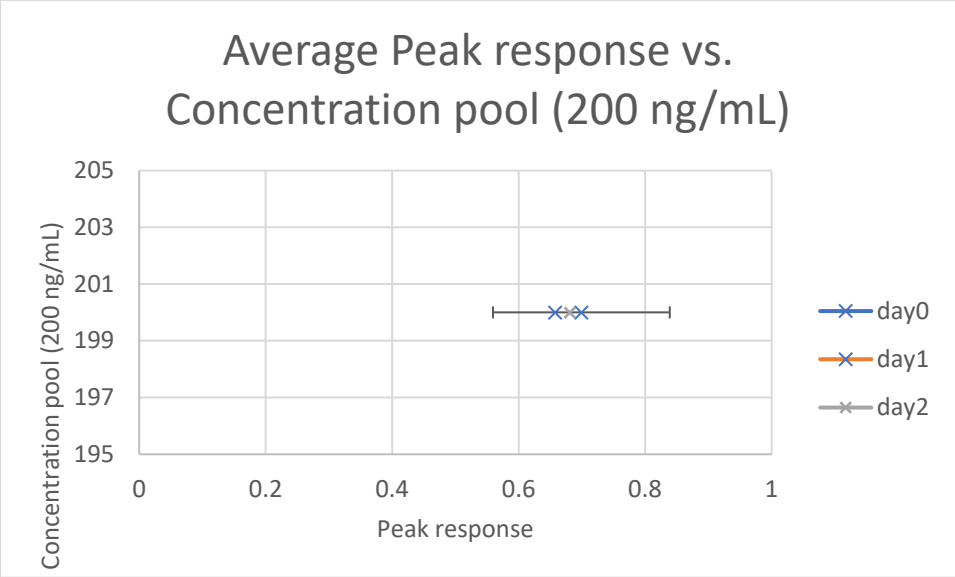


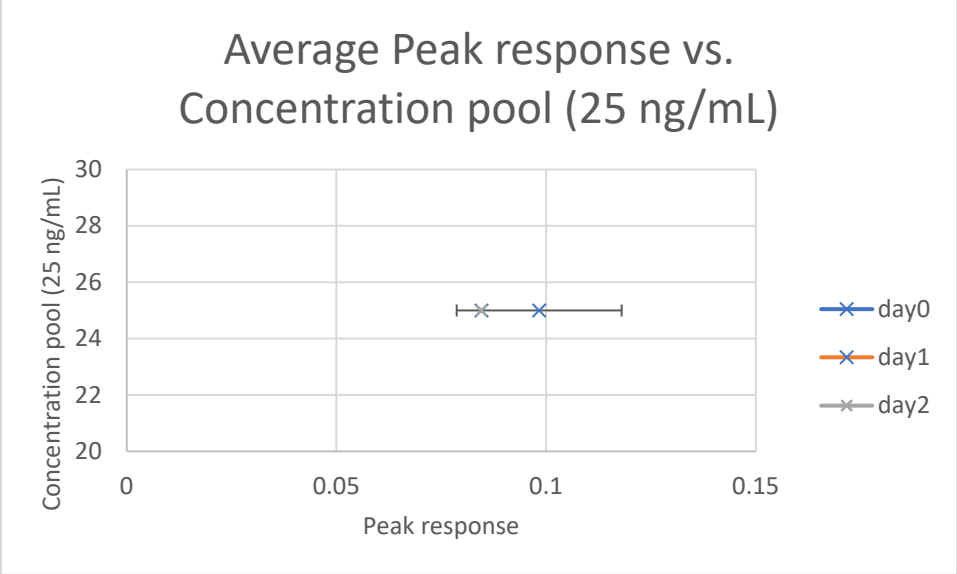




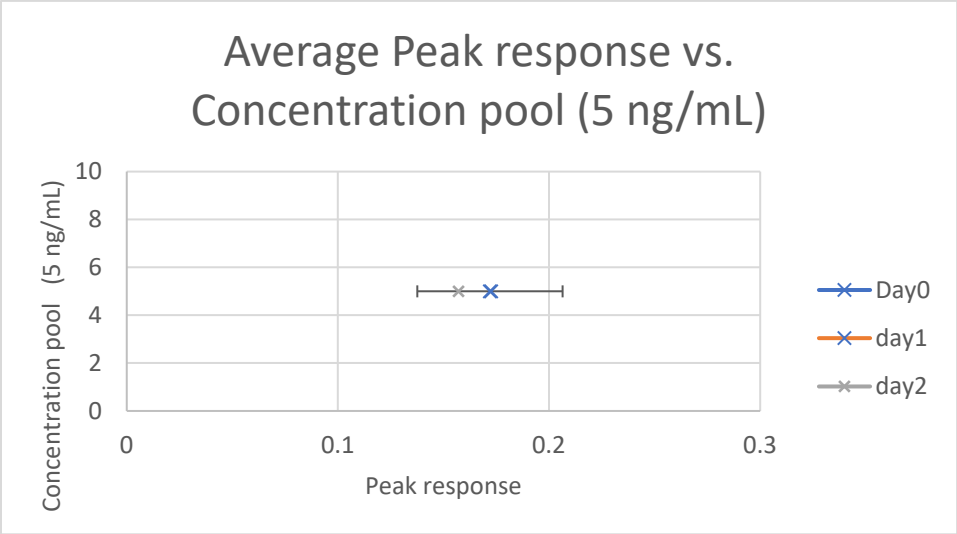
Stability Graphs 177-179 (urine): Alpha hydroxy triazolam

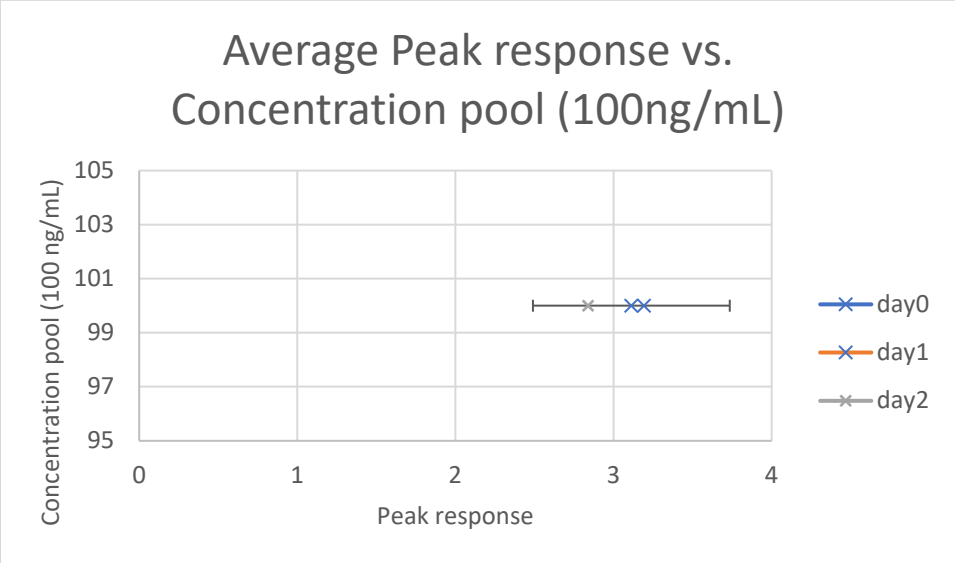


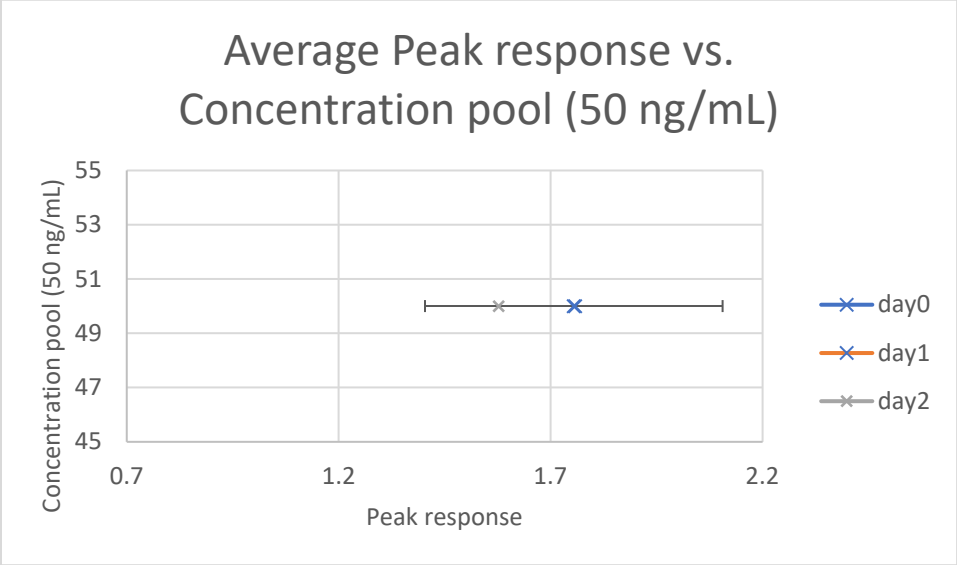




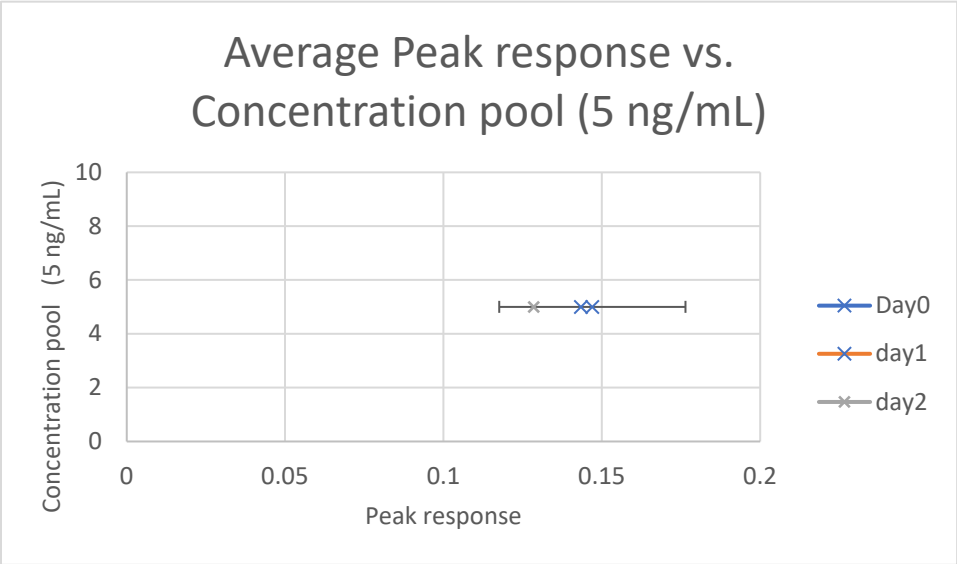
Stability Graphs 180-182 (urine): Bromazepam

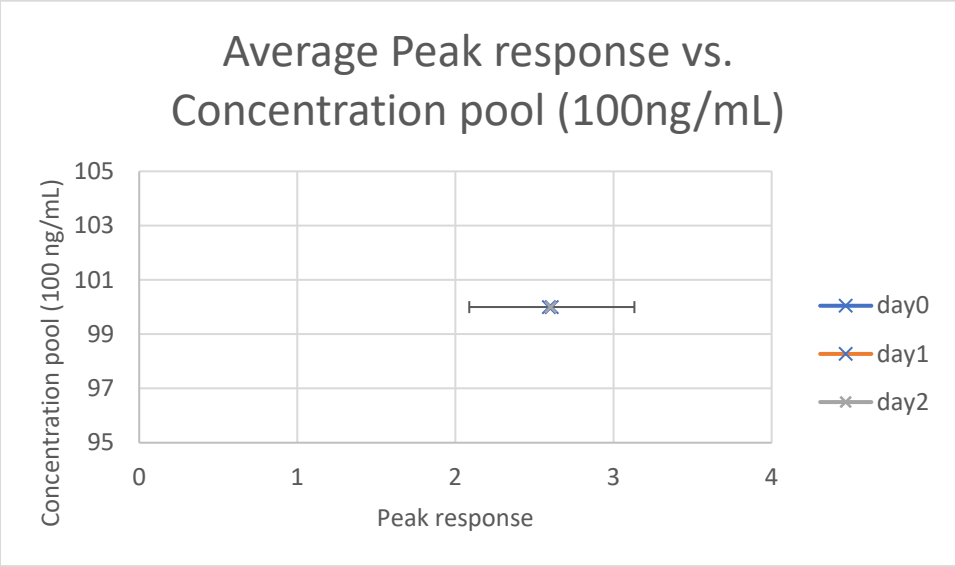


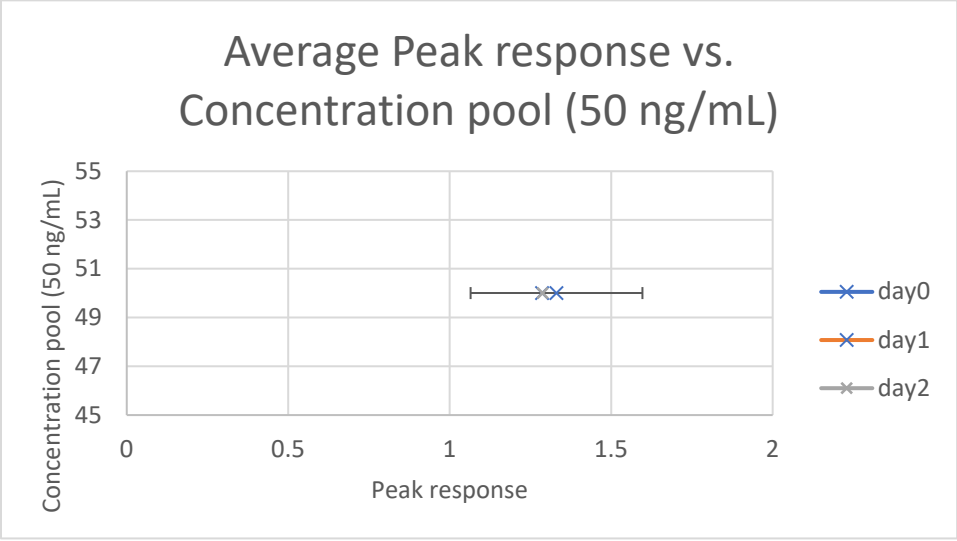




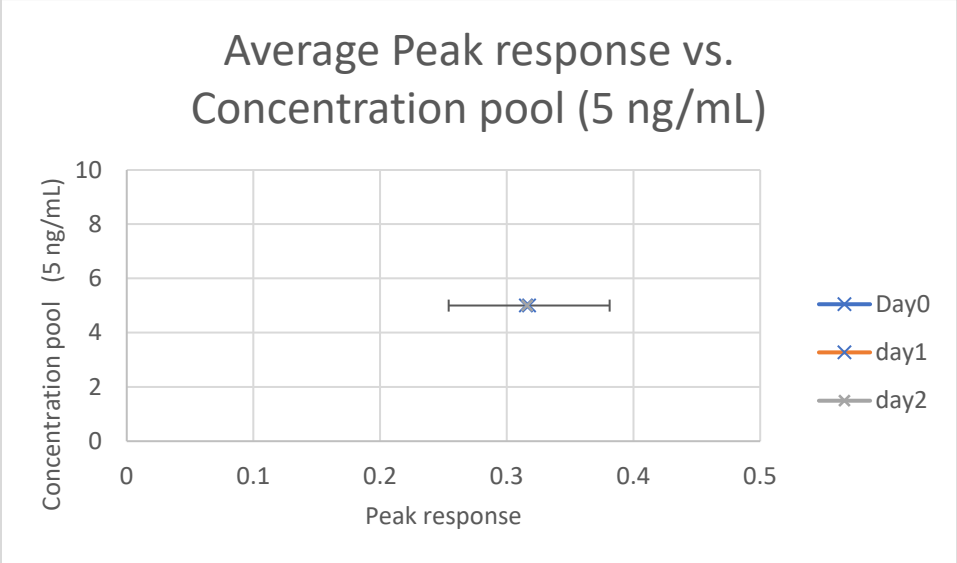
Stability Graphs 183-185 (urine): Bromazolam

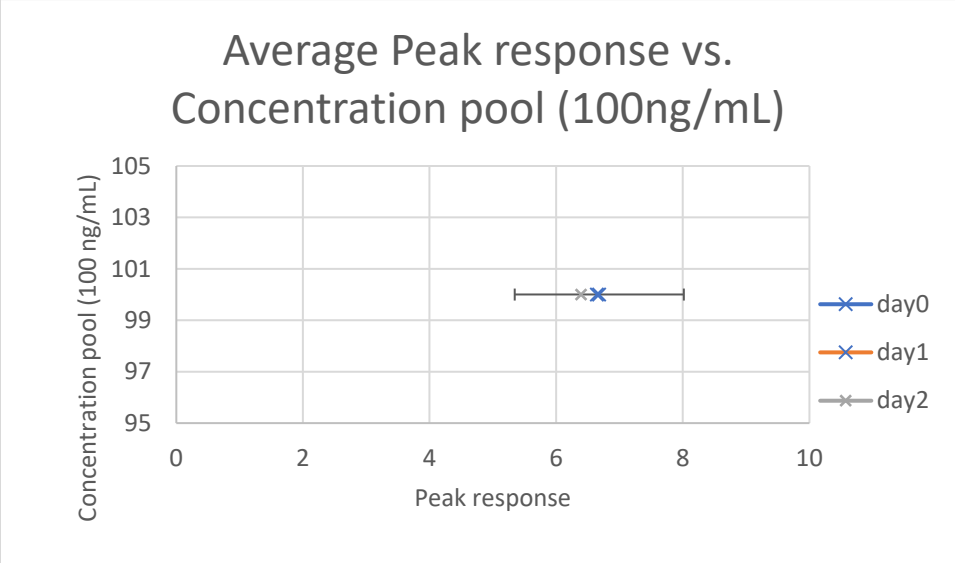


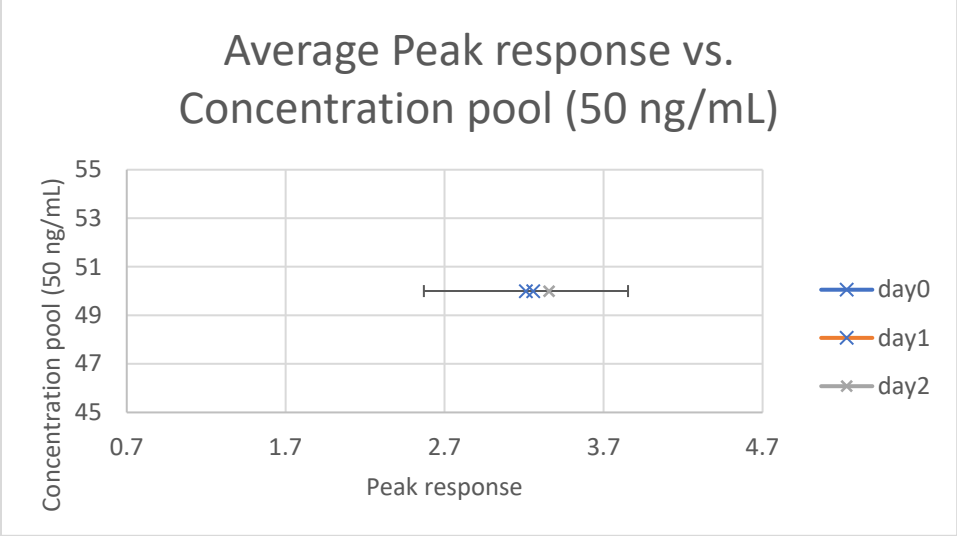




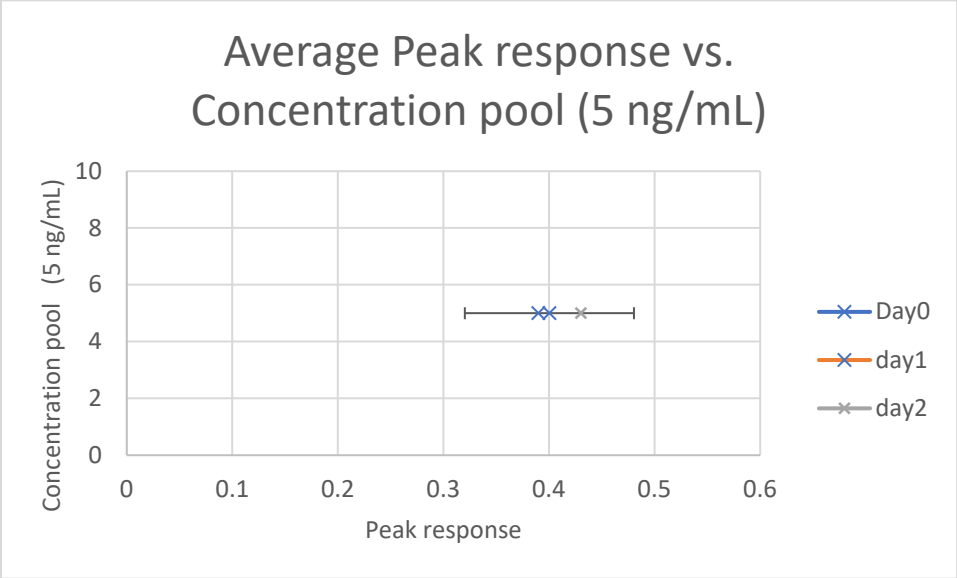
Stability Graphs 186-188 (urine): Chlordiazepoxide

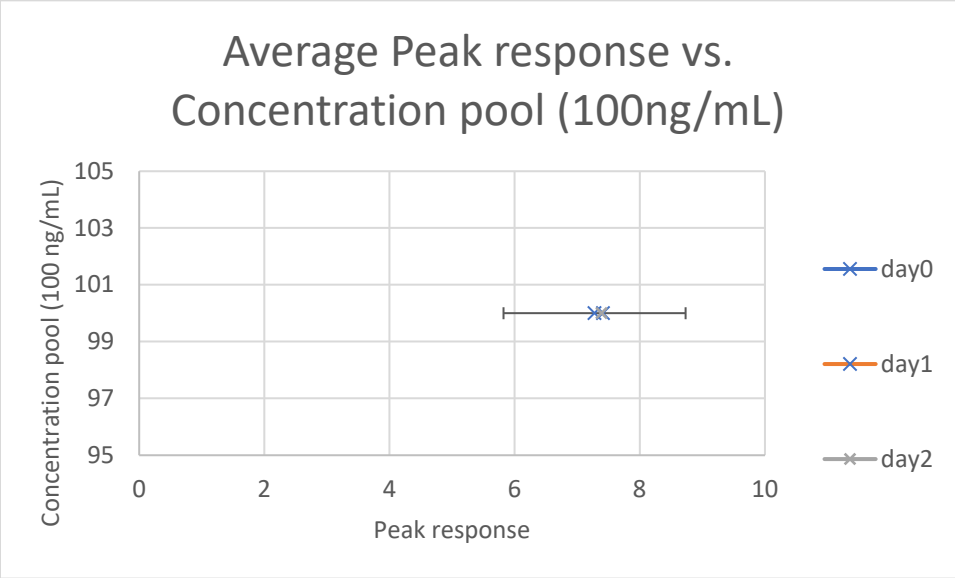


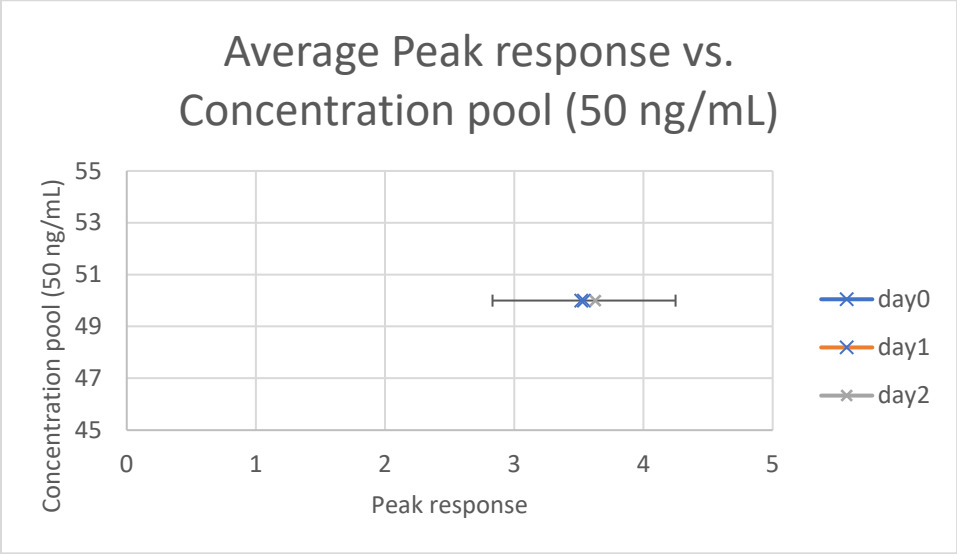




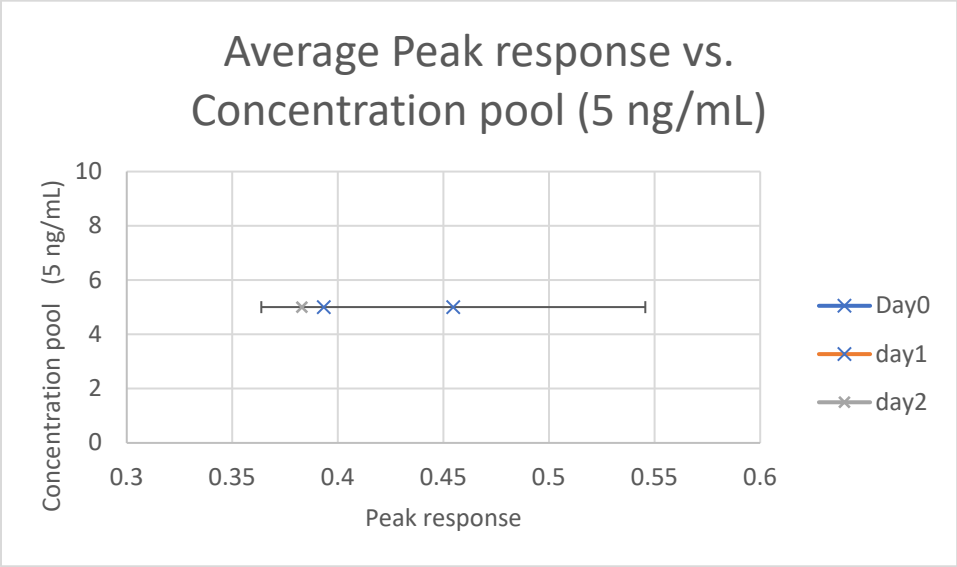
Stability Graphs 189-191 (urine): Clobazam

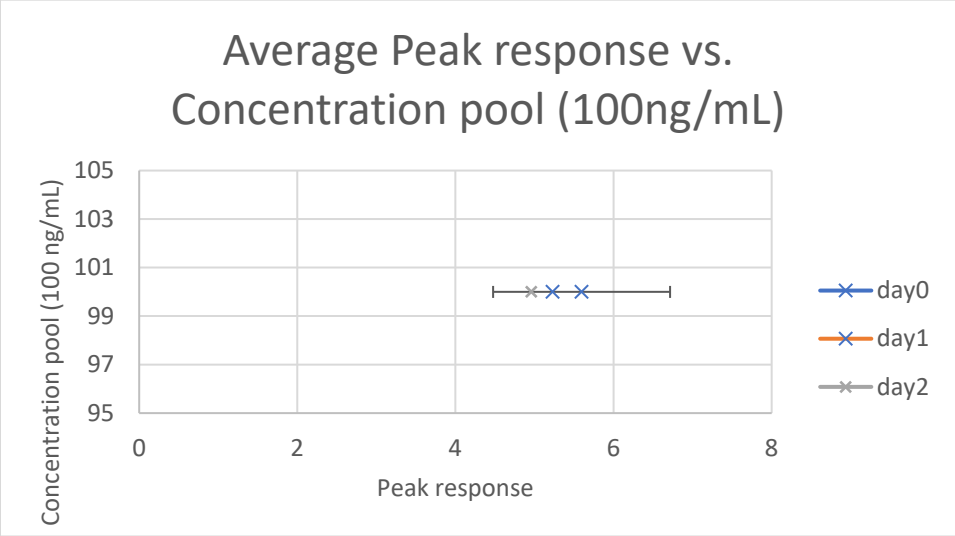


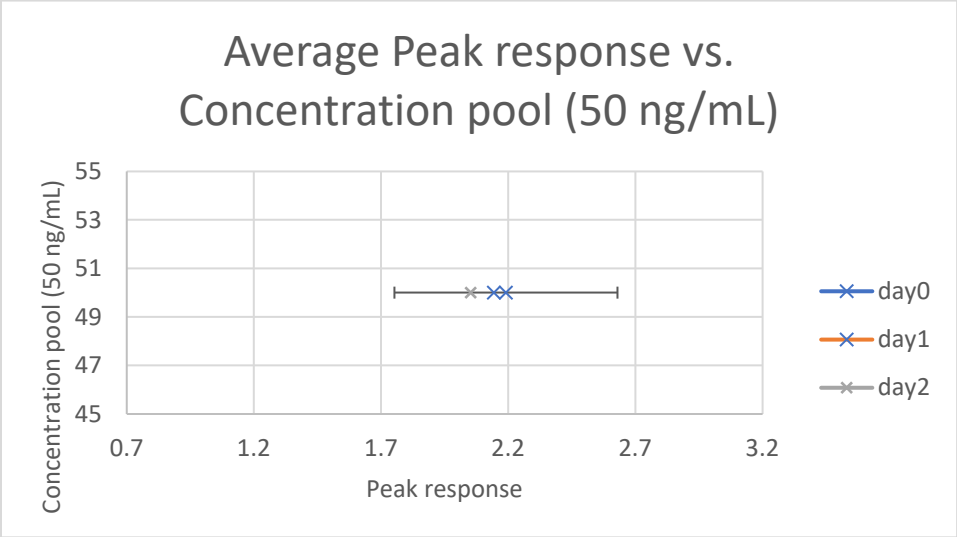




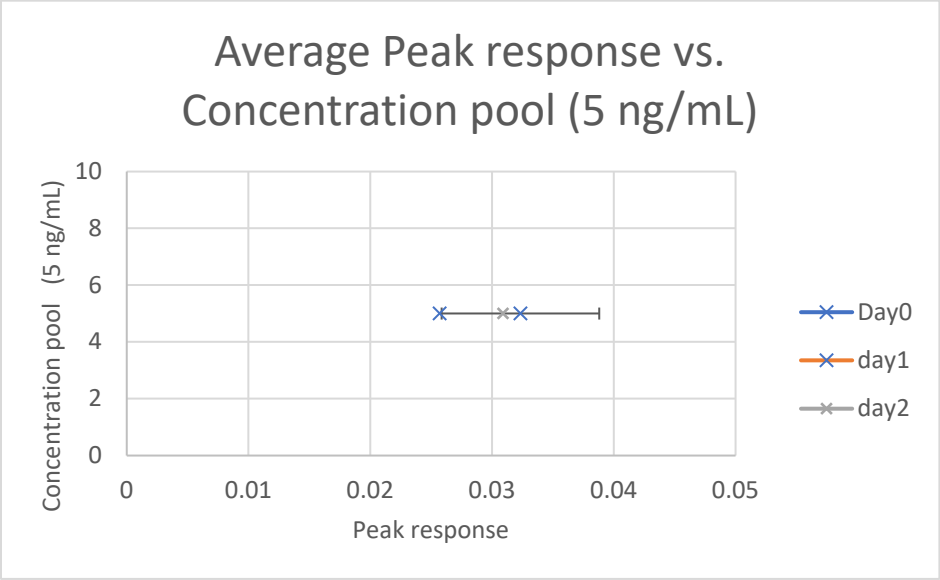
Stability Graphs 192-194 (urine): 2 ethyl hydroxy flurazepam

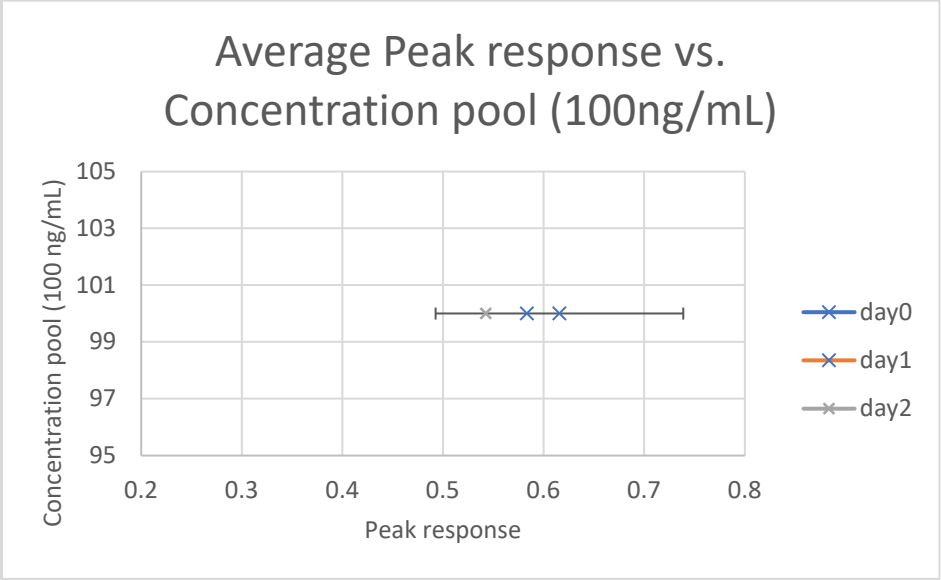


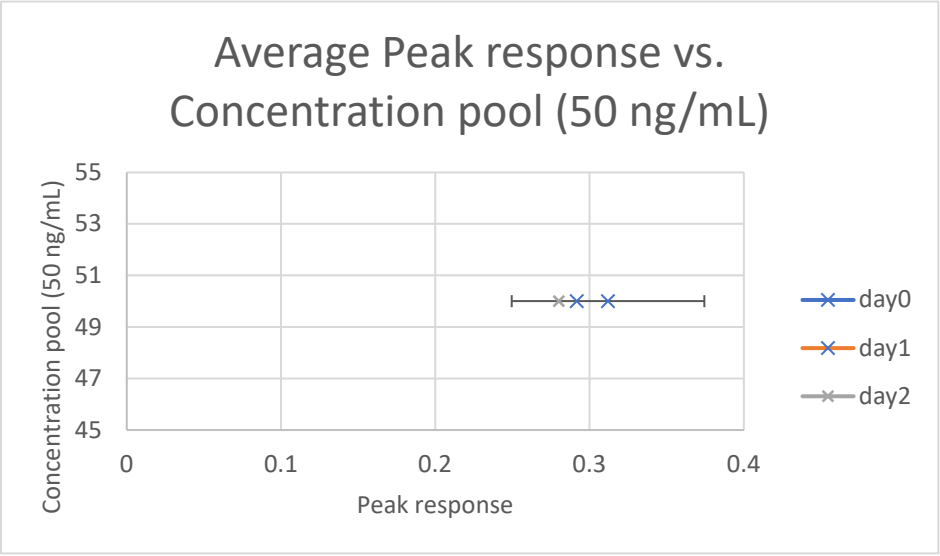




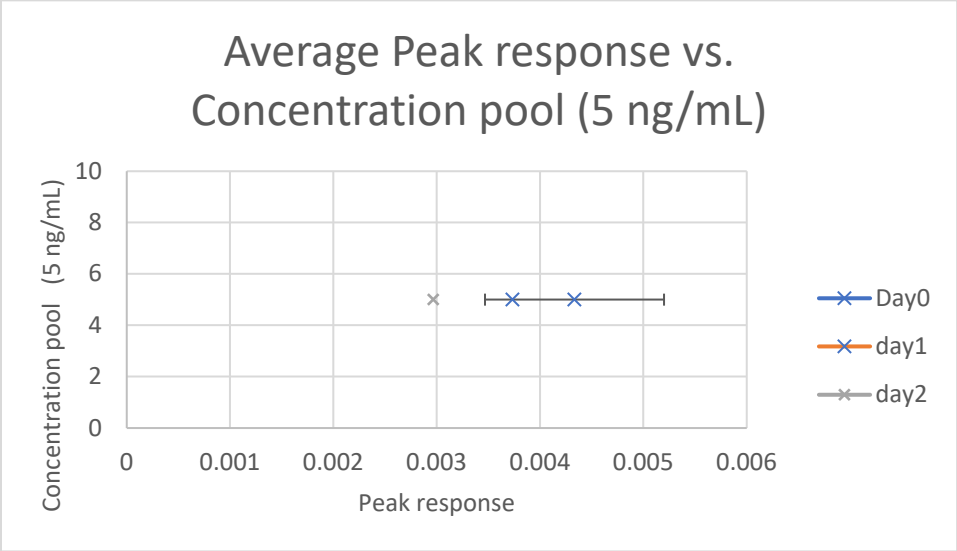
Stability Graphs 195-197 (urine): Clonazolam

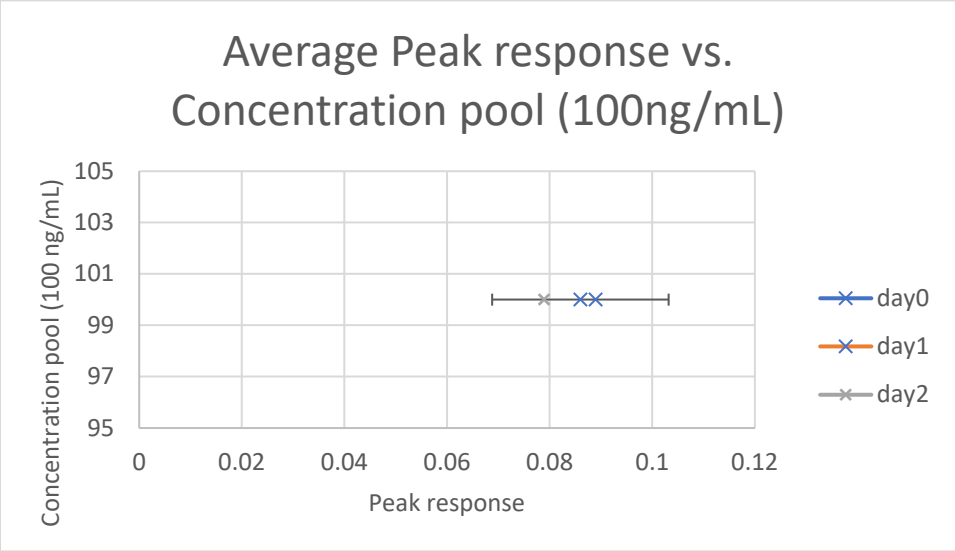


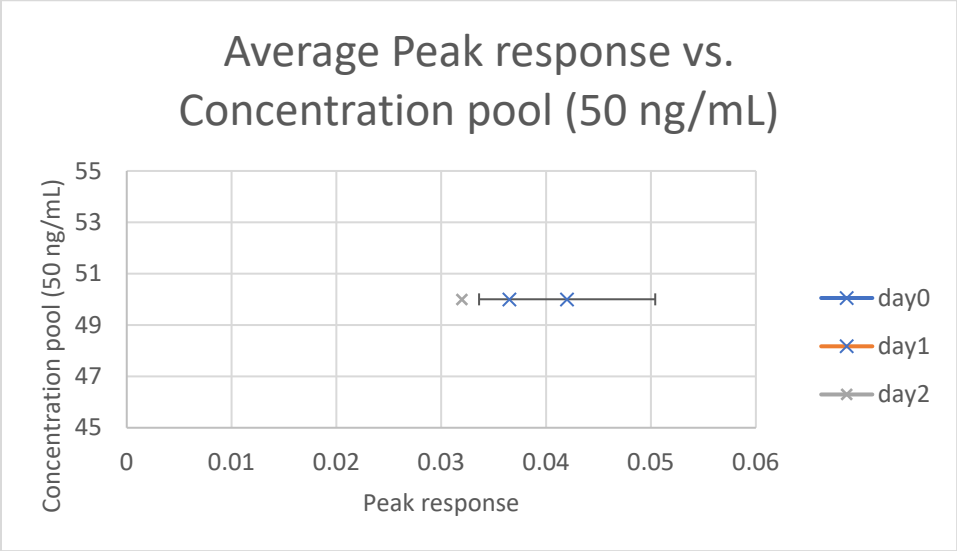




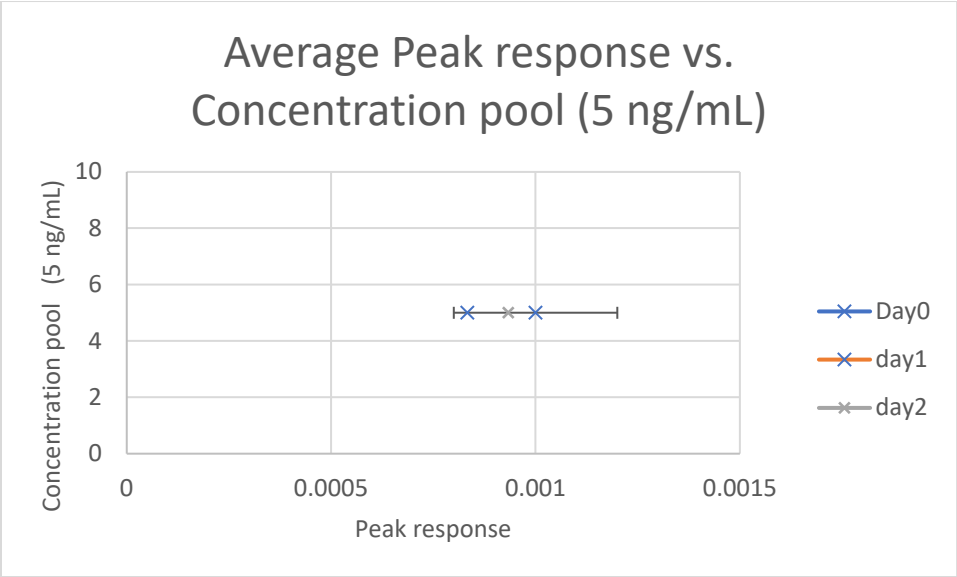
Stability Graphs 198-200 (urine): 8-amino clonazolam

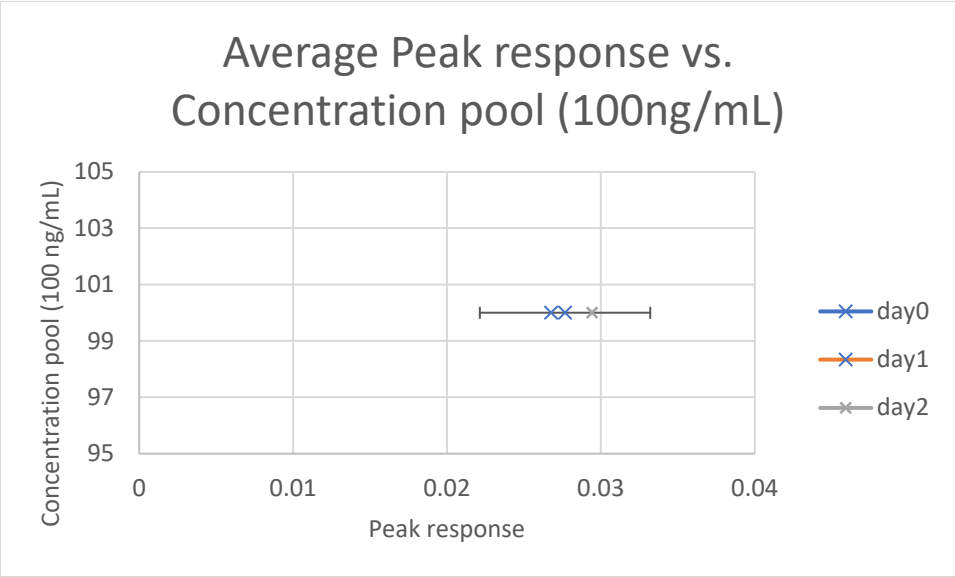


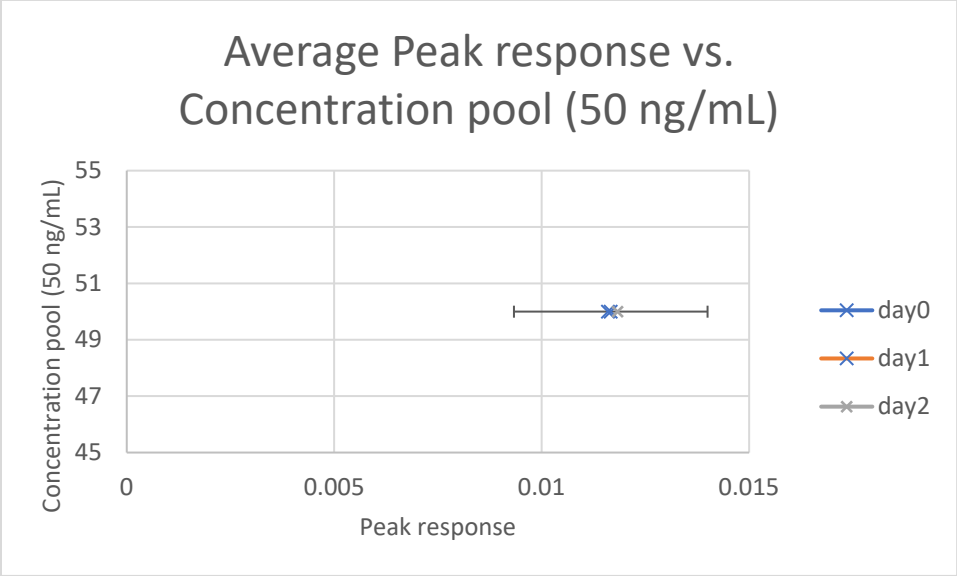




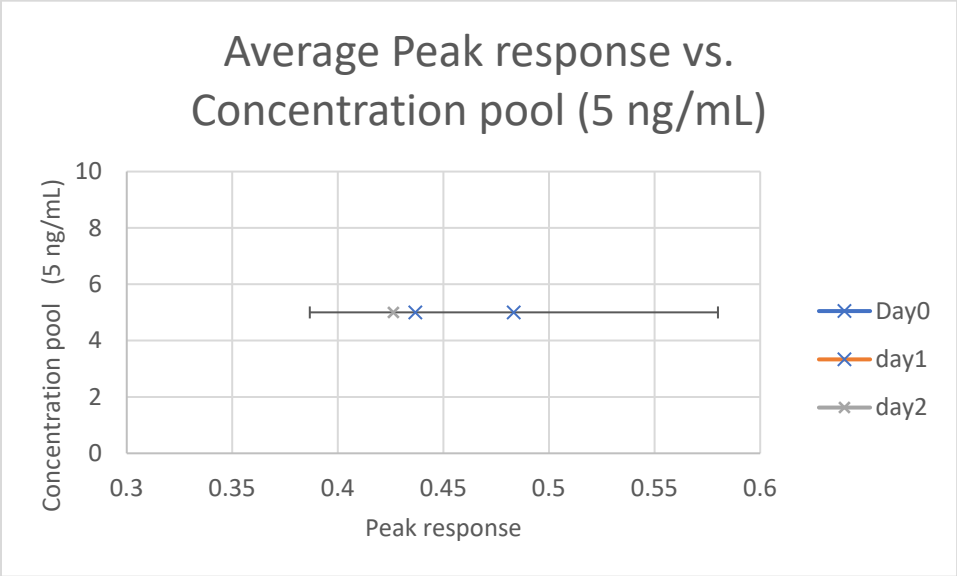
Stability Graphs 201-203 (urine): Alpha hydroxy clonazolam

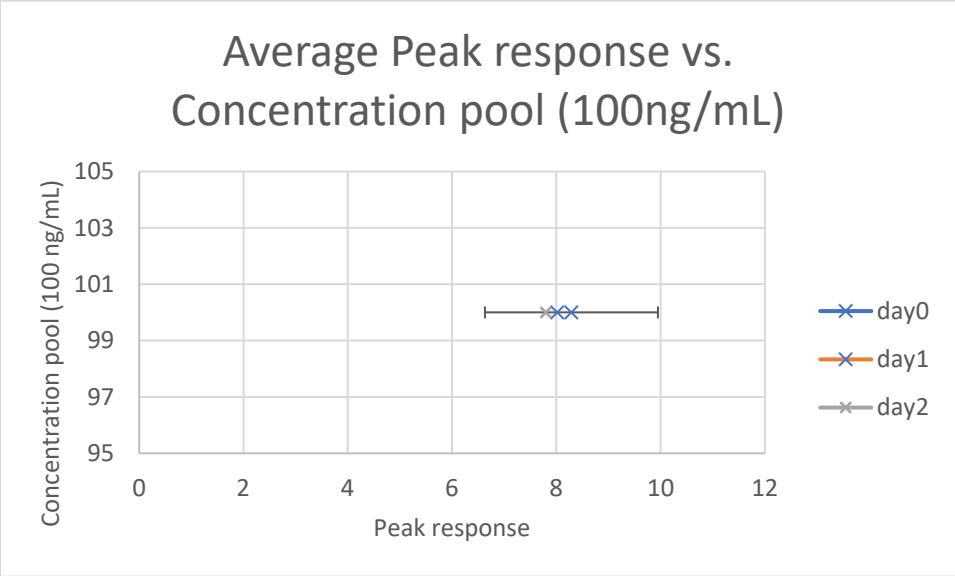


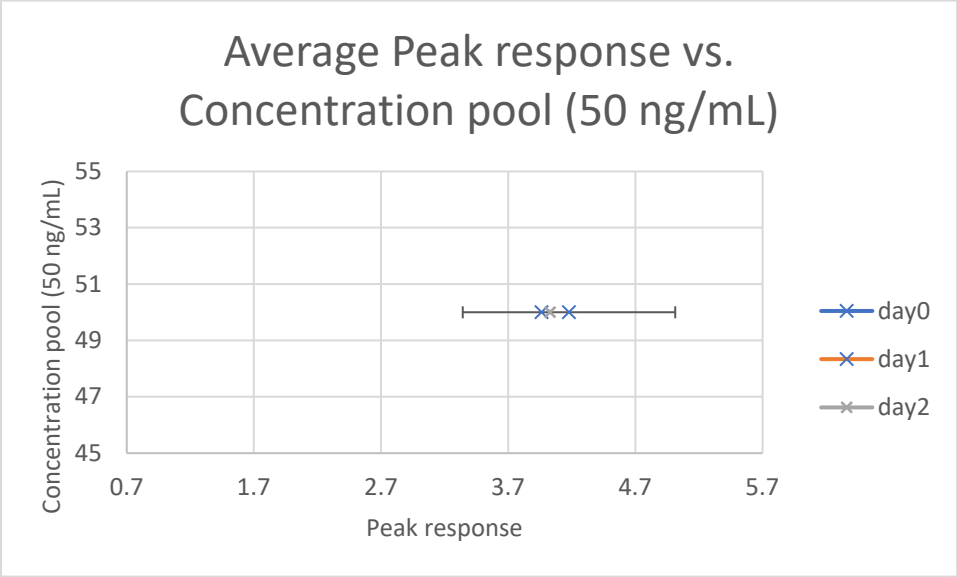




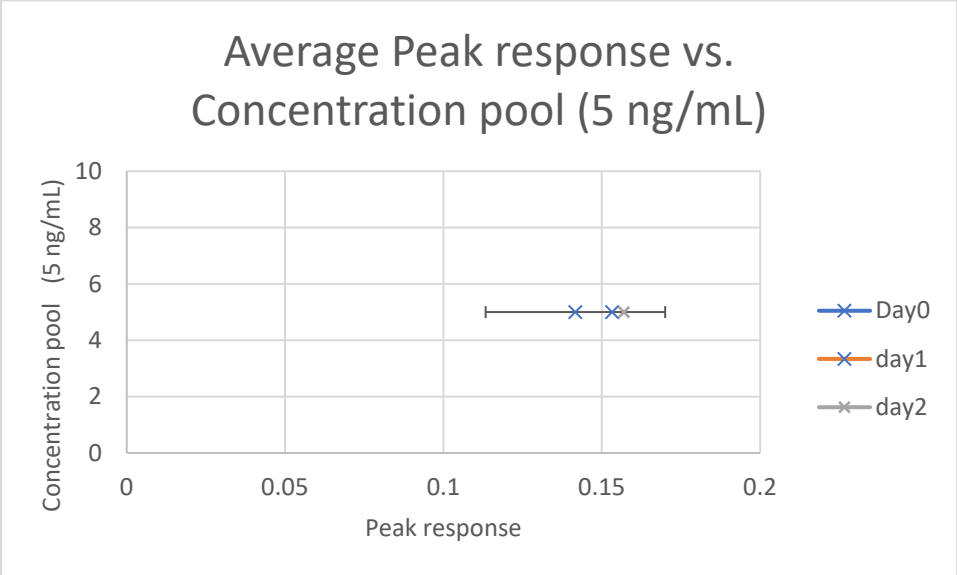
Stability Graphs 204-206 (urine): Delorazepam

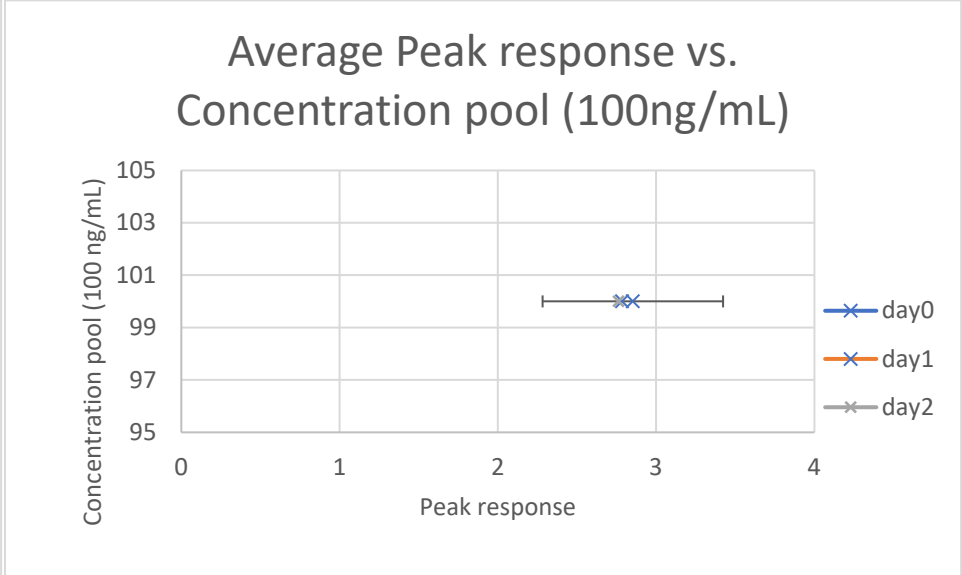


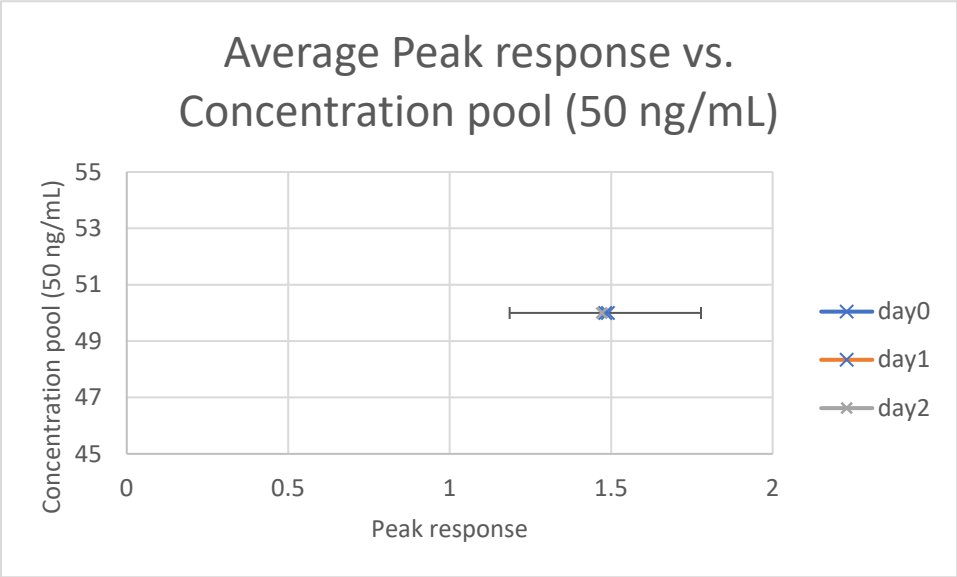




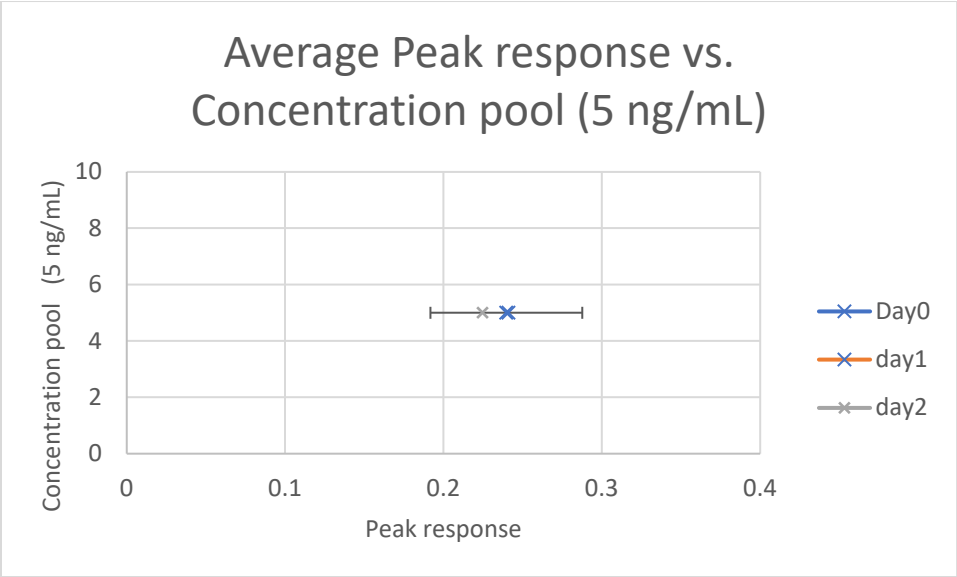
Stability Graphs 207-209 (urine): Demoxepam

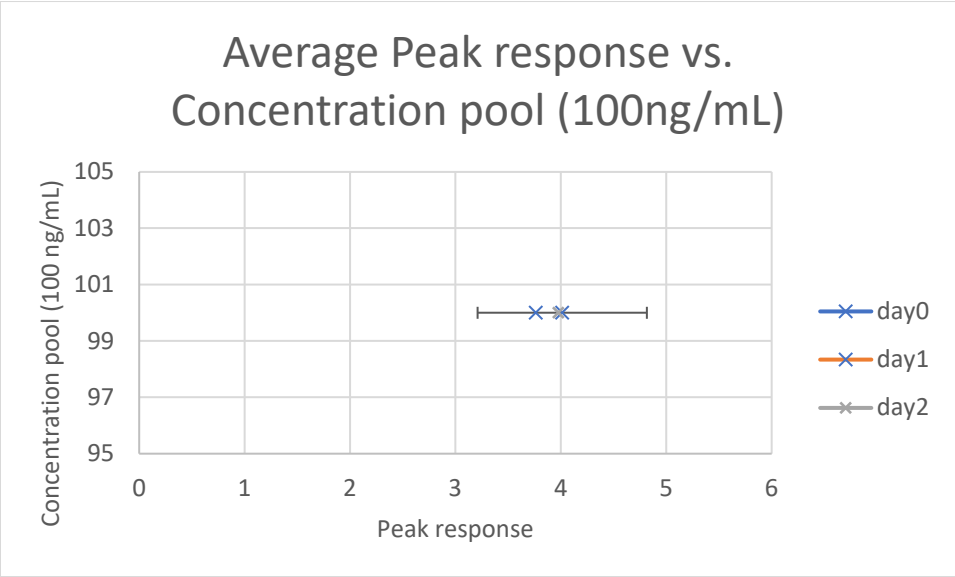


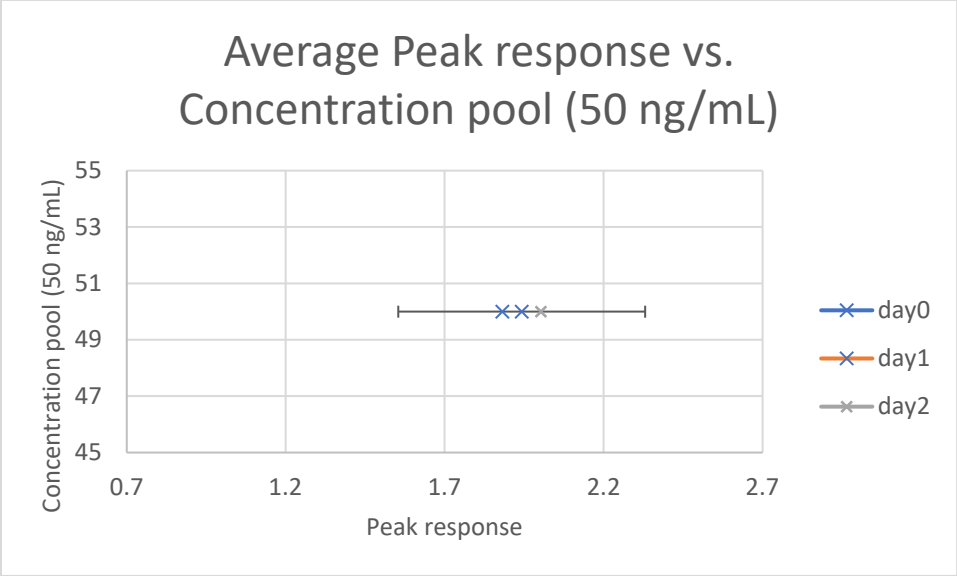




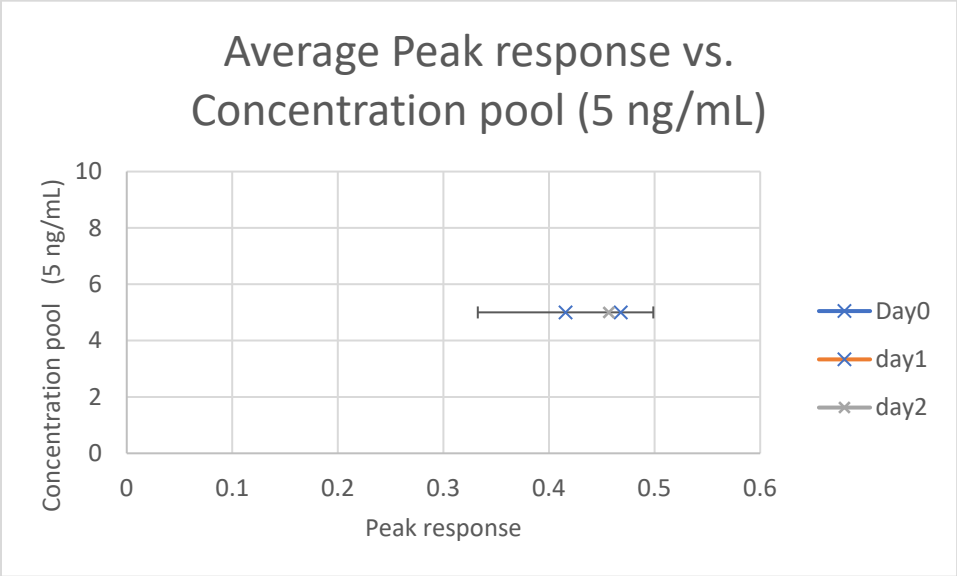
Stability Graphs 210-212 (urine): Diclazepam

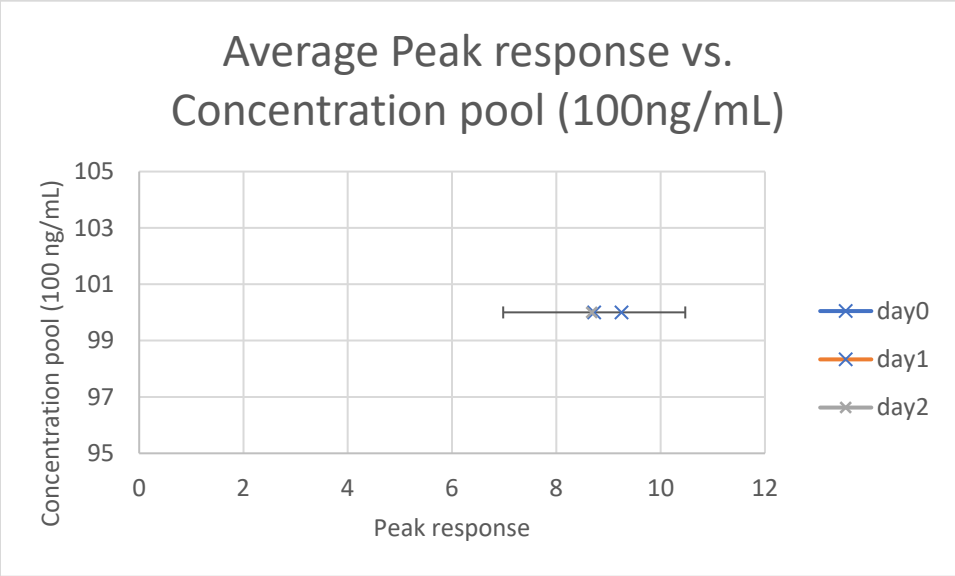


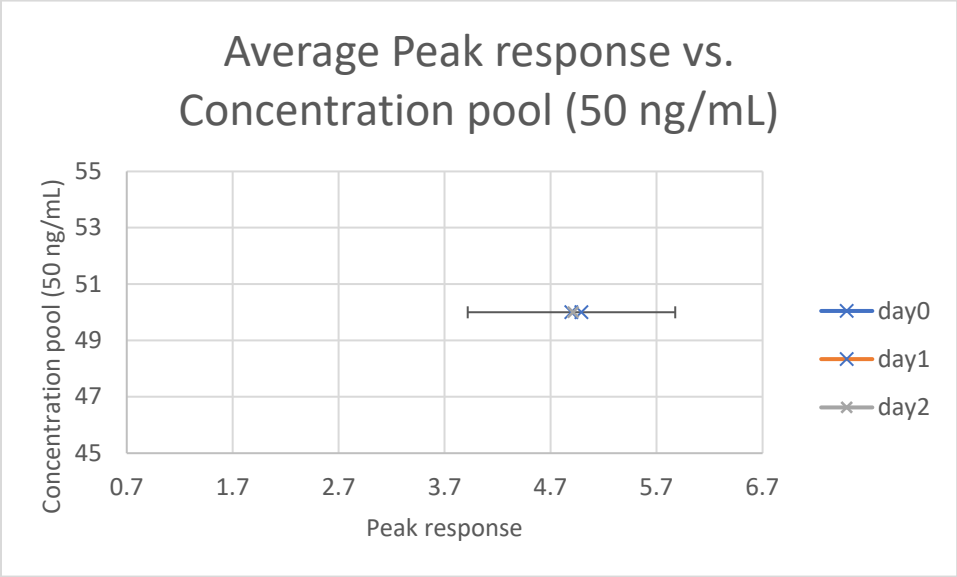




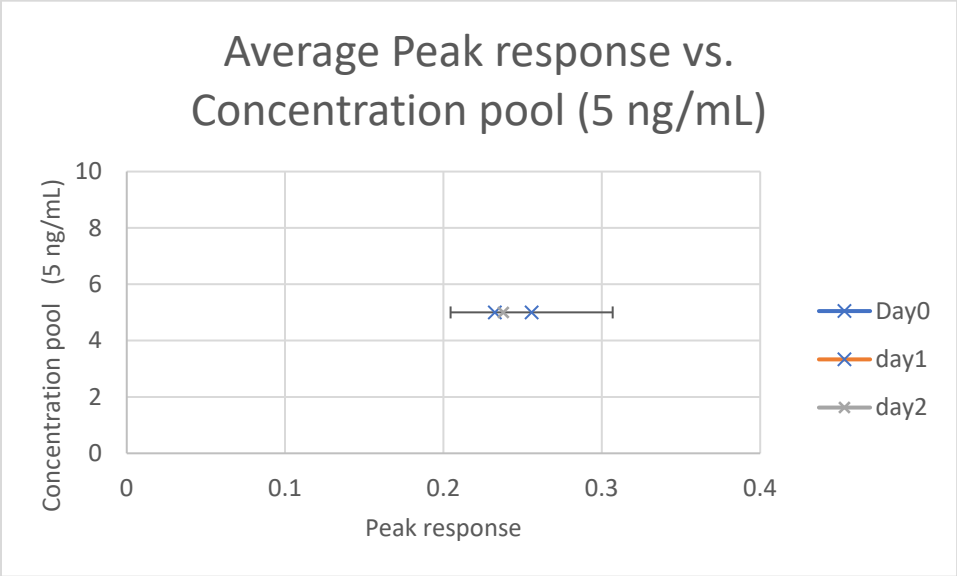
Stability Graphs 213-215 (urine): Estazolam

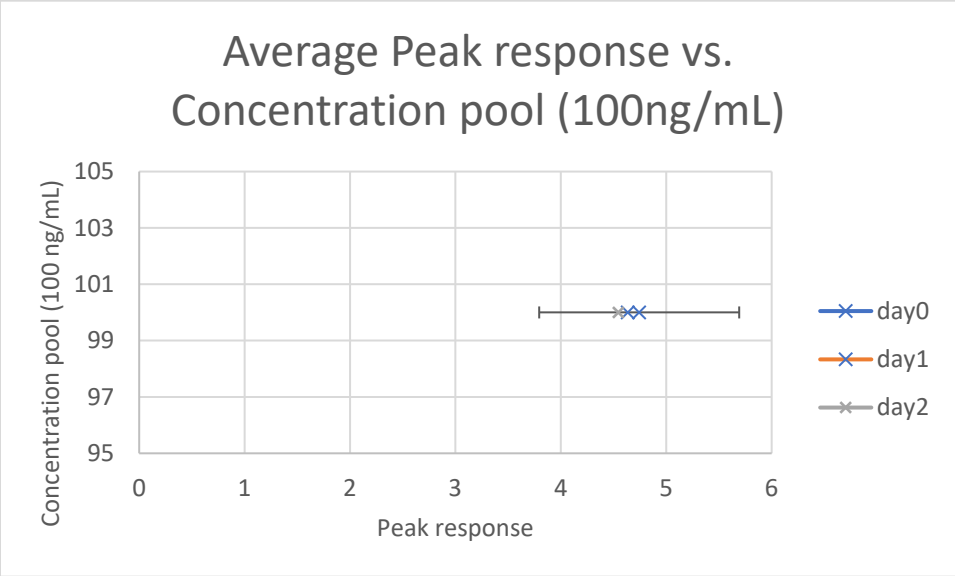


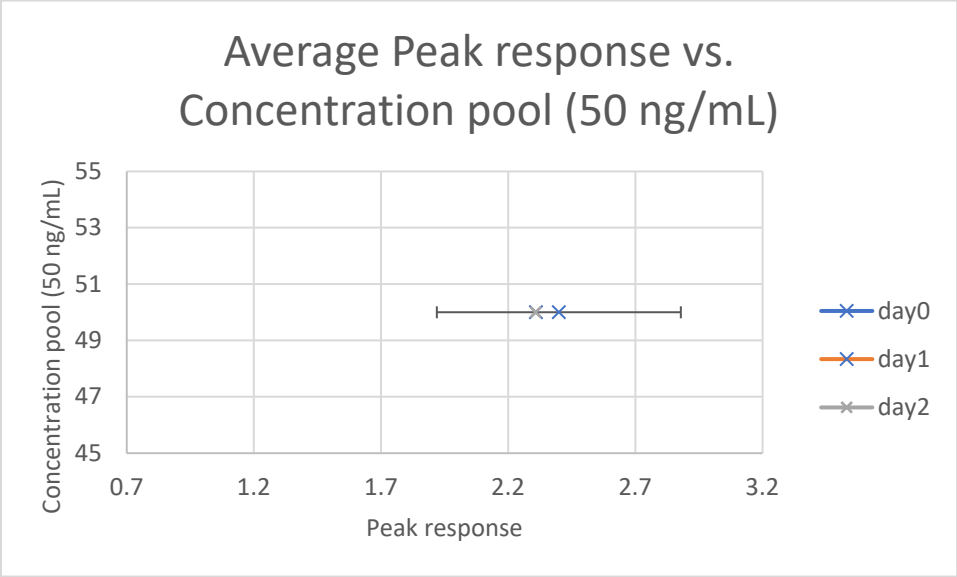




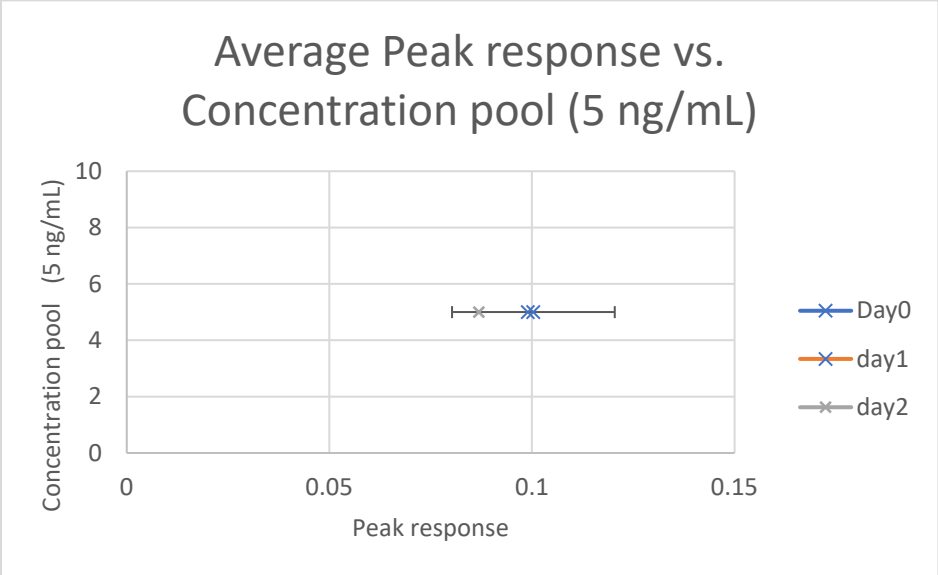
Stability Graphs 216-218 (urine): Flualprazolam

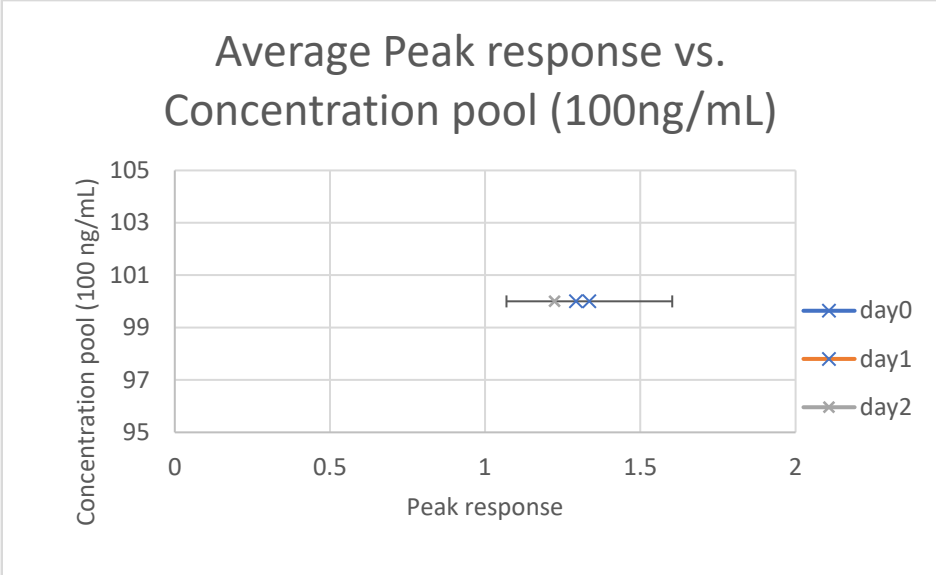


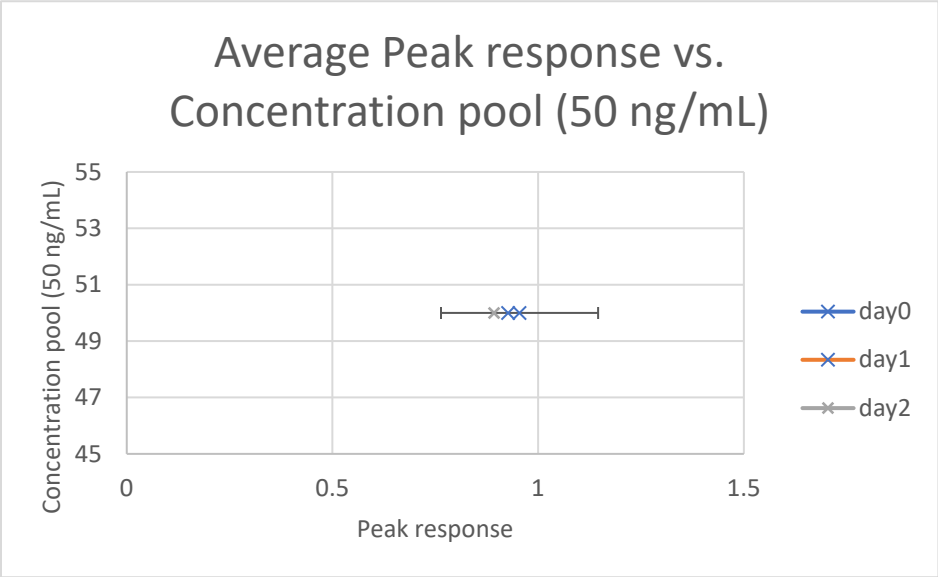




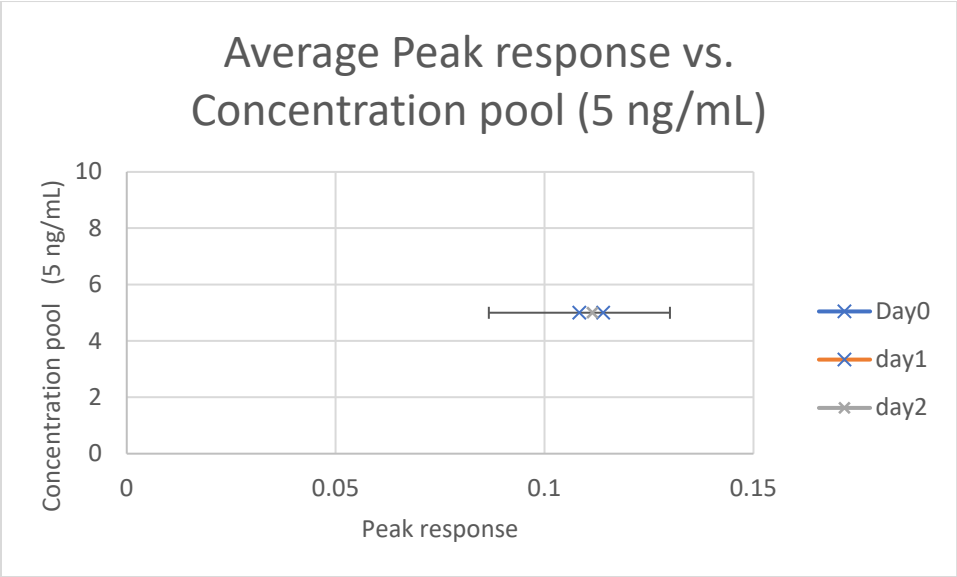
Stability Graphs 219-221 (urine): Flubromazepam

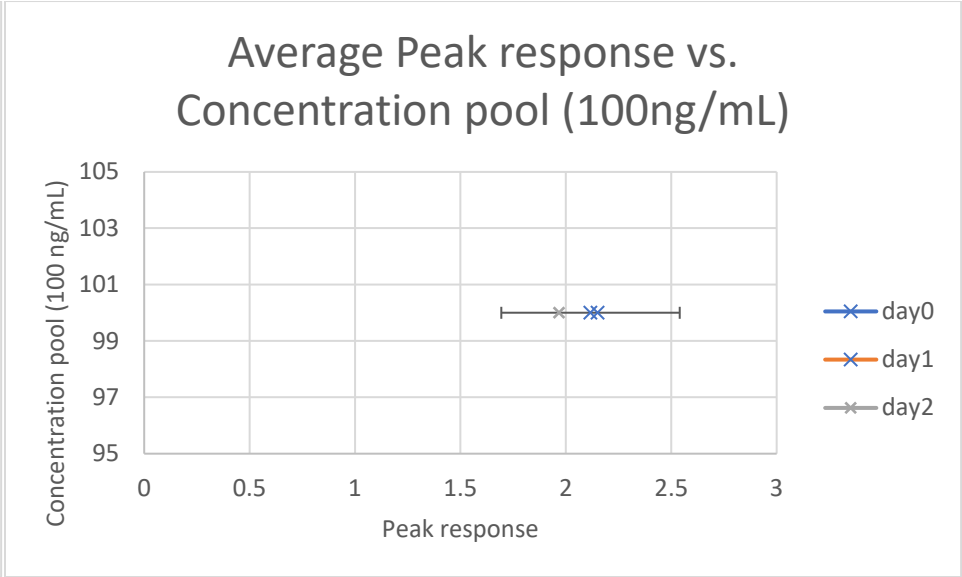


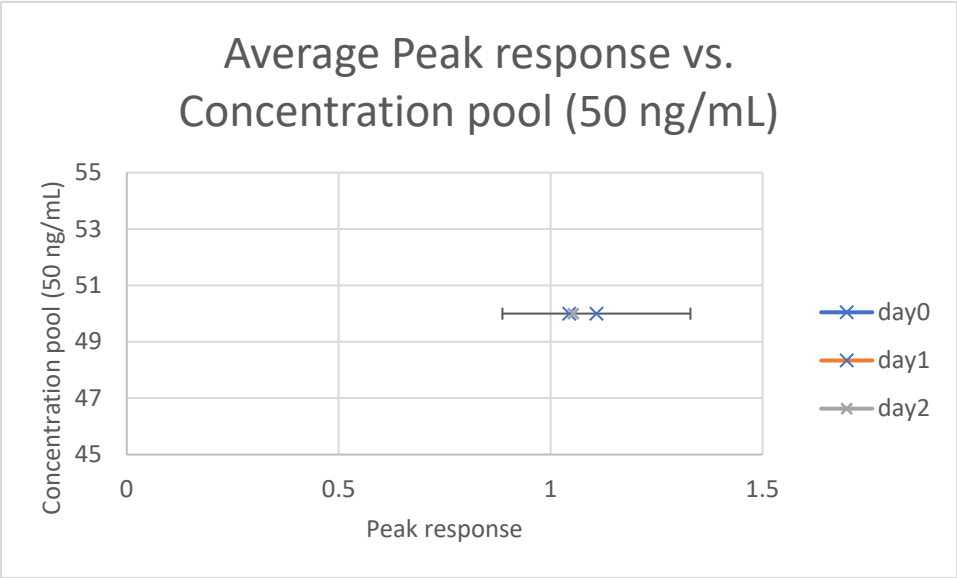




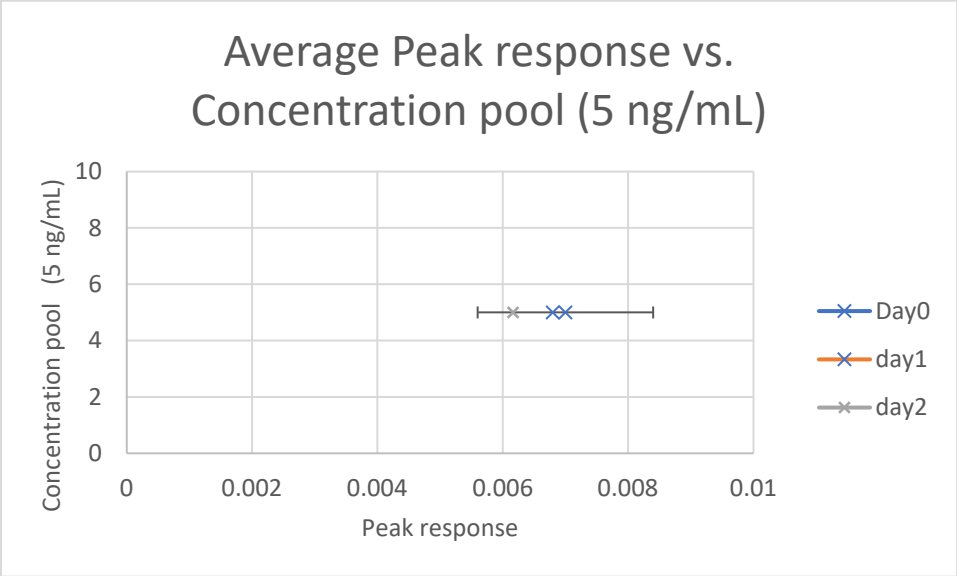
Stability Graphs 222-224 (urine): Flubromazolam

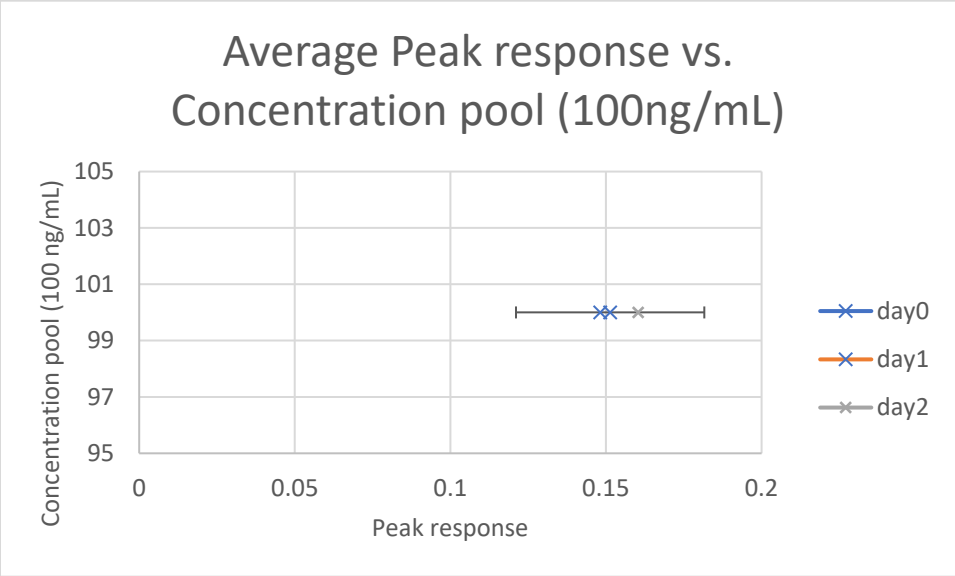


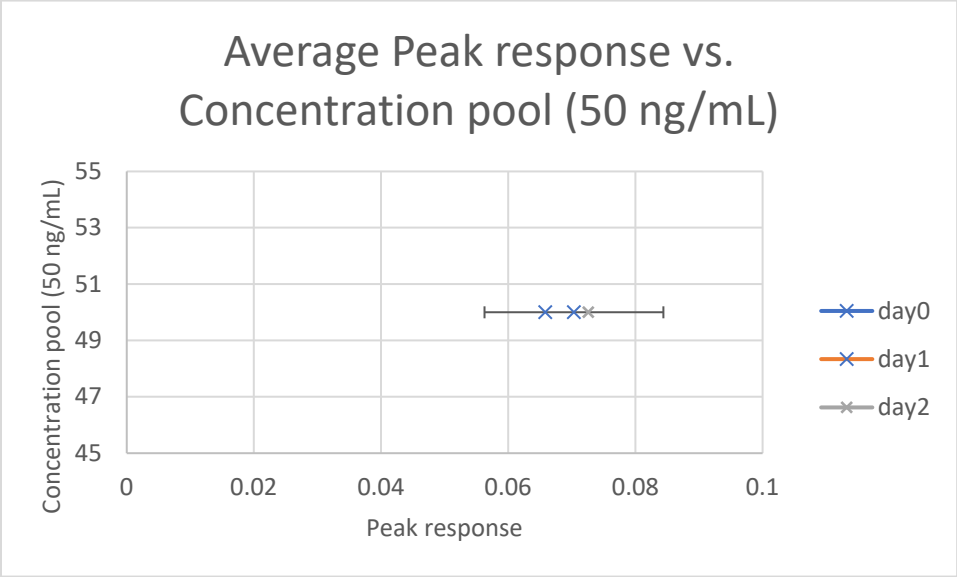




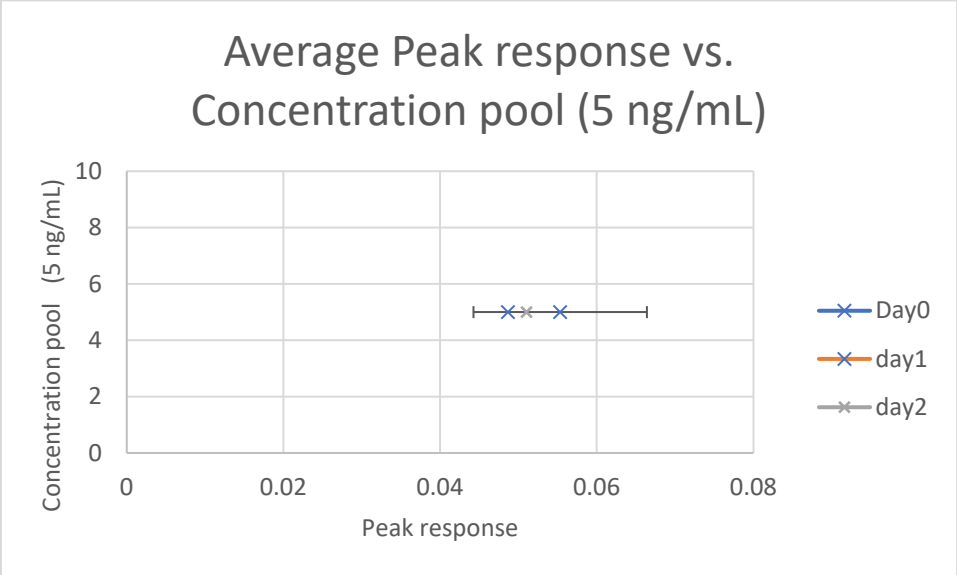
Stability Graphs 225-227 (urine): Alpha hydroxy Flubromazolam

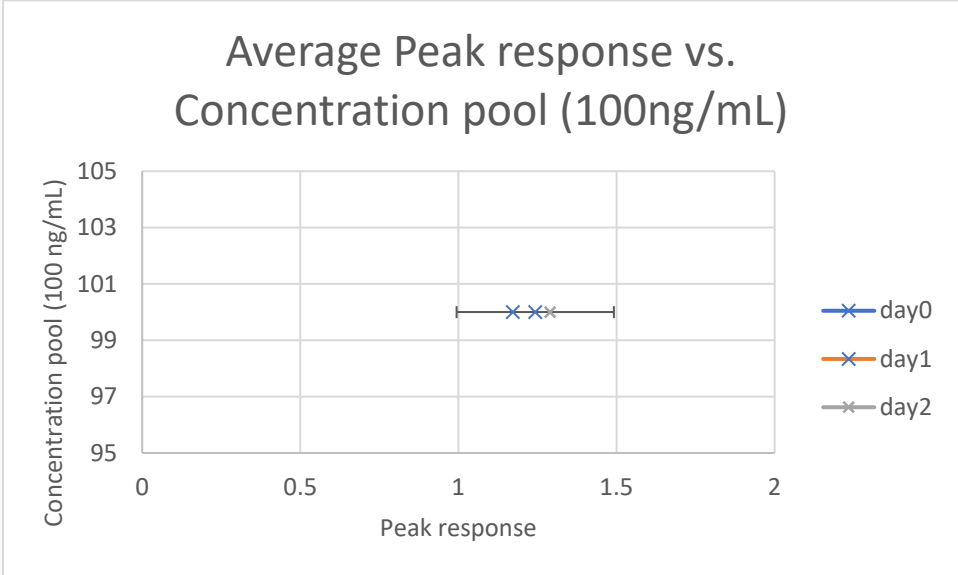


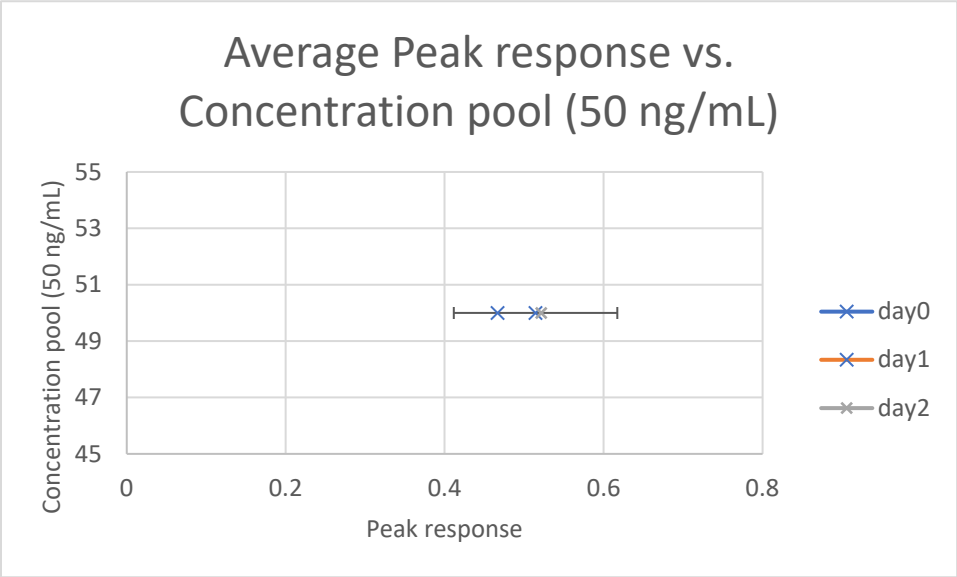




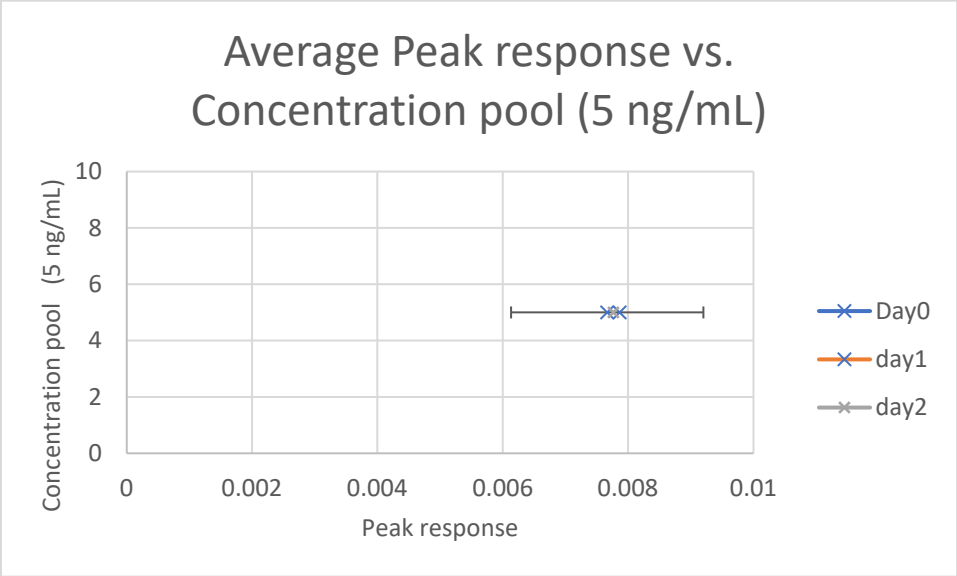
Stability Graphs 228-230 (urine): Flurazepam

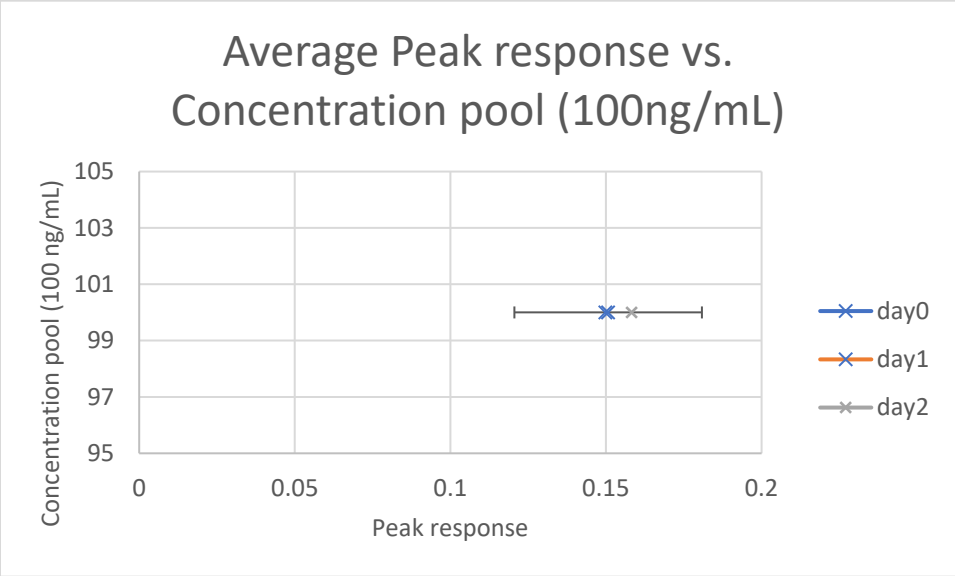


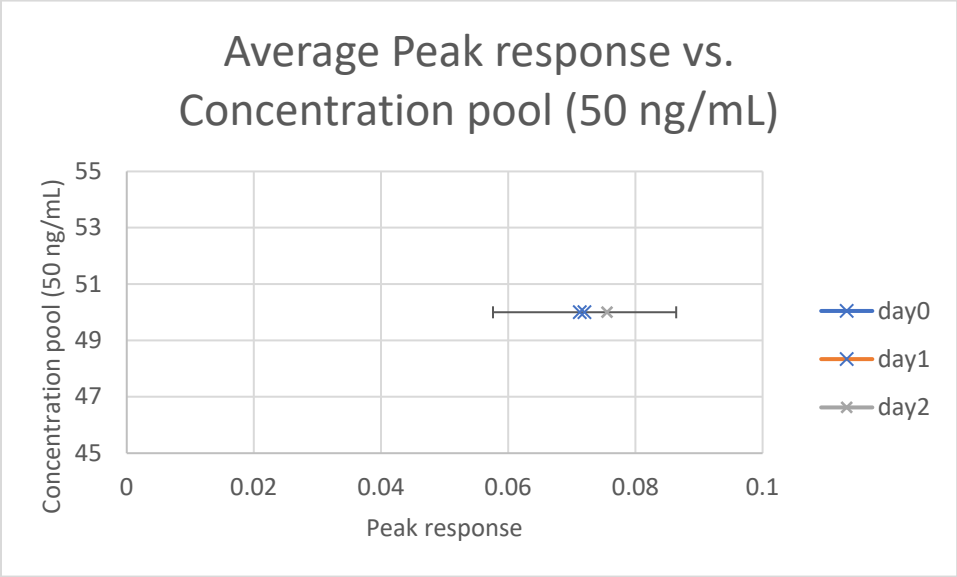




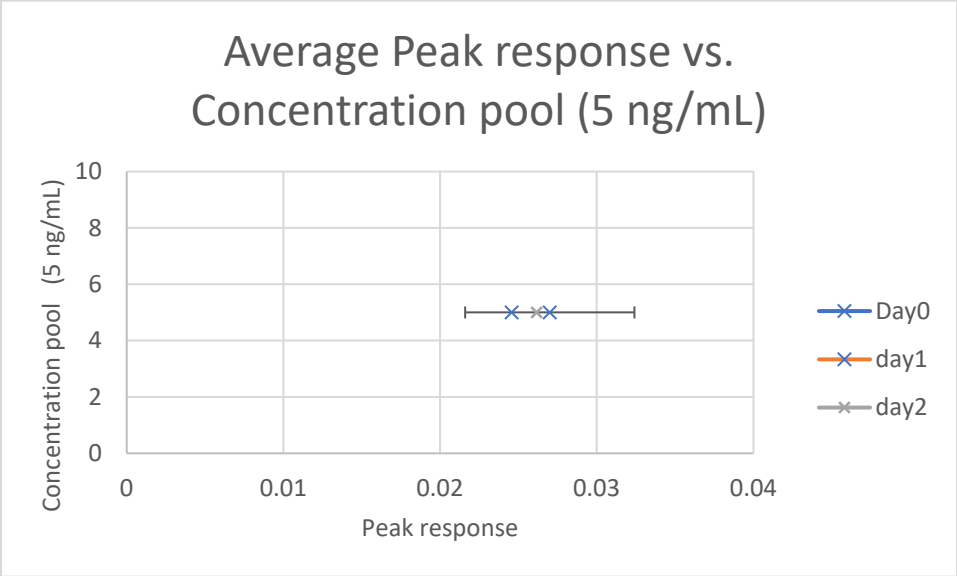
Stability Graphs 231-233 (urine): Lormetazepam

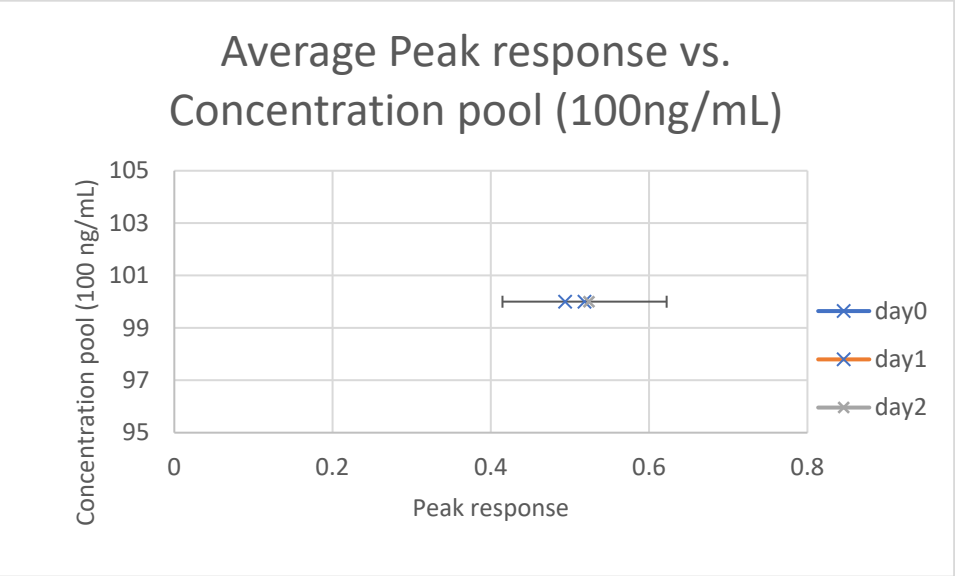


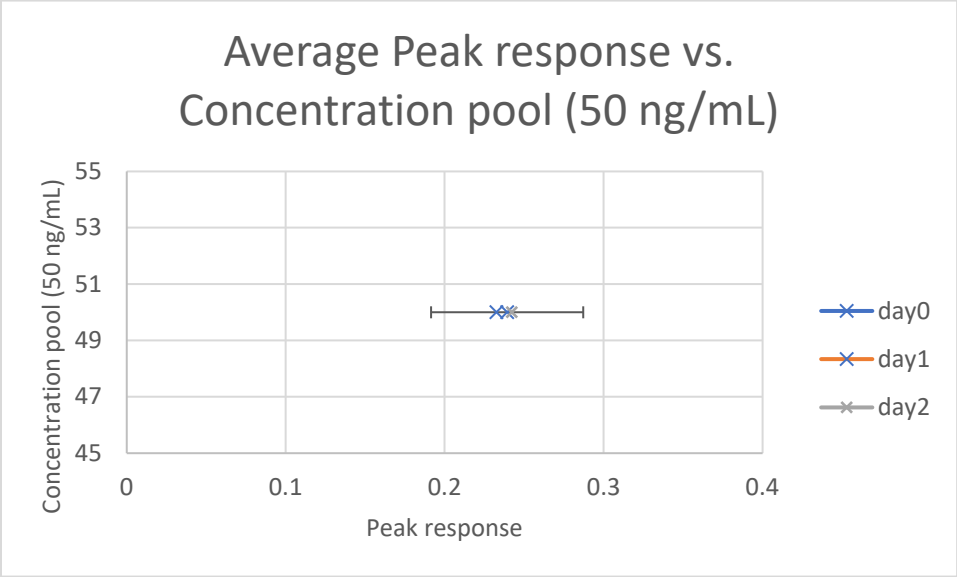




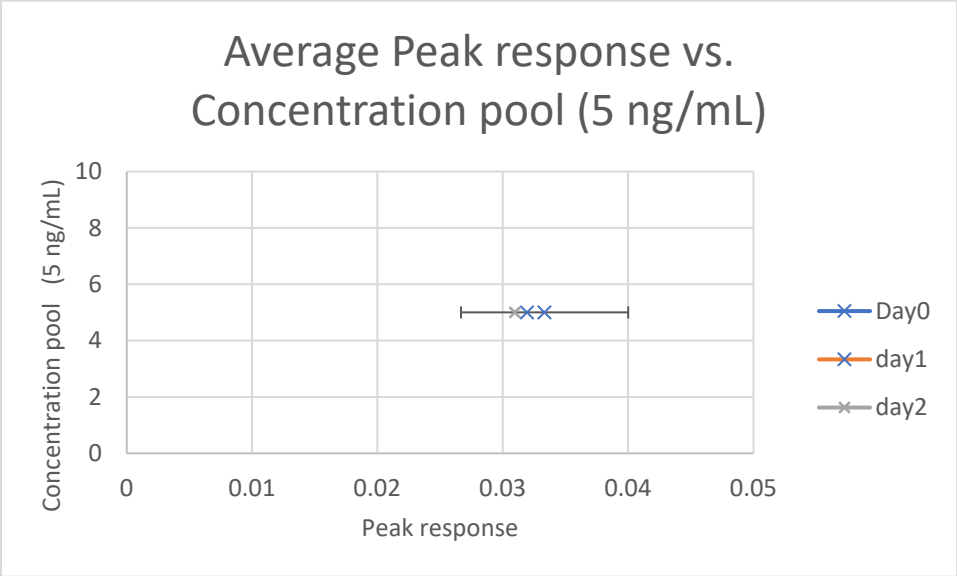
Stability Graphs 234-236 (urine): Nimetazepam

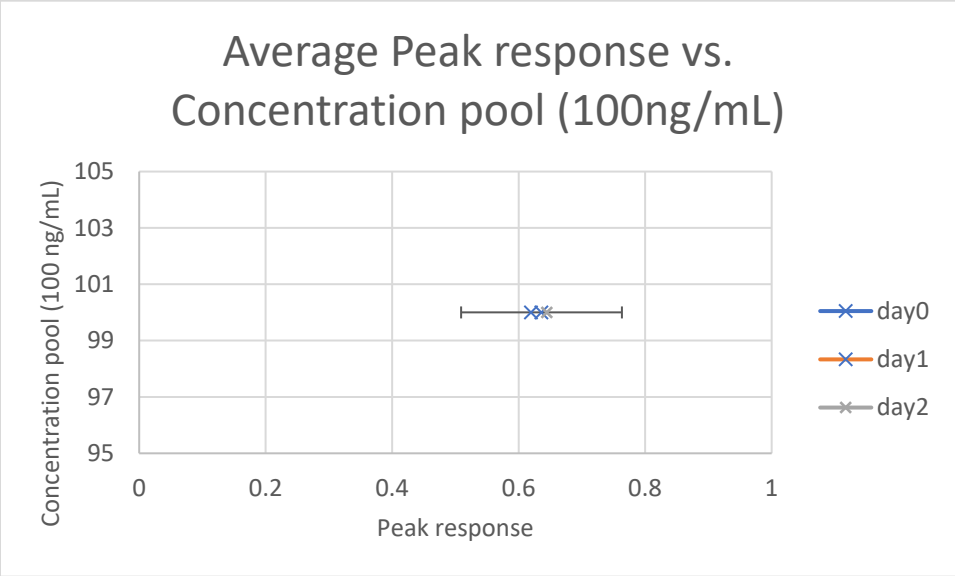


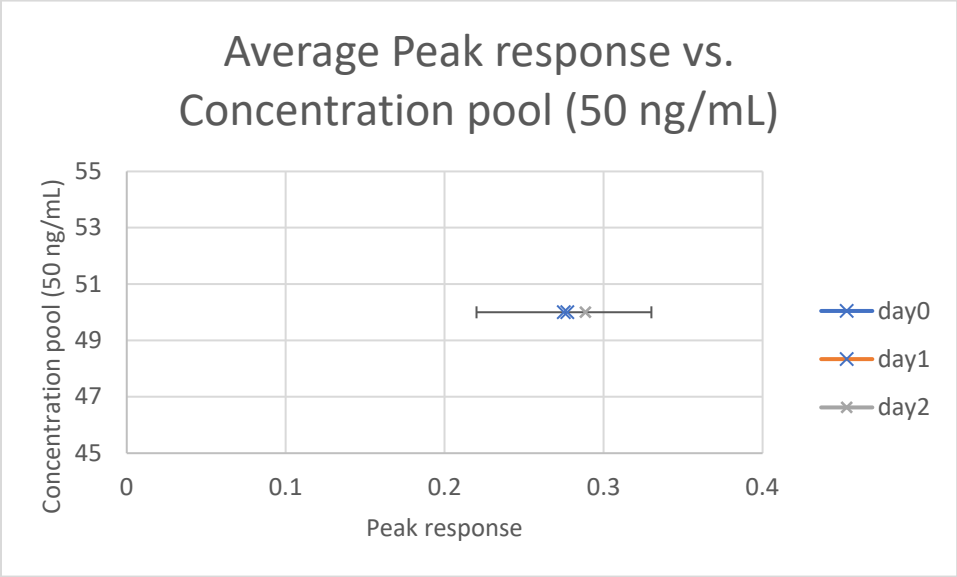




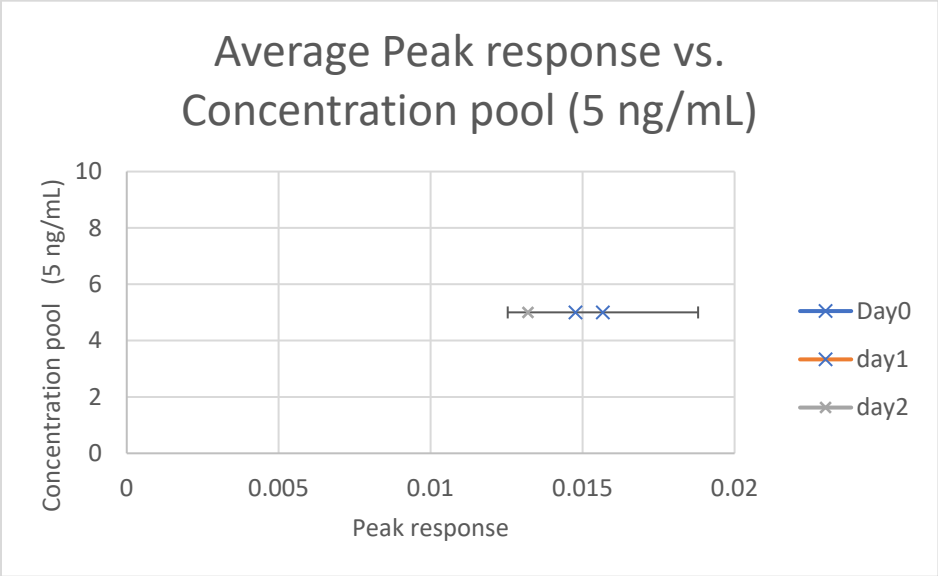
Stability Graphs 237-239 (urine): Nitrazepam

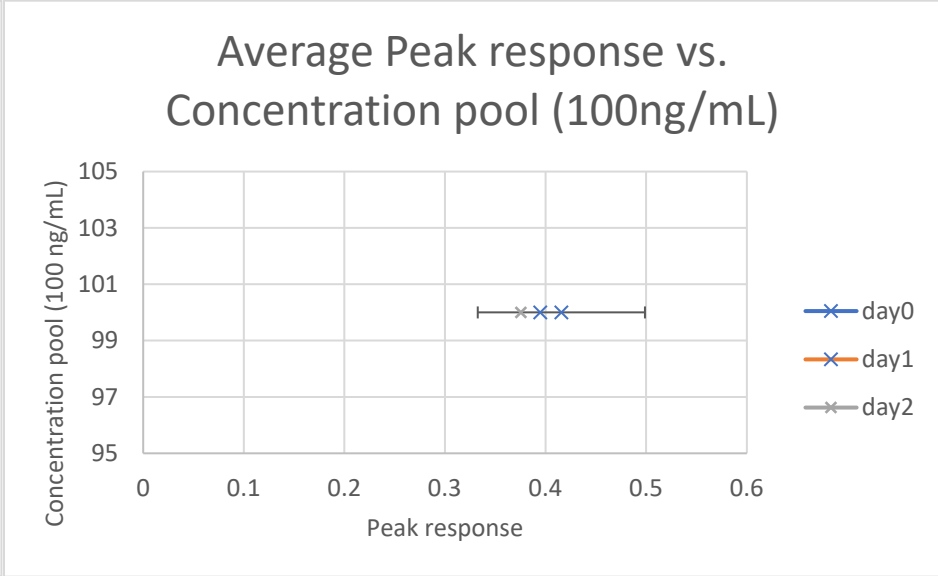


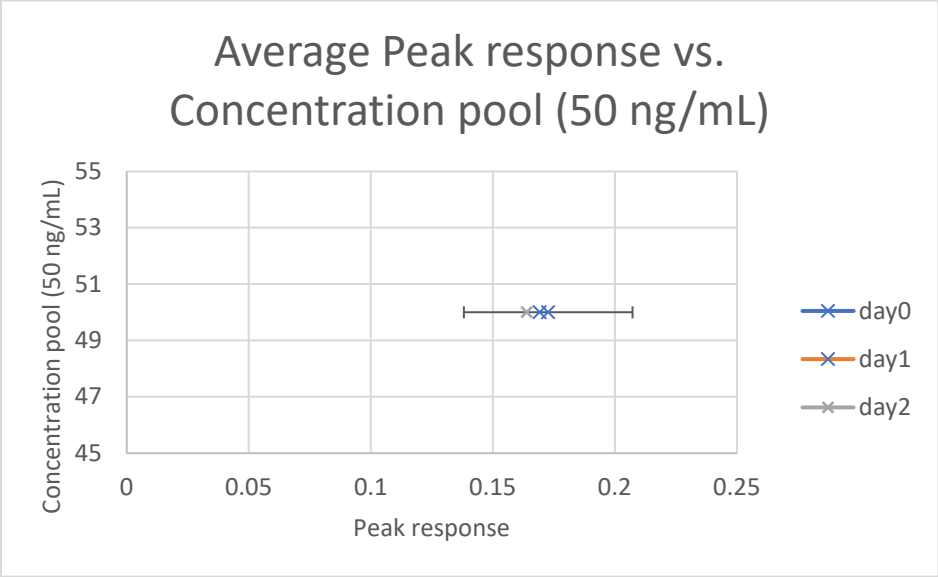




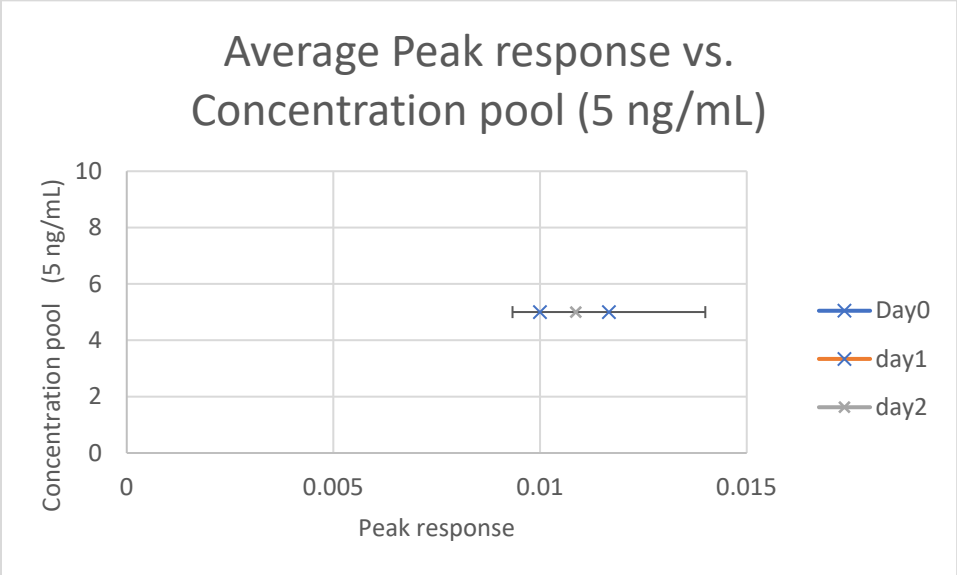
Stability Graphs 240-242 (urine): Norchlordiazepoxide

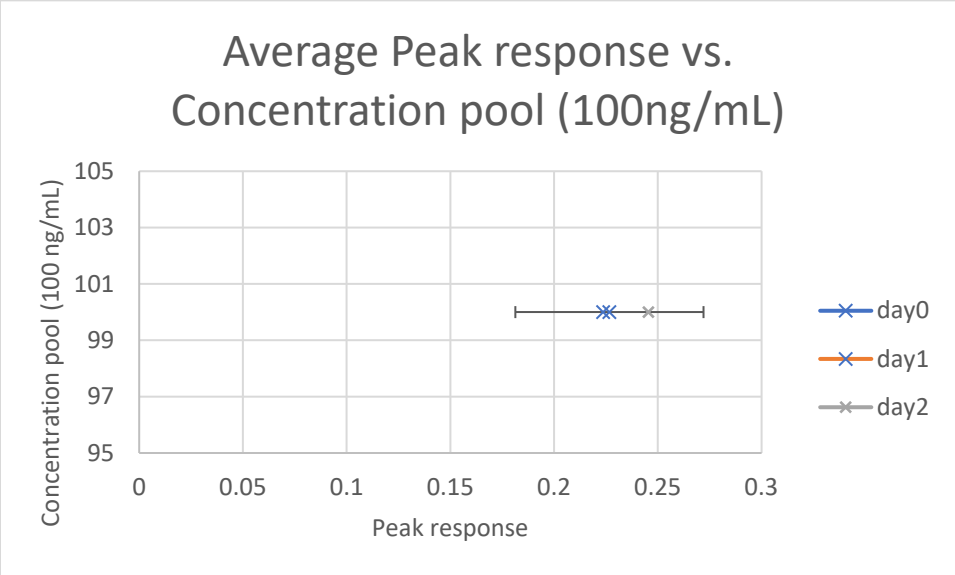


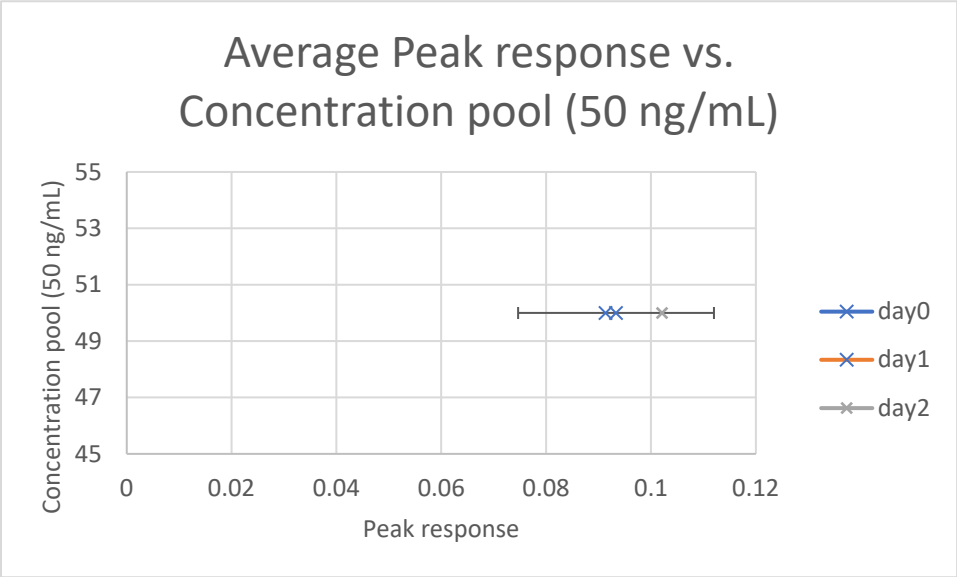




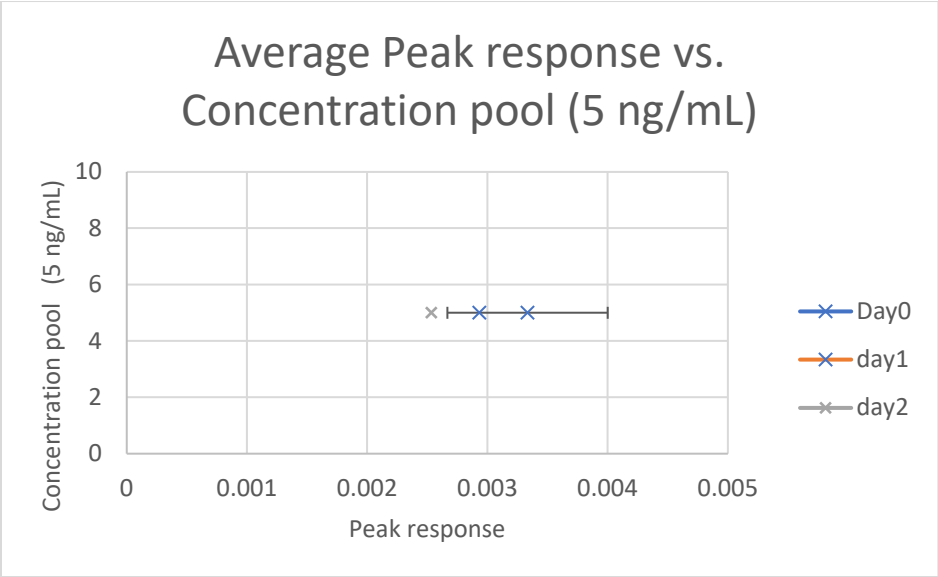
Stability Graphs 243-245 (urine): Phenazepam

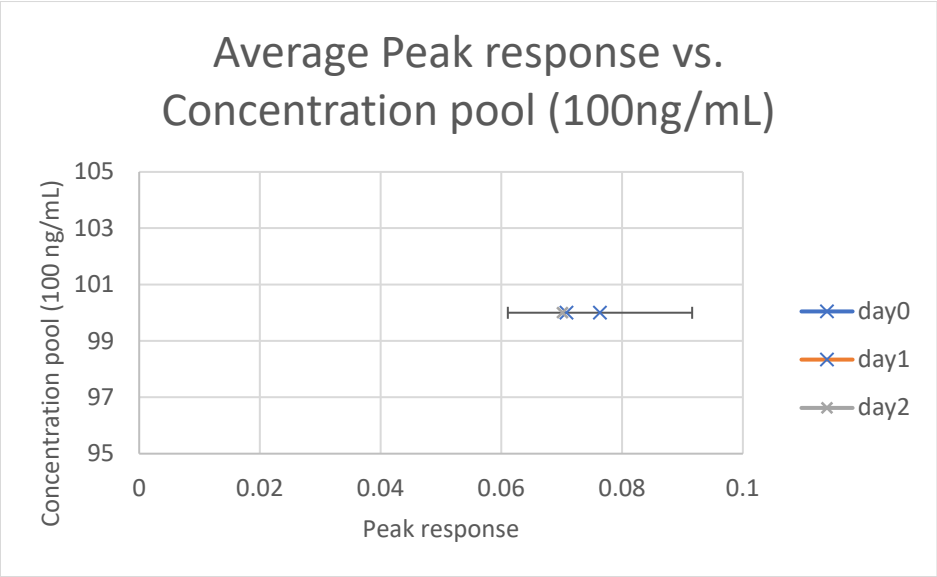


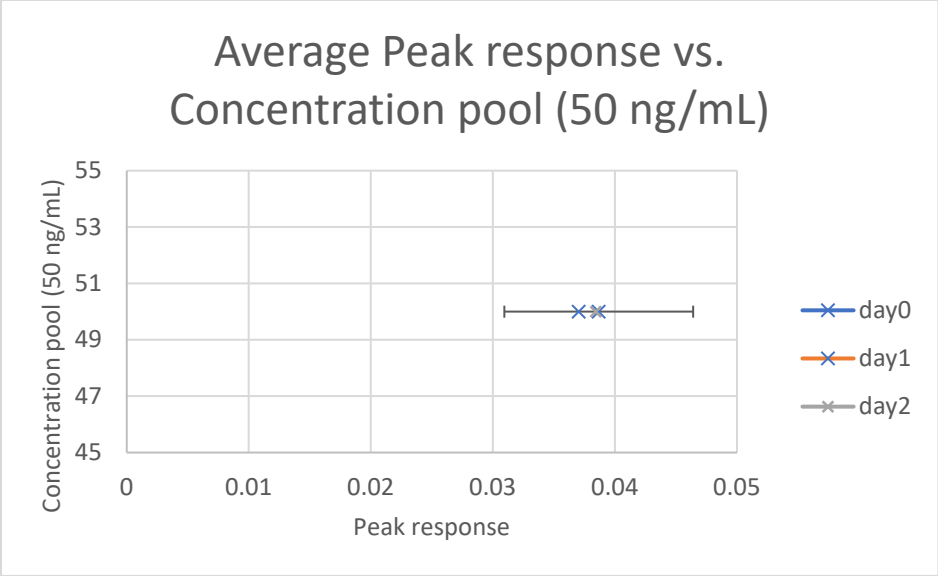




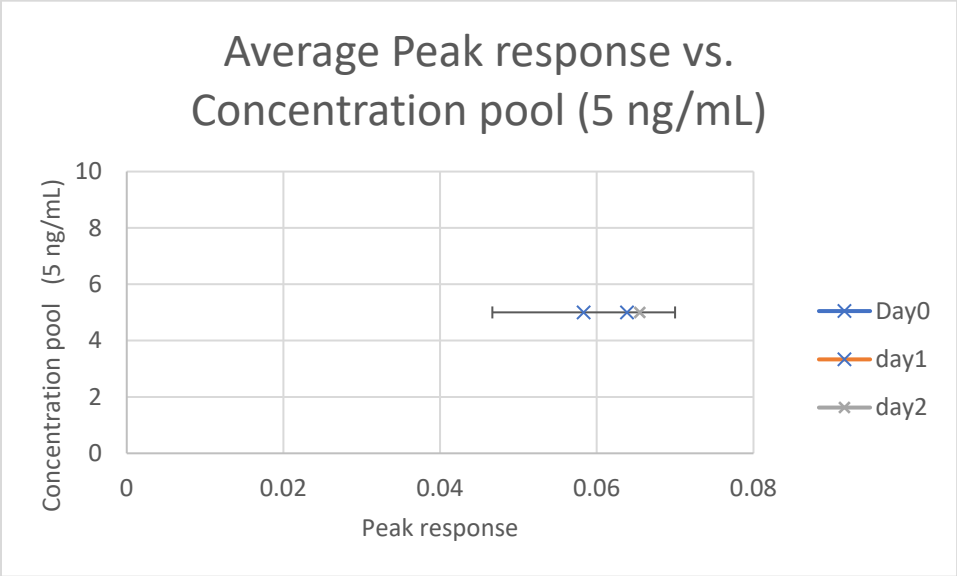
Stability Graphs 246-248 (urine): Pyrazolam

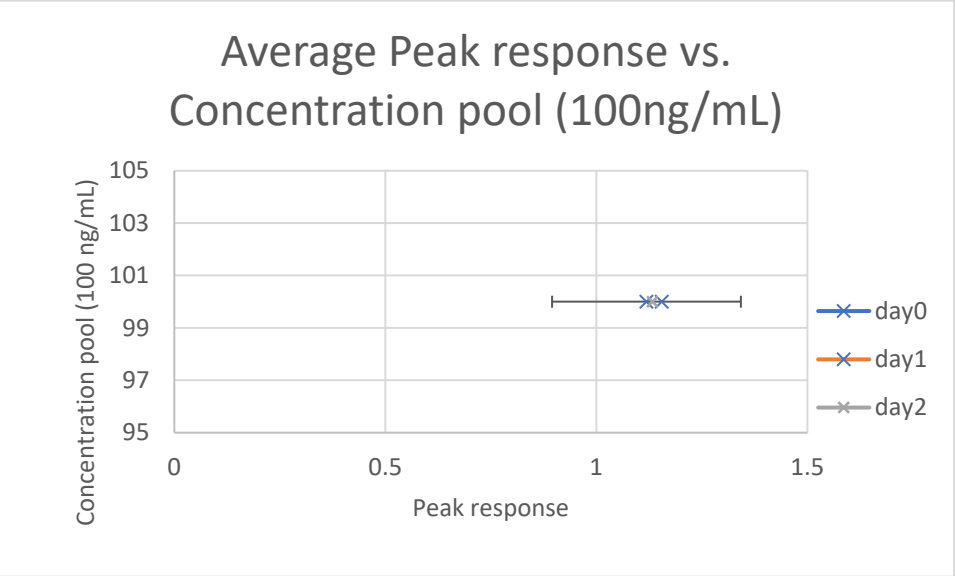


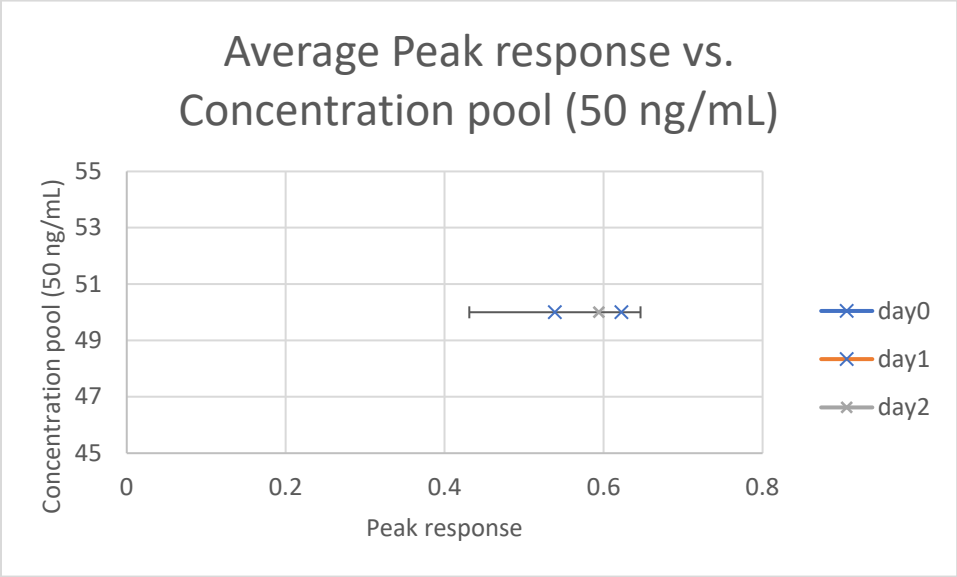




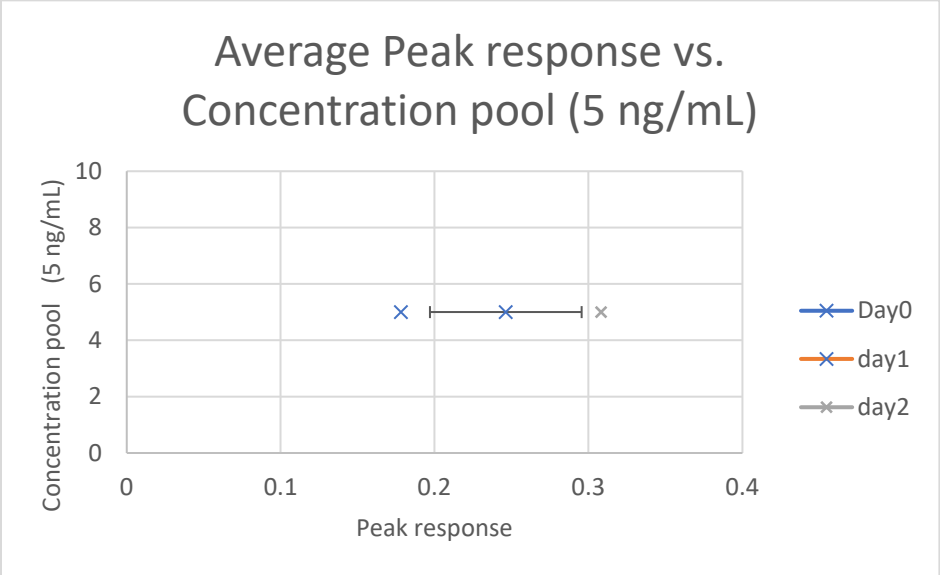
Stability Graphs 249-251 (urine): Zaleplon

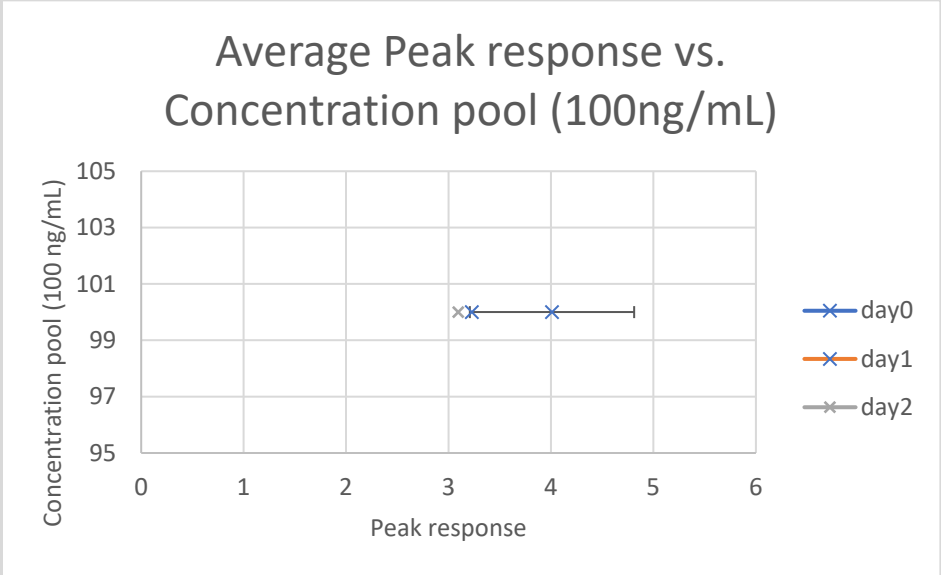


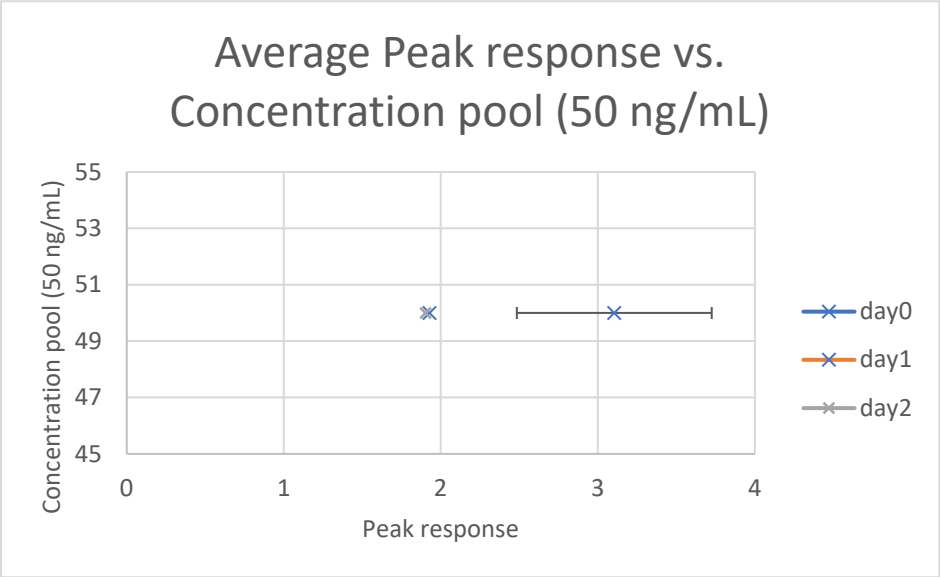




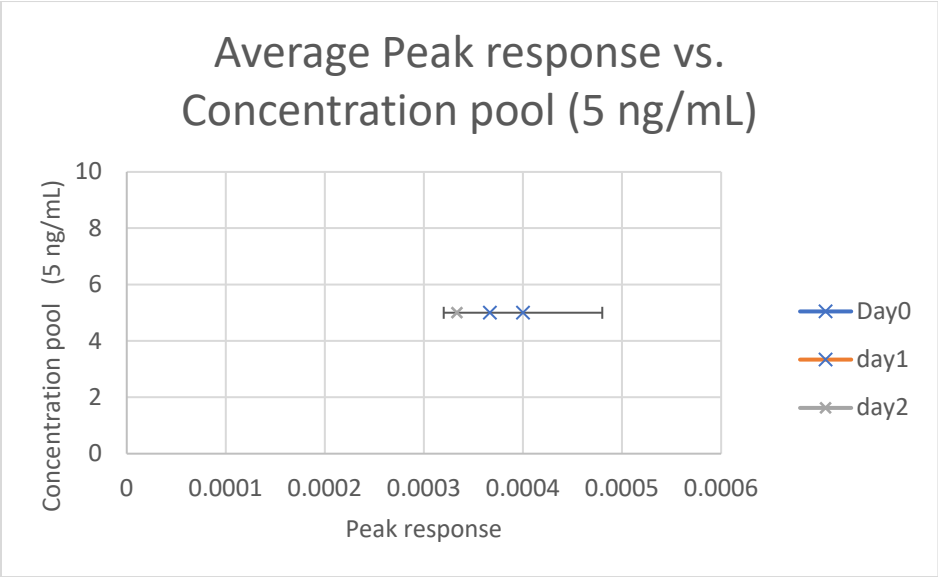
Stability Graphs 252-254 (urine): Zopiclone

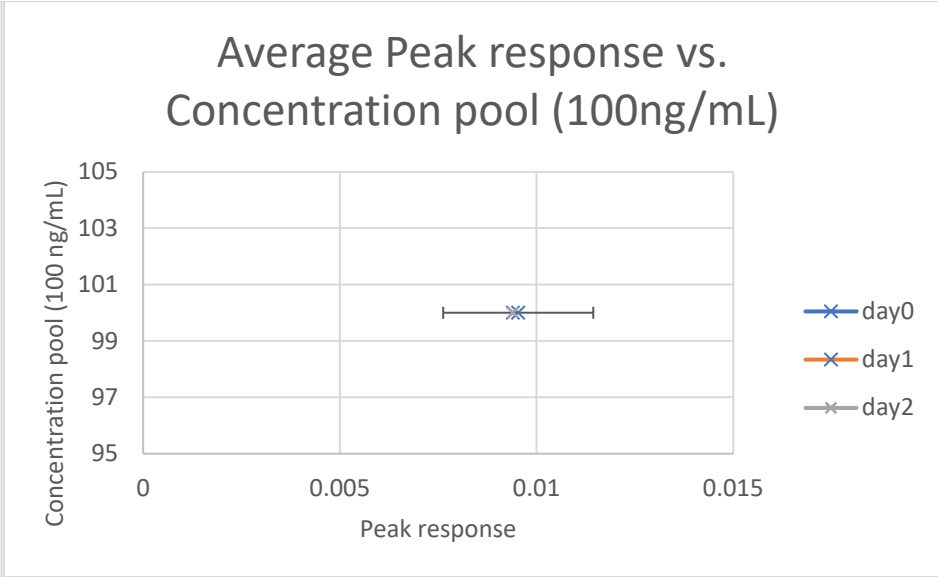






Stability Graphs 255-257 (urine): Adinazolam





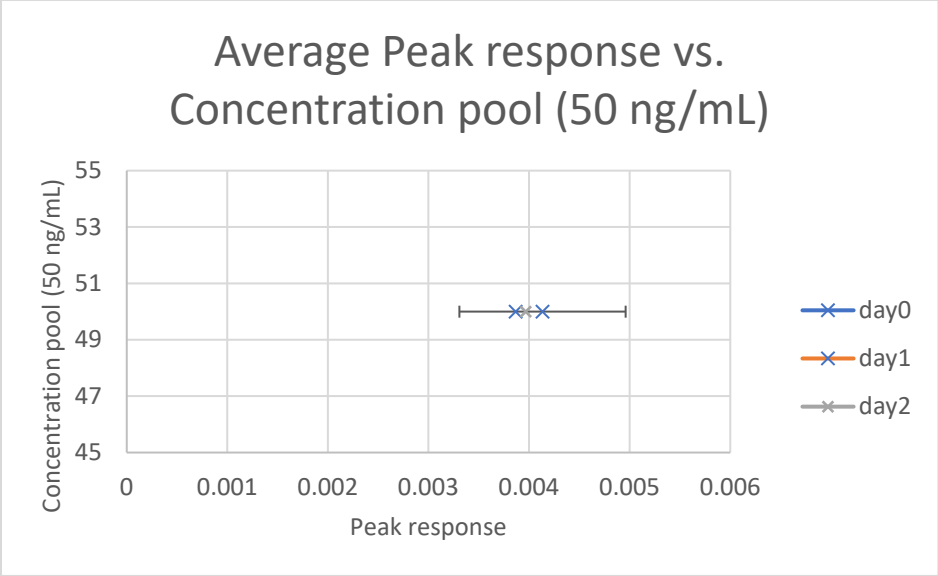


Table 8 - Summary of bias and precision for dilution integrity of quantitative analytes. Red/black numbers just show the grouping of three different calculations

	7-amino clonazepam	Alprazolam	Clonazepam	Etizolam	Lorazepam	Zolpidem	OH-Alprazolam	Diazepam	Nordiazepam	Oxazepam	Temazepam
Bias (undiluted)	-1.72	-7.92	-7.92	-17.42	1.30	-4.85	-3.08	-1.38	-1.05	2.92	-9.54
Bias (1:9)	-7.98	-11.00	-15.91	-24.22	-10.53	-9.61	-10.79	-5.18	-2.92	-6.06	-11.20
Bias (1:4)	-5.45	-7.59	-11.81	-21.37	-4.88	-5.11	-5.59	-1.02	0.21	-2.57	-9.10
Bias (1:1)	-1.72	-7.78	-10.26	-20.17	-2.96	-2.79	-3.50	-1.87	-0.98	-0.97	-10.35
Within run CV (1:9)	3.35	4.01	4.73	5.32	7.27	5.32	5.92	4.63	4.69	4.67	4.61
Within run CV (1:4)	2.75	4.48	4.89	4.81	6.48	5.58	4.14	3.71	4.65	5.18	4.35
Within run CV (1:1)	4.44	4.48	4.00	3.40	4.47	6.02	3.81	3.99	3.38	4.11	4.09
Between run CV (1:9)	9.64	10.89	21.35	12.32	18.80	10.19	17.44	16.05	17.37	16.59	19.44
Between run CV (1:4)	6.40	9.30	14.86	9.95	14.34	6.48	9.52	10.17	11.21	13.75	13.67
Between run CV (1:1)	6.02	9.25	11.16	10.59	10.94	7.94	8.29	8.35	9.84	8.30	8.76

Table 9- 7-amino clonazepam undiluted vs. diluted concentrations

PC 1 **(15 ug/L)**	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Rep 1	146.5	141.83	135.63	133.47	122.47
Rep 2	145.49	136.83	142.52	124.15	131.21
Rep 3	144.51	129.26	135.32	128.46	130.22
PC 2 **(30 ug/L)**	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Rep 1	145.225	131.305	147.895	139.05	133.875
Rep 2	141.115	134.175	138.025	130.975	131.245
Rep 3	144.1	140.555	142.165	137.695	133.08
PC 3 **(75 ug/L)**	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Rep 1	139.704	137.204	146.334	145.462	132.354
Rep 2	144.706	148.06	139.962	147.122	145.948
Rep 3	150.474	132.91	147.686	139.53	129.88
PC 4 **(150 ug/L)**	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Rep 1	151.102	144.614	137.681	164.339	139.329
Rep 2	0	0	0	0	0
Rep 3	0	0	0	0	0

Acceptable range: 120-180 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 10- Alprazolam undiluted vs. diluted concentrations

PC 1 (15 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	146.54	145.76	128.88	128.08	136.18	137.38
Rep 2	142.67	136.29	123.86	120.98	119.44	133.22
Rep 3	139.68	138	129.08	121.16	130.8	145.07
PC 2 (30 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	140.025	134.92	119.15	135.975	136.165	155.475
Rep 2	148.54	141.86	132.045	132.745	138.845	144.805
Rep 3	137.695	136.465	143.37	131.91	134.07	151.075
PC 3 (75 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	137.052	135.202	126	146.424	136.724	142.696
Rep 2	138.894	136.576	127.522	127.508	135.196	161.208
Rep 3	137.45	139.656	132.556	144.734	138.948	145.588
PC 4 (150 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	142.501	139.385	125.044	131.118	141.288	149.381
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 120-180 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 11 - Clonazepam undiluted vs. diluted concentrations

PC 1 (15 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	145.72	139.5	110.41	135.29	113.13	143.46
Rep 2	137.68	127.76	105.66	115.07	107.34	138.46
Rep 3	144.95	131.52	108.84	115.16	108.09	142.31
PC 2 (30 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	133.56	134.345	119.04	127.52	115.315	149.56
Rep 2	135.09	134.015	137.48	124.29	111.81	145.735
Rep 3	137.775	129.735	146.93	135.11	115.49	148.45
PC 3 (75 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	135.318	139.758	128.256	139.166	127.442	147.036
Rep 2	136.258	141.254	130.658	125.52	128.502	155.818
Rep 3	137.118	133.806	122.71	135.746	118.214	140.52
PC 4 (150 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	140.454	141.211	117.916	128.476	151.459	149.198
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 120-180 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 12- Diazepam undiluted vs. diluted concentrations

PC 1 (50 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	547.13	529.56	436.84	501.79	438.27	508.37
Rep 2	513.78	485.78	419.49	437.16	425.11	511.86
Rep 3	504.61	479.23	416.95	435.01	424.26	518.87
PC 2 (100 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	519.27	504.26	456.195	490.165	448.16	545.98
Rep 2	515.935	496.885	505.48	476.485	439.74	510.19
Rep 3	499.005	506.485	528.91	500.025	445.16	519.67
PC 3 (250 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	501.696	504.91	473.414	495.474	473.886	503.668
Rep 2	519.686	522.664	484.194	448.706	475.158	540.814
Rep 3	516.134	491.238	459.594	492.094	439.85	488.208
PC 4 (500 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	518.062	497.2	429.852	457.4	533.401	522.707
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 400-600 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 13 - Etizolam undiluted vs. diluted concentrations

PC 1 (15 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	125.9	114.64	103.66	126.5	110.89	118.67
Rep 2	112.2	117.07	108.89	110.39	107.7	123.45
Rep 3	122.64	116.07	96.14	116.87	97.6	116.76
PC 2 (30 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	121.64	115.195	104.7	115.585	111.55	134.53
Rep 2	123.235	114.86	113.935	120.37	106.37	123.65
Rep 3	114.59	120.64	124.615	124.18	108.97	124.33
PC 3 (75 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	125.044	120.15	108.932	120.992	111.394	123.098
Rep 2	122.042	126.342	108.46	118.476	119.586	133.796
Rep 3	127.912	123.23	108.472	125.362	109.76	122.334
PC 4 (150 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	128.518	125.901	105.867	121.698	125.747	135.451
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 120-180 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 14 - Lorazepam undiluted vs. diluted concentrations

PC 1 (15 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	159.62	152.86	128.96	145.95	115.4	142.59
Rep 2	140.67	130.04	117.8	127.18	117.91	136.69
Rep 3	152.38	144.04	120.19	115.85	113.63	154.01
PC 2 (30 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	153.615	136.13	128.175	141.32	114.825	154.485
Rep 2	153.545	150.215	152.66	141.365	125.735	144.7
Rep 3	147.38	142.065	166.925	144.785	123.99	146.26
PC 3 (75 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	154.986	148.704	139.1	151.6	131.178	147.3
Rep 2	153.82	151.104	147.54	135.54	137.842	157.156
Rep 3	159.764	146.494	132.064	154.494	125.902	145.566
PC 4 (150 ug/L)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	159.556	148.461	140.11	137.495	162.287	163.813
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 120-180 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 15 - Nordiazepam undiluted vs. diluted concentrations

PC 1 (50 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	555.6	519.54	435.86	514.98	452.53	538.38
Rep 2	516.14	480.27	426.24	455.84	442.45	529.19
Rep 3	536.55	504.37	423.7	426.25	429.19	550.03
PC 2 (100 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	518.22	496.435	425.215	480.465	470.175	557.445
Rep 2	527.535	501.98	500.1	464.23	461.22	546.415
Rep 3	511.225	505.55	521.06	511.56	468.92	550.955
PC 3 (250 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	508.064	498.426	443.822	503.092	477.868	521.804
Rep 2	512.958	514.9	464.318	456.456	505.378	547.888
Rep 3	517	492.942	440.29	509.71	480.6	516.22
PC 4 (500 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	518.927	506.514	419.373	456.251	535.013	532.426
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 400-600 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 16- Oxazepam undiluted vs. diluted concentrations

PC 1 (50 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	552.76	508.29	459.14	494.04	420.54	491.31
Rep 2	505.68	459.89	428.9	452.6	398.07	492.27
Rep 3	535.73	483.8	422.45	428.11	410.99	509.82
PC 2 (100 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	506.695	480.655	447.295	494.4	425.33	519.21
Rep 2	524.18	480.035	515.97	463.7	412.82	517.81
Rep 3	504.52	474.25	557.775	509.035	417.265	517.77
PC 3 (250 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	519.922	478.358	478.926	512.612	479.798	488.336
Rep 2	527.754	505.506	491.848	478.022	487.94	528.596
Rep 3	541.468	476.694	461.93	528.88	442.458	483.432
PC 4 (500 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	554.17	496.917	465.975	471.254	559.227	540.147
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 400-600 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 17 - Temazepam undiluted vs. diluted concentrations

PC 1 (50 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	506.17	491.87	421.35	468.82	392.38	499.86
Rep 2	496.93	444.89	399.87	426.2	372.49	491.97
Rep 3	493.38	443.01	393.7	399.48	362.89	486.3
PC 2 (100 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	486.965	455.435	420.635	443.275	412.52	513.61
Rep 2	490.84	464.465	454.83	425.675	389.155	479.775
Rep 3	477.295	451.44	500.195	444.345	387.825	482.555
PC 3 (250 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	476.12	446.058	447.94	467.76	430.554	456.378
Rep 2	461.394	473.022	432.816	426.844	426.55	479.752
Rep 3	486.688	433.736	420.066	463.238	389.738	449.688
PC 4 (500 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	460.527	460.808	416.292	418.514	474.568	483.111
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 400-600 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 18 - Zolpidem undiluted vs. diluted concentrations

PC 1 (15 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	145.9	158.11	130.67	137.79	136.98	137.33
Rep 2	147.03	133.91	129.33	132.07	123.24	130.18
Rep 3	145.43	136.33	126.2	121.91	131.51	136.72
PC 2 (30 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	139.615	139.815	133.04	138.08	140.21	142.53
Rep 2	140.875	140.545	148.315	139.555	135.755	138.205
Rep 3	143.36	150.14	166.505	144.43	147.045	134.06
PC 3 (75 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	140.566	153.556	157.296	153.946	132.612	144.174
Rep 2	147.62	160.6	145.32	135.43	129.074	150.516
Rep 3	147.73	145.206	142.072	153.508	151.264	134.144
PC 4 (150 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	145.928	143.206	142.194	135.293	143.196	146.54
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 120-180 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Table 19 - Alpha hydroxy alprazolam undiluted vs. diluted concentrations

PC 1 (15 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	149.58	154.12	132.35	132.55	124.71	147.57
Rep 2	145.7	137.81	122.79	113.13	120.24	138.6
Rep 3	159.88	131.38	117.2	121.08	118.48	141.5
PC 2 (30 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	148.965	150.06	135.55	133.07	131.645	155.775
Rep 2	146.88	144.315	142.035	131.59	132.88	137.935
Rep 3	143.945	142.745	154.75	134.175	131.14	151.745
PC 3 (75 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	143.26	144.372	134.992	150.208	141.254	155.554
Rep 2	148.712	148.432	142.644	131.08	143.368	163.81
Rep 3	146.866	143.826	135.868	142.984	138.894	149.296
PC 4 (150 ug/L)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	148.758	146.413	131.57	135.073	157.242	153.196
Rep 2	0	0	0	0	0	0
Rep 3	0	0	0	0	0	0

Acceptable range: 120-180 µg/L

PC 1= 1:9 dilution

PC 2 = 1:4 dilution

PC 3 = 1:1 dilution

PC 4 = undiluted

Graph 258 - Recoveries of extracted quantitative analytes from the dilution integrity experiments

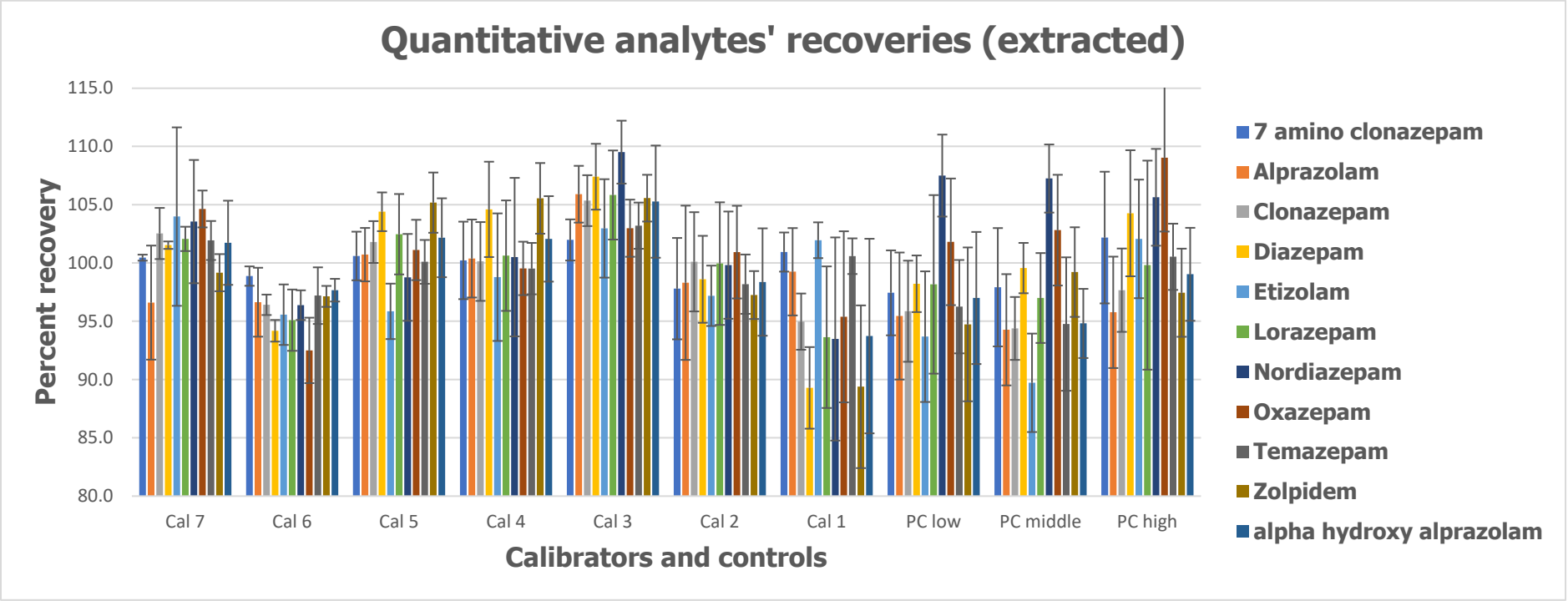


Table 20 - Average percent recoveries of extracted calibrators and positive controls.

Replicate total	Drug/metabolite	Cal 7	Cal 6	Cal 5	Cal 4	Cal 3	Cal 2	Cal 1	PC low	PC middle	PC high
5	7 amino clonazepam	100.5	98.9	100.6	100.2	102.0	97.8	100.9	97.4	97.9	102.2
6	Alprazolam	96.6	96.6	100.7	100.4	105.9	98.3	99.3	95.5	94.3	95.8
6	Clonazepam	102.5	96.4	101.8	100.1	105.4	100.1	95.0	95.9	94.4	97.7
6	Diazepam	101.5	94.2	104.4	104.6	107.4	98.6	89.3	98.2	99.6	104.3
6	Etizolam	104.0	95.6	95.9	98.8	103.0	97.2	102.0	93.7	89.7	102.1
6	Lorazepam	102.1	95.1	102.5	100.6	105.8	100.0	93.6	98.2	97.0	99.8
6	Nordiazepam	103.6	96.4	98.8	100.5	109.5	99.8	93.5	107.5	107.3	105.6
6	Oxazepam	104.6	92.5	101.1	99.5	103.0	100.9	95.4	101.8	102.8	109.0
6	Temazepam	101.9	97.2	100.1	99.5	103.2	98.2	100.6	96.3	94.8	100.5
6	Zolpidem	99.2	97.1	105.2	105.6	105.6	97.3	89.4	94.7	99.2	97.5
6	alpha hydroxy alprazolam	101.7	97.7	102.2	102.1	105.3	98.4	93.7	97.0	94.8	99.0

Graph 259 - Neat recoveries of quantitative analytes

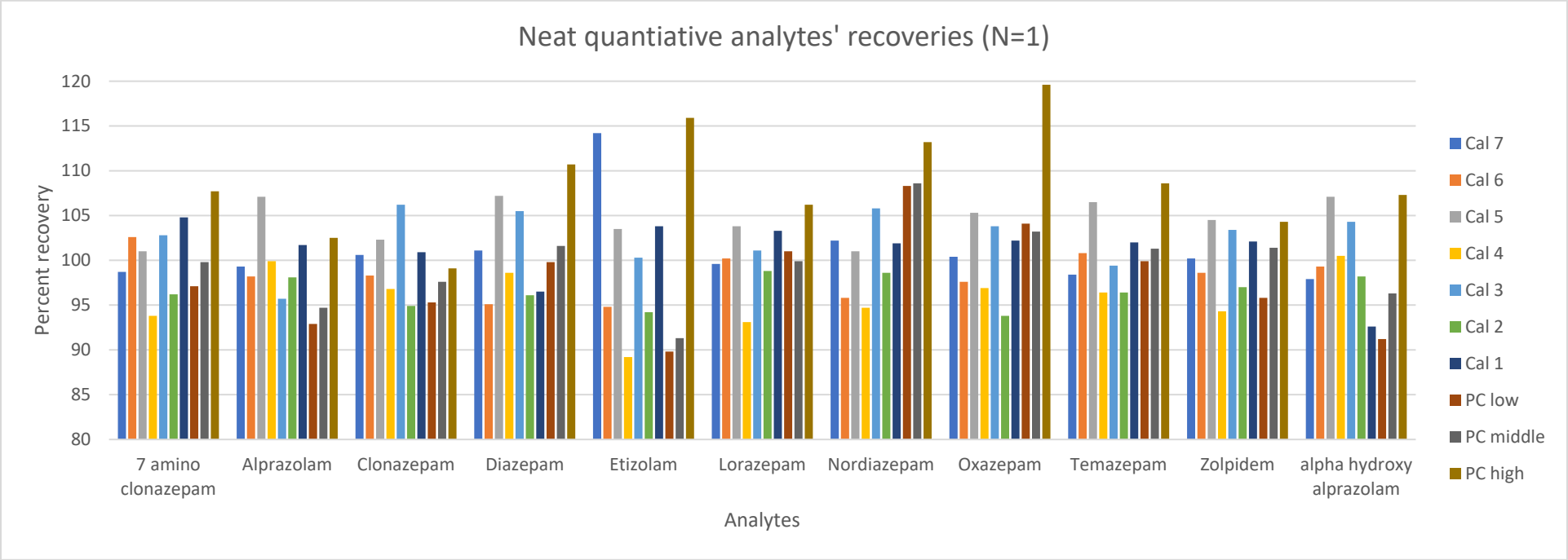


Table 21 - Neat percent recoveries of calibrators and positive controls

Drug/metabolite	Cal 7	Cal 6	Cal 5	Cal 4	Cal 3	Cal 2	Cal 1	PC low	PC medium	PC high
7 amino clonazepam	98.7	102.6	101	93.8	102.8	96.2	104.8	97.1	99.8	107.7
Alprazolam	99.3	98.2	107.1	99.9	95.7	98.1	101.7	92.9	94.7	102.5
Clonazepam	100.6	98.3	102.3	96.8	106.2	94.9	100.9	95.3	97.6	99.1
Diazepam	101.1	95.1	107.2	98.6	105.5	96.1	96.5	99.8	101.6	110.7
Etizolam	114.2	94.8	103.5	89.2	100.3	94.2	103.8	89.8	91.3	115.9
Lorazepam	99.6	100.2	103.8	93.1	101.1	98.8	103.3	101	99.9	106.2
Nordiazepam	102.2	95.8	101	94.7	105.8	98.6	101.9	108.3	108.6	113.2
Oxazepam	100.4	97.6	105.3	96.9	103.8	93.8	102.2	104.1	103.2	119.6
Temazepam	98.4	100.8	106.5	96.4	99.4	96.4	102	99.9	101.3	108.6
Zolpidem	100.2	98.6	104.5	94.3	103.4	97	102.1	95.8	101.4	104.3
alpha hydroxy alprazolam	97.9	99.3	107.1	100.5	104.3	98.2	92.6	91.2	96.3	107.3

Graph 260 – Post extraction addition analyte recoveries

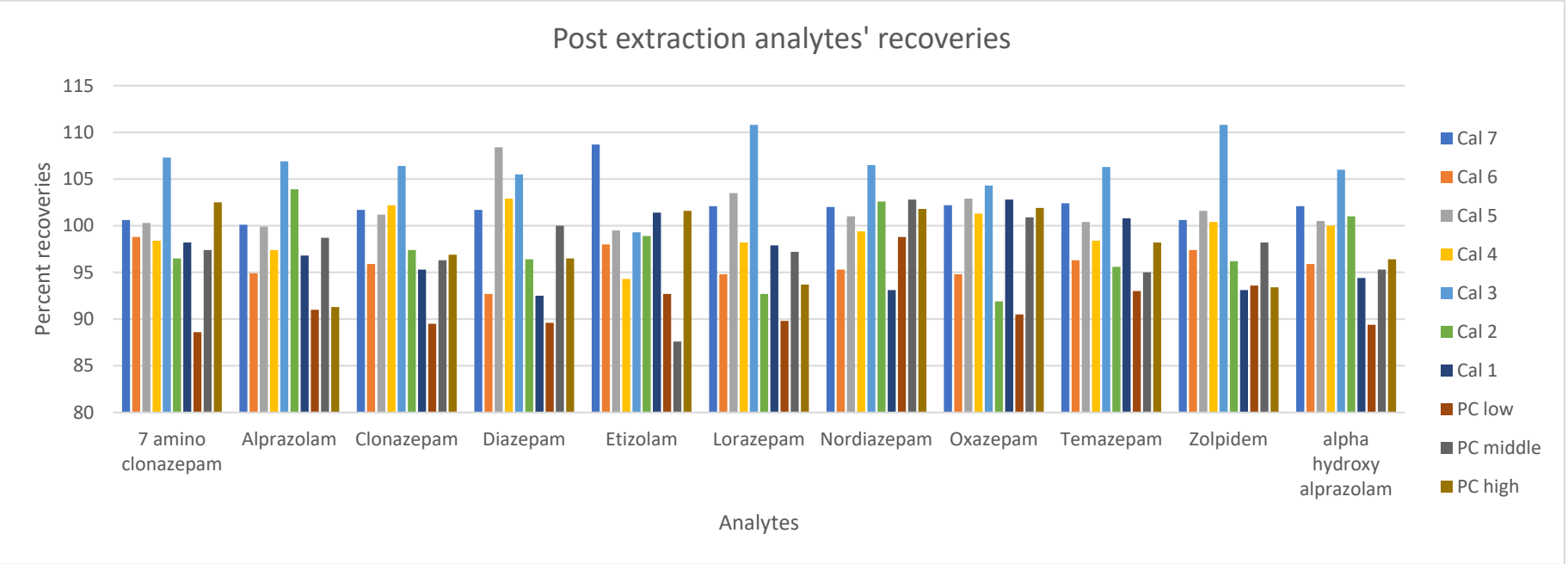


Table 22 - Post extraction percent recoveries of analytes

Drug/metabolite	Cal 7	Cal 6	Cal 5	Cal 4	Cal 3	Cal 2	Cal 1	PC low	PC medium	PC high
7 amino clonazepam	100.6	98.8	100.3	98.4	107.3	96.5	98.2	88.6	97.4	102.5
Alprazolam	100.1	94.9	99.9	97.4	106.9	103.9	96.8	91	98.7	91.3
Clonazepam	101.7	95.9	101.2	102.2	106.4	97.4	95.3	89.5	96.3	96.9
Diazepam	101.7	92.7	108.4	102.9	105.5	96.4	92.5	89.6	100	96.5
Etizolam	108.7	98	99.5	94.3	99.3	98.9	101.4	92.7	87.6	101.6
Lorazepam	102.1	94.8	103.5	98.2	110.8	92.7	97.9	89.8	97.2	93.7
Nordiazepam	102	95.3	101	99.4	106.5	102.6	93.1	98.8	102.8	101.8
Oxazepam	102.2	94.8	102.9	101.3	104.3	91.9	102.8	90.5	100.9	101.9
Temazepam	102.4	96.3	100.4	98.4	106.3	95.6	100.8	93	95	98.2
Zolpidem	100.6	97.4	101.6	100.4	110.8	96.2	93.1	93.6	98.2	93.4
alpha hydroxy alprazolam	102.1	95.9	100.5	100	106	101	94.4	89.4	95.3	96.4

Table 23 - Analyst competency assignments with expected results from CAP

Competency assignments				Year	Sample	Drugs	Target concentration (ng/mL)	All method mean	S.D.
LJM				2022	5	no benzos			
	EDW			2022	3	Zolpidem	180	186.69	35.22
		ANW		2021	2	7-amino clonazepam	200	174.84	24.86
						Clonazepam	25	25.67	3.13
		ALR		2020	1	Lorazepam	250	241.68	27.85
	EDW		GC/MS	2019	2	7-amino clonazepam	100	110.09	15.32
						Clonazepam	20	20.18	2.31
LJM	EDW		GC/MS		4	7-amino flunitrazepam	50		
		ANW/ ALR		2018	4	alprazolam	60		
						a-OH alprazolam	100		

Table 24 - Summary of CAP acceptable results. The acceptable range was ±30% of the target concentration or two times the standard deviation, whichever was greater. Per CAP participate summary from their respective years *

Sample		Matrix	30% of mean	All method Mean minus 2 SD	All method mean plus 2 SD	All method mean minus 30%	All method mean plus 30%	Limit of acceptability calculated by CAP	
5	No benzos	Blood							
3	Zolpidem	Blood	56.007	116.25	257.13	130.68	242.70	116.2	257.2
2	7-amino clonazepam	Blood	52.452	125.12	224.56	122.39	227.29		
	Clonazepam	Blood	7.701	19.41	31.93	17.97	33.37	17.9	33.4
1	Lorazepam	Blood	72.504	185.98	297.38	169.18	314.18	169.1	314.2
2	7-amino clonazepam	Blood	33.027	79.45	140.73	77.06	143.12		
	Clonazepam	Blood	6.054	15.56	24.8	14.13	26.23	14.1	26.3
4	7-amino flunitrazepam	Urine							
4	alprazolam	Urine							
	a-OH alprazolam	Urine							

*Those highlighted in green are acceptable results unless specified in the last two columns. Notice that CAP limit of acceptability is truncated to one decimal point.

Table 25 - Summary of analyst competency assignments, and their results.

Competency assignments				Year	Sample	Sample Type	Drugs	Results (µg/L)	
								GC/MS	LC/MS/MS
LJM				2022	5	Blood	no benzodiazepines		None detected
	EDW			2022	3	Blood	Zolpidem		180
		ANW		2021	2	Blood	7-amino clonazepam		130
							Clonazepam		26
		ALR		2020	1	Blood	Lorazepam		220
	EDW		GC/MS	2019	2	Blood	7-amino clonazepam	Confirmed	83
							Clonazepam	23	18
LJM	EDW		GC/MS		4	Urine	7-amino flunitrazepam	Confirmed	Confirmed
		ANW/ALR		2018	4	Urine	Alprazolam		Confirmed
							a-OH alprazolam		Confirmed