Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

Determination of prescribed and designer benzodiazepines and metabolites in influent wastewater

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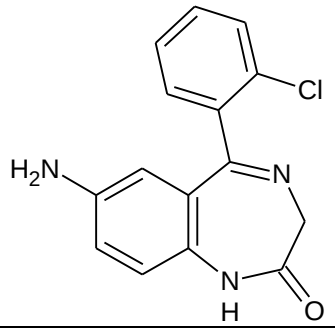
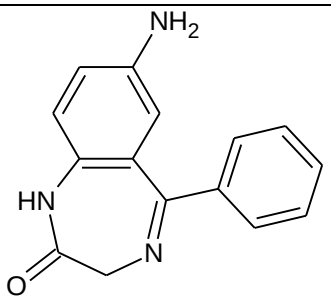
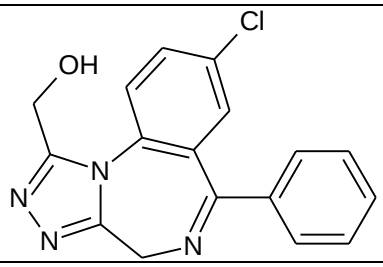
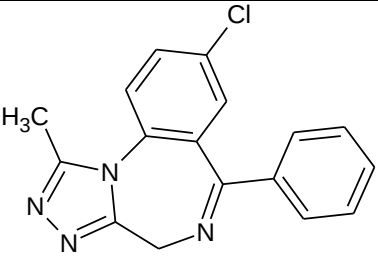
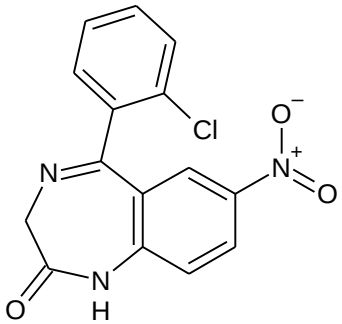
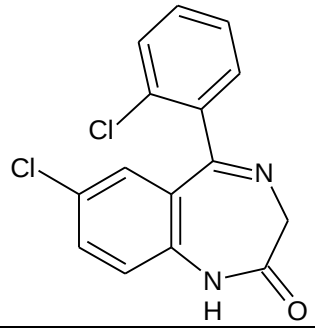
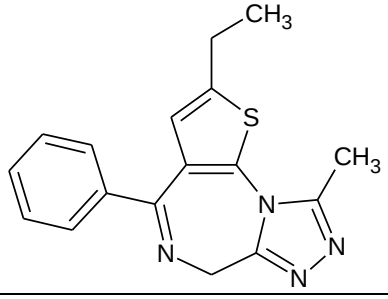
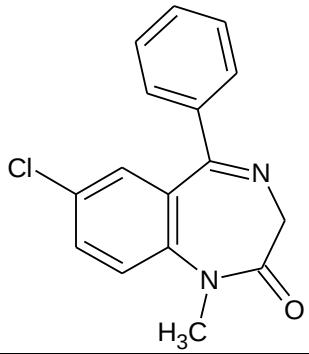
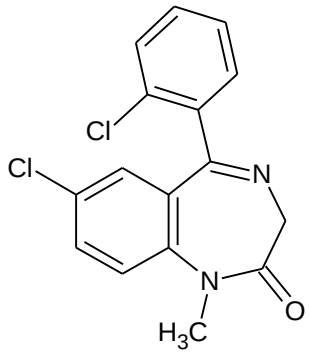
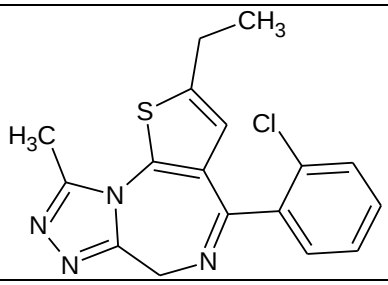
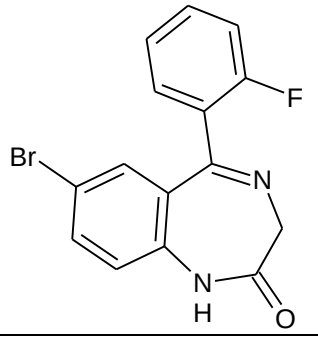
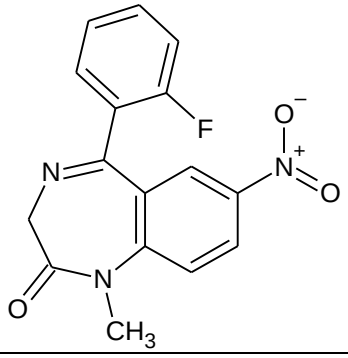
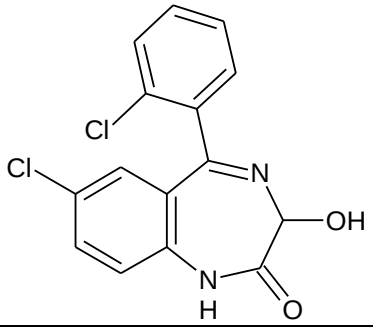
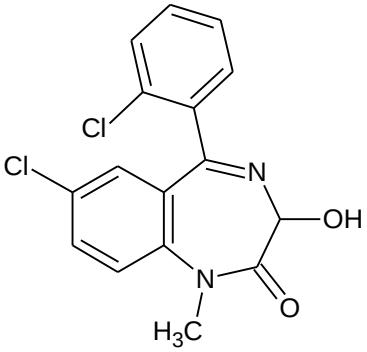
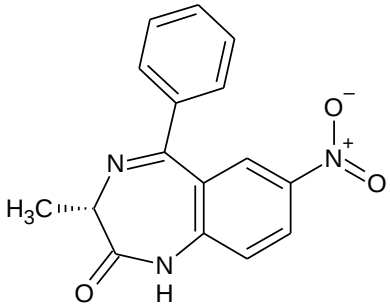
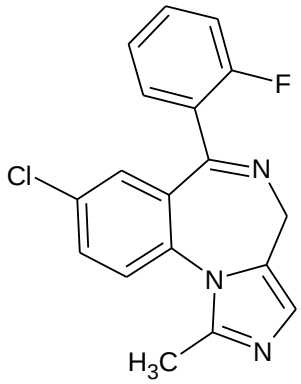
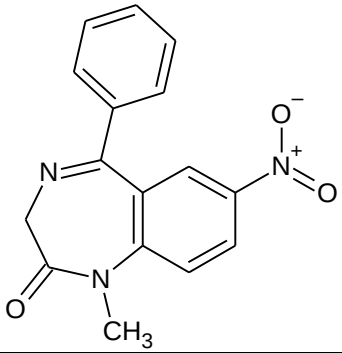
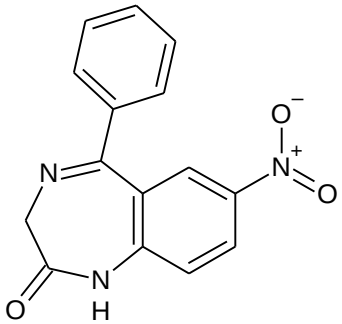
Table S1: Structures of all compounds in the method and compound class (designer benzodiazepine, prescribed benzodiazepine or metabolite)

Compound	Structure	Compound class
7-Amino Clonazepam		Metabolite of clonazepam
7-Amino Nimetazepam		Metabolite of nimetazepam
Alpha-hydroxy Alprazolam		Metabolite of alprazolam
Alprazolam		Prescribed benzodiazepine
Clonazepam		Prescribed benzodiazepine

Delorazepam		Metabolite of diclazepam
Deschloroetizolam		Metabolite of etizolam
Diazepam		Prescribed benzodiazepine
Diclazepam		Designer benzodiazepine
Etizolam		Designer benzodiazepine

Flubromazepam		Designer benzodiazepine
Flunitrazepam		Prescribed benzodiazepine
Lorazepam		Prescribed benzodiazepine
Lormetazepam		Illicit benzodiazepine

Meclonazepam		Designer benzodiazepine
Midazolam		Prescribed benzodiazepine
Nimetazepam		Illicit benzodiazepine
Nitrazepam		Prescribed benzodiazepine

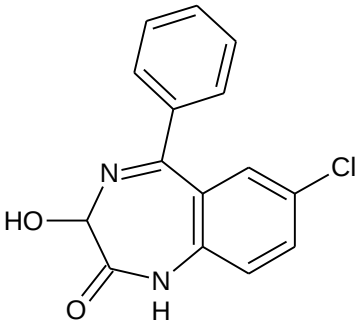
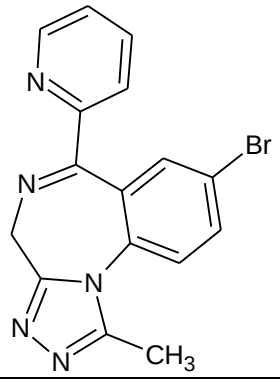
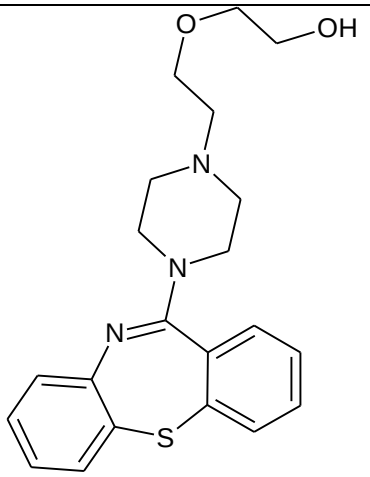
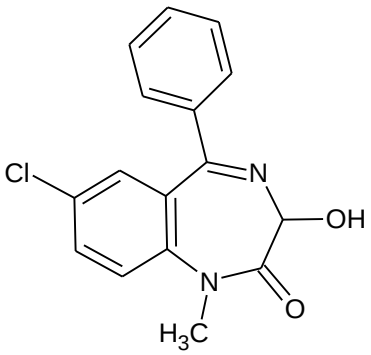
Oxazepam		Prescribed benzodiazepine
Pyrazolam		Designer benzodiazepine
Quetiapine		Non-benzodiazepine antipsychotic
Temazepam		Prescribed benzodiazepine

Table S2: Flow rates (ML) and population for all four wastewater treatment plants.

Date	WWTP A	WWTP B	WWTP C	WWTP D
	Population: 728,759	Population: 75,225	Population: 212,309	Population: 155,604
27-Nov	171.7	23.1	31.7	51.1
28-Nov	171.8	22.9	31.2	50.4
29-Nov	168.8	23.1	31.8	50.2
30-Nov	159.2	23.5	33.7	51.5
1-Dec	152.9	23.3	34.3	52.3
2-Dec	168.6	23.1	33.6	51.5
3-Dec	167.1	23.1	33.2	50.8
25-Dec	139.4	22.0	38.2	46.5
26-Dec	138.7	22.0	30.9	47.3
27-Dec	151.7	27.8	30.7	47.9
28-Dec	149.5	21.8	30.6	47.7
29-Dec	138.9	21.7	30.6	45.9
30-Dec	153.8	22.0	30.8	47.9
31-Dec	158.9	22.3	30.6	49.5
1-Jan	158.9	21.4	29.7	44.8
2-Jan	158.9	21.9	30.5	47.4

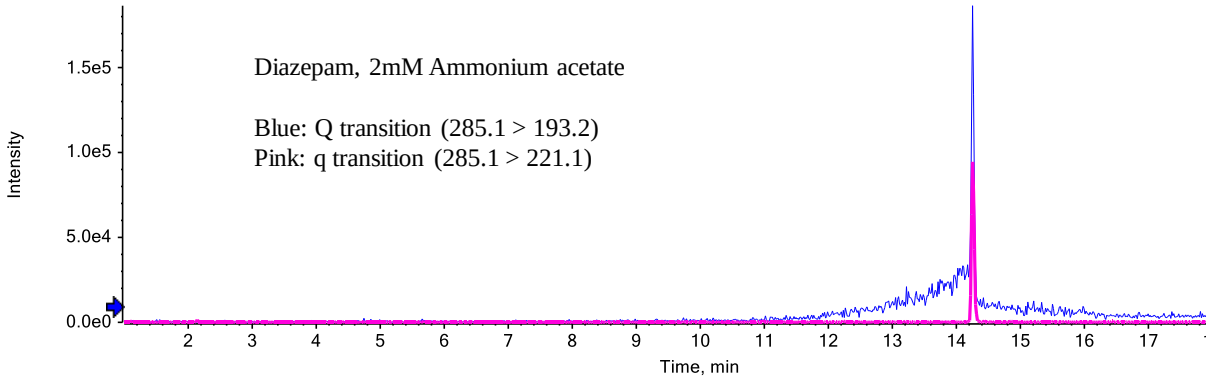


Figure S1: Poor peak shape of diazepam with ammonium acetate as a buffer. The pink peak is the confirmatory transition (285.1 > 221.1) and the blue peak is the quantification transition (285.1 > 193.2).

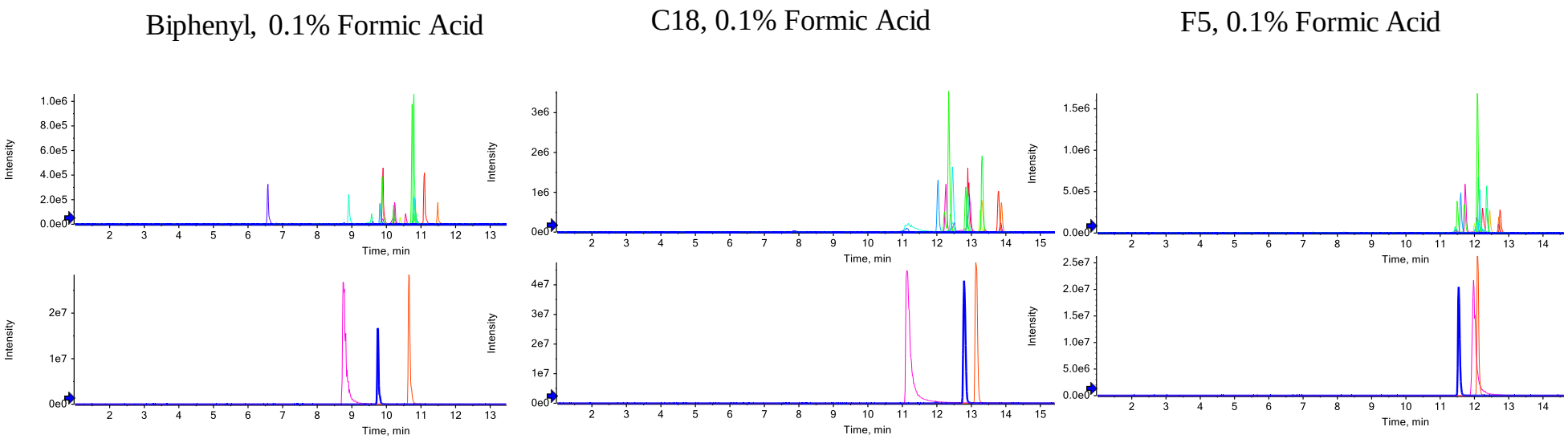
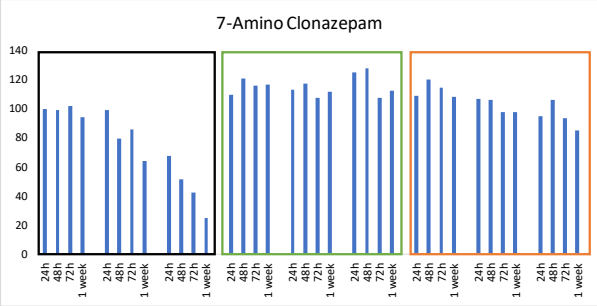
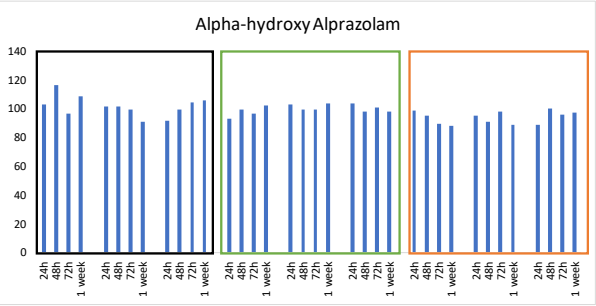
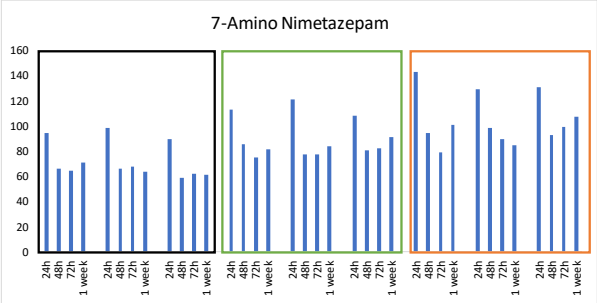
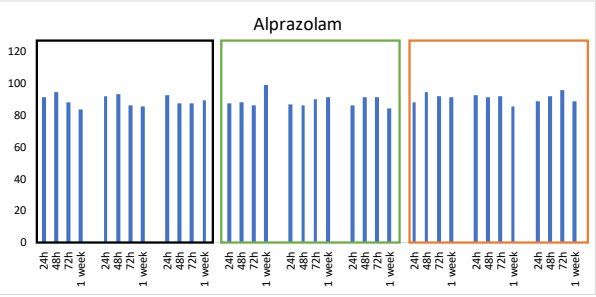


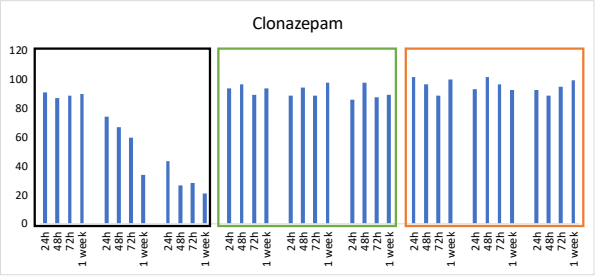
Figure S2: Chromatographic separation of all analytes with a biphenyl (LEFT), C18 (MIDDLE) and F5 (RIGHT) column. The separation of quetiapine (pink), oxazepam (blue) and temazepam (red) are shown separately at the bottom due to their higher concentrations compared to the other analytes in the method.

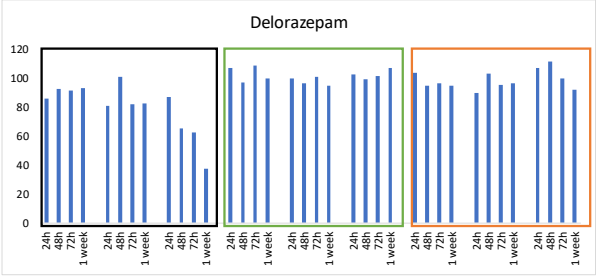

7-Amino Clonazepam

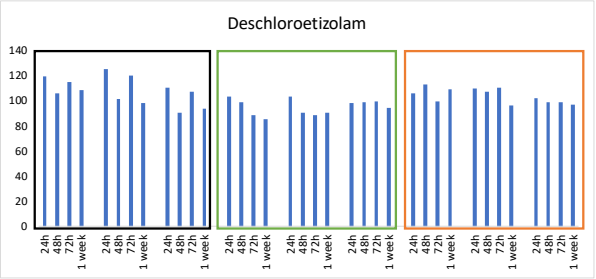

Alpha-hydroxy Alprazolam

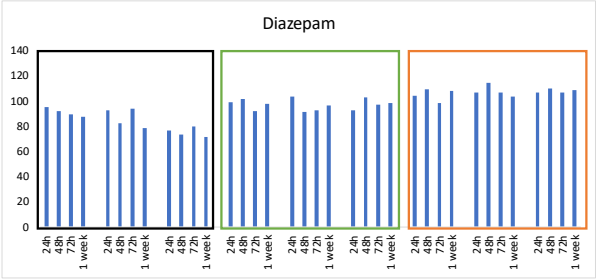

7-Amino Nimetazepam

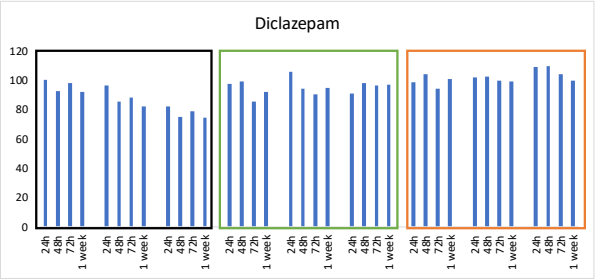

Alprazolam

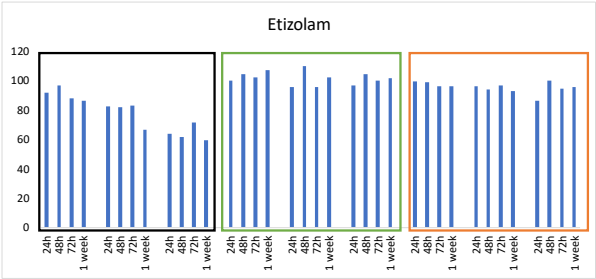

Clonazepam

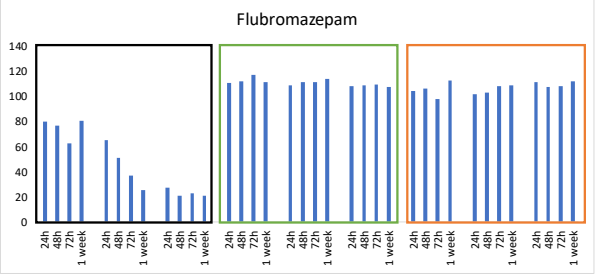

Delorazepam

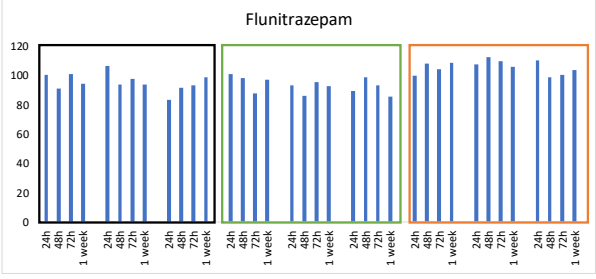

Deschloroetizolam


Diazepam


Diclazepam


Etizolam


Flubromazepam


Flunitrazepam

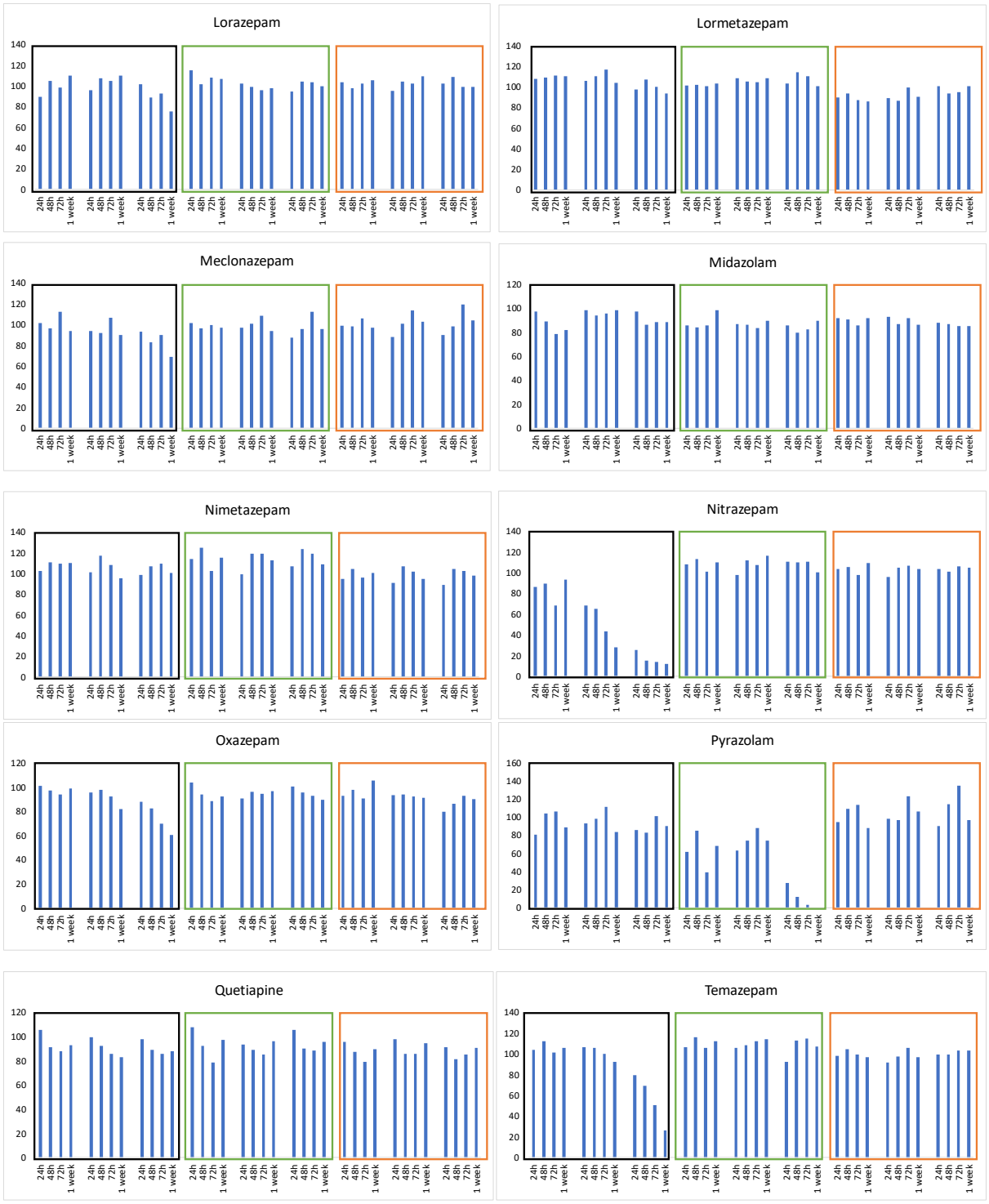
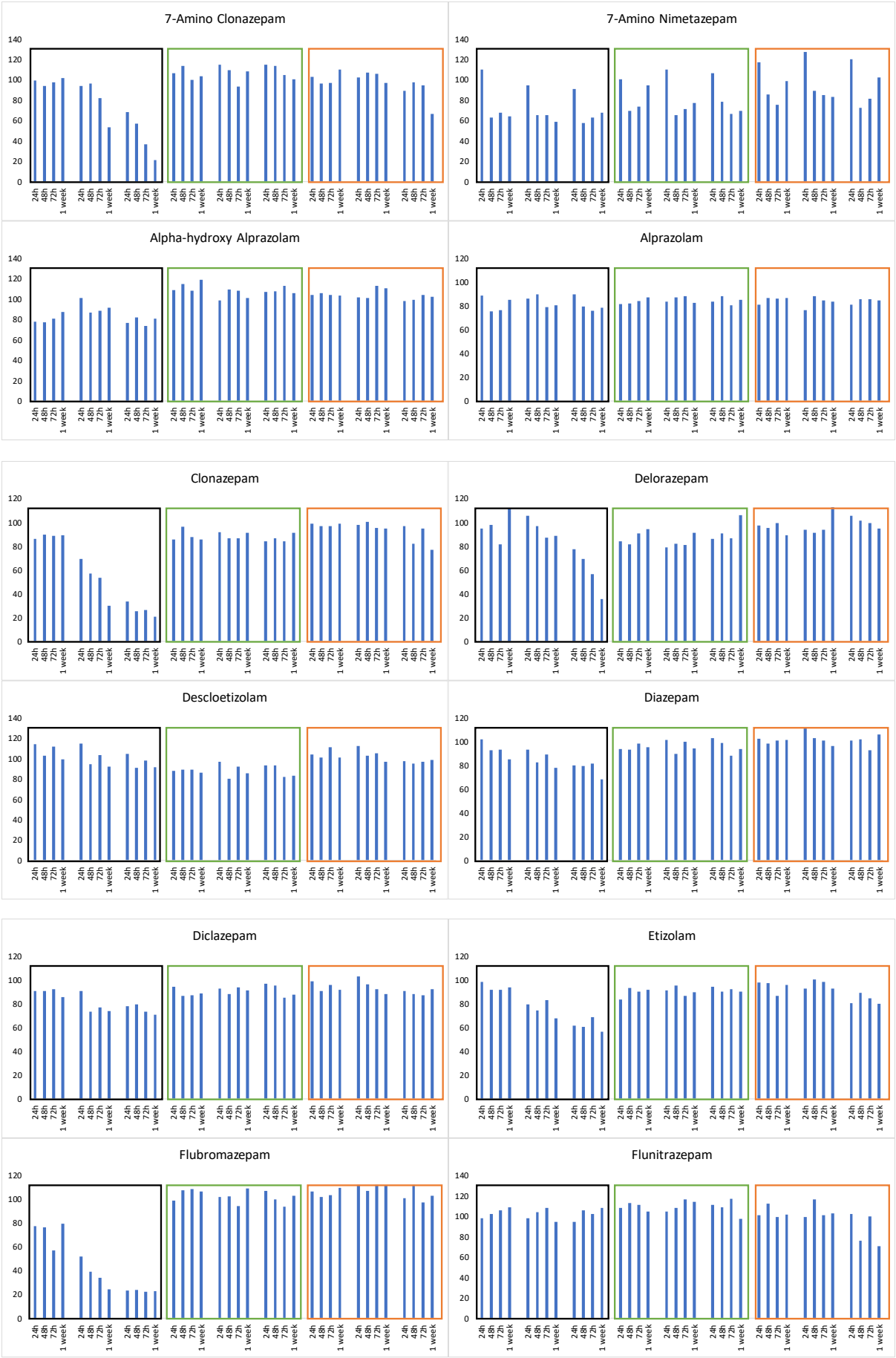


Figure S3: Stability of all compounds at LQC level in filtered wastewater for up to 1 week. Black square indicates acid preservation, green square indicates raw wastewater (no preservation) and the orange square indicates sodium metabisulfite preservation. Within each square, the lines are in blocks of four, reflecting the temperature at which they were stored: -20 °C (LEFT), 4 °C (MIDDLE) and room temperature (RIGHT). All time periods are normalised to the initial t=0 point.


7-Amino Clonazepam
7-Amino Nimetazepam
Alpha-hydroxy Alprazolam
Alprazolam
Clonazepam
Delorazepam
Descloetizolam
Diazepam
Diclazepam
Etizolam
Flubromazepam
Flunitrazepam

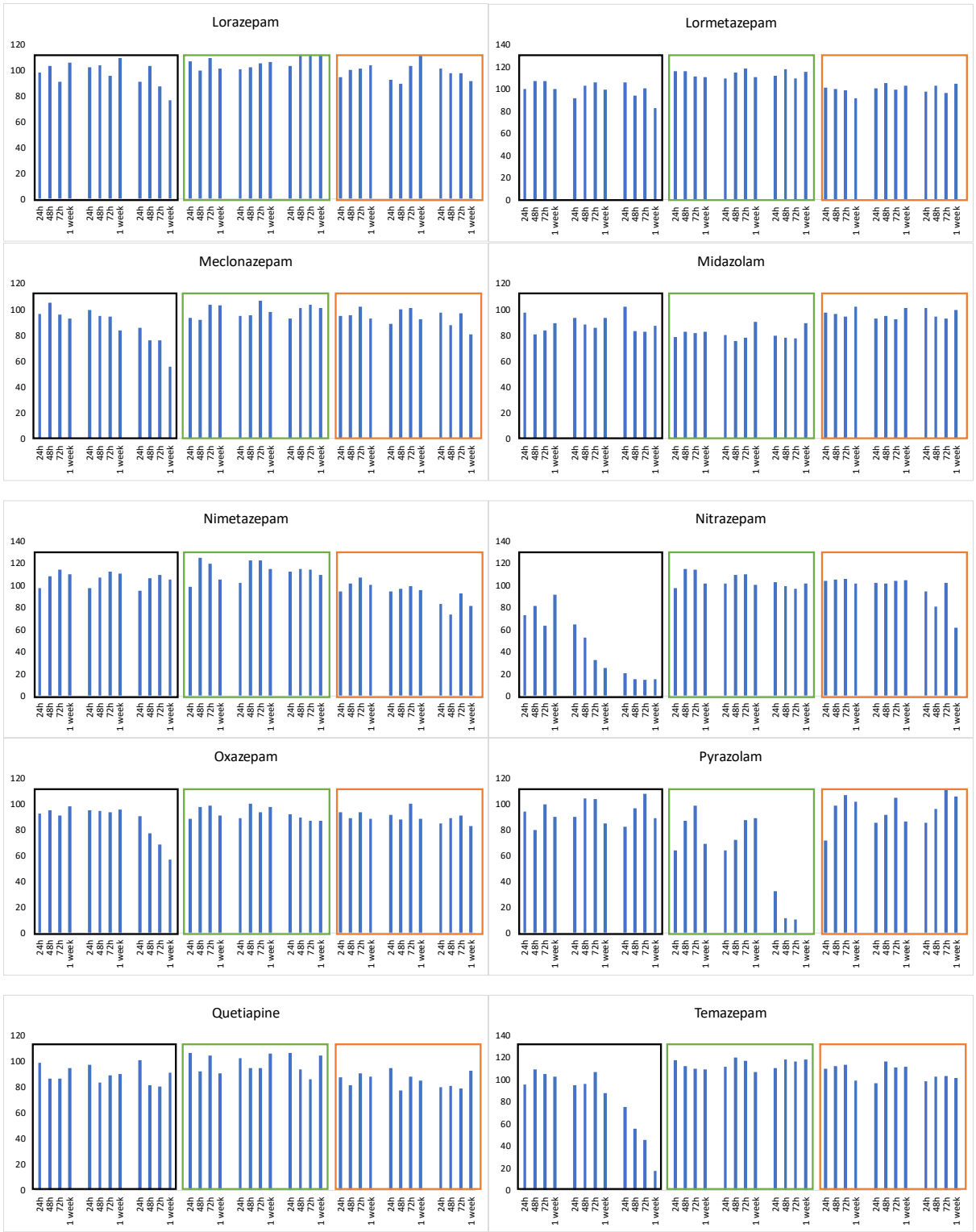


Figure S4: Stability of all compounds at HQC level in filtered wastewater for up to 1 week. Black square indicates acid preservation, green square indicates raw wastewater (no preservation) and the orange square indicates sodium metabisulfite preservation. Within each square, the lines are in blocks of four, reflecting the temperature at which they were stored: -20 °C (LEFT), 4 °C (MIDDLE) and room temperature (RIGHT). All time periods are normalised to the initial t=0 point.

Table S3: Excreted mass loads (mg/day/1000 people) for compounds found in influent wastewater samples from four wastewater treatment plants in South Australia over the 2019-20 Christmas period

Compound	WWTP	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu
		25-Dec	26-Dec	27-Dec	28-Dec	29-Dec	30-Dec	31-Dec	1-Jan	2-Jan
Alpha-hydroxy alprazolam	A	0.38	0.54	0.48	0.45	0.39	0.54	0.45	0.43	0.54
	B	0.58	0.7	0.7	0.59	0.77	0.62	0.73	0.44	0.55
	C	0.33	0.46	0.47	0.35	0.4	0.49	0.28	0.35	0.44
	D	D	D	D	D	D	0.41	D	D	D
Alprazolam	A	0.28	0.27	0.32	0.2	0.24	0.27	0.23	0.18	0.3
	B	0.27	0.42	0.49	0.39	0.31	0.37	0.27	0.44	0.4
	C	0.28	0.18	0.23	0.31	0.25	0.21	0.22	0.17	0.24
	D	0.12	0.08	0.14	0.09	0.12	0.23	0.16	0.14	0.14
Diazepam	A	1.08	1.03	1.02	0.87	1.34	0.87	0.72	0.81	1.42
	B	2.33	1.3	1.67	1.29	0.93	1.42	1.00	0.93	0.95
	C	1.44	1.04	0.95	1.23	1.12	1.21	1.07	0.9	0.85
	D	1.01	1.32	1.28	1.32	1.38	1.08	1.06	1.04	1.00
Etizolam	A	0.03	0.01	0.02	0.02	0.01	0.03	0.02	0.01	0.03
	B	0.19	0.06	0.07	0.04	0.02	0.03	0.04	0.03	0.02
	C	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
	D	0.01	0.03	0.01	0.01	0.01	0.02	0.01	0.02	0.01
Lorazepam	A	2.12	1.88	2.07	2.08	1.87	2.38	1.96	2.04	2.35
	B	2.27	2.16	2.87	2.84	2.93	2.22	2.30	2.00	2.43
	C	2.27	1.9	2.01	2.06	1.83	1.79	1.74	1.98	2.06
	D	2.79	2.34	2.46	2.84	2.79	1.8	2.65	3.2	3.4
Lormetazepam	A	0.85	0.62	0.67	0.72	0.72	1.18	1.09	1.03	0.95
	B	1.18	1.08	1.33	0.76	1.21	1.31	1.13	1.06	1.05
	C	1.17	1.01	0.81	0.91	0.88	1.08	0.76	0.98	0.87
	D	0.94	0.95	0.87	0.91	0.89	0.88	0.87	0.9	0.88
Midazolam	A	D	D	D	D	D	D	D	D	D
	B						D		D	D
	C		D	D						
	D	D	D	D	D	D	0.31	D	D	D
Oxazepam	A	123.37	126.32	117.2	126.2	93.26	125.93	101.36	116.63	134.59
	B	136.55	186.35	197.65	162.45	154.23	173.21	163.73	171.55	173.96
	C	177.5	168.78	146.59	154.07	166.54	165.41	148.98	161.82	176.4
	D	118.42	119.76	154.58	128.91	114.47	159.01	123.32	165.95	151.69
Quetiapine	A	2.49	4.7	12.19	8.08	21.41	8.62	8.66	7.45	12.97
	B	33.1	37.55	39.71	40.54	21.58	28.33	38.42	27.78	28.99
	C	26.26	21.75	22.73	21.51	27.71	18.73	8.63	16.85	23.25
	D	4.43	5.24	9.7	10.57	5.18	29.48	9.13	4.99	4.97
Temazepam	A	55.39	45.33	51	49.03	34.91	79.2	67.58	62.59	83.36
	B	72.83	74.15	96.29	122.13	97.03	89.45	112.69	106.74	113.47
	C	73.63	72.5	64.35	71.26	72.19	82.67	69.86	96.51	96.97
	D	57.27	75.22	75.49	77.88	86.54	70.06	86.74	101.27	99.16

D: Detected only (below LOQ)

Table S4: Excreted mass loads (mg/day/1000 people) for compounds found in influent wastewater samples from four wastewater treatment plants in South Australia in November - December 2019

Compound	WWTP	Wed	Thu	Fri	Sat	Sun	Mon	Tue
		27-Nov	28-Nov	29-Nov	30-Nov	1-Dec	2-Dec	3-Dec
Alpha-hydroxy alprazolam	A	0.36	0.27	0.28	0.31	0.31	0.29	0.26
	B	0.47	0.36	0.45	0.40	0.33	0.41	0.37
	C	0.27	0.31	0.26	0.30	0.31	0.36	0.25
	D	0.20	0.22	0.24	0.35	0.28	0.29	0.22
Alprazolam	A	0.32	0.20	0.20	0.25	0.18	0.24	0.29
	B	0.40	0.34	0.23	0.32	0.34	0.30	0.26
	C	0.21	0.22	0.19	0.20	0.14	0.22	0.19
	D	0.19	0.20	0.20	0.20	0.17	0.17	0.16
Diazepam	A	1.79	0.95	1.06	1.12	1.21	1.87	1.10
	B	1.63	1.13	1.16	0.86	1.05	0.79	0.98
	C	1.28	1.20	3.18	1.32	1.08	0.91	5.09
	D	1.23	1.07	0.88	0.94	0.96	0.94	0.97
Etizolam	A	0.07	0.09	0.08	0.13	0.07	0.11	0.05
	B	0.07	0.08	0.11	0.13	0.13	0.10	0.15
	C	0.07	0.04	0.07	0.04	0.09	0.06	0.05
	D	0.14	0.13	0.08	0.07	0.09	0.09	0.08
Lorazepam	A	3.01	2.65	2.42	2.09	2.16	2.67	2.05
	B	2.16	2.03	1.87	2.61	2.20	2.45	2.32
	C	2.02	1.80	1.80	1.92	2.02	1.53	1.75
	D	3.01	2.62	3.00	3.59	3.14	3.27	3.53
Lormetazepam	A	0.70	0.68	0.69	0.69	0.60	0.66	0.64
	B	0.82	0.86	1.11	0.91	0.93	0.80	0.89
	C	0.56	0.69	0.56	0.61	0.66	0.56	0.64
	D	0.79	0.71	0.68	0.80	0.65	0.71	0.72
Midazolam	A	D	D	D	D	D	D	D
	B							
	C						D	
	D	D	D	D	D	D	D	
Oxazepam	A	122.35	133.87	137.71	120.93	132.95	130.22	127.09
	B	169.26	157.81	167.34	146.46	165.29	167.10	165.86
	C	166.21	150.52	156.27	181.63	170.02	160.07	151.81
	D	140.48	148.94	153.92	145.62	141.20	140.15	147.39
Quetiapine	A	40.38	33.81	36.40	33.43	36.64	39.58	31.83
	B	35.67	35.76	34.24	83.87	78.98	47.28	35.18
	C	81.68	46.27	44.13	46.12	43.67	43.39	44.44
	D	32.30	31.30	32.46	33.85	33.56	29.23	26.41
Temazepam	A	74.00	74.63	70.24	74.00	66.28	64.41	62.87
	B	86.24	75.36	85.63	82.21	77.47	81.38	83.36
	C	66.01	64.33	68.81	73.60	71.78	65.47	65.13
	D	74.87	82.71	83.90	87.09	78.97	79.17	76.12

D: Detected only (below LOQ)