Occurrence and Mass Loading of Synthetic Opioids, Synthetic Cathinones, and Synthetic Cannabinoids in Wastewater Treatment Plants in Four U.S. Communities

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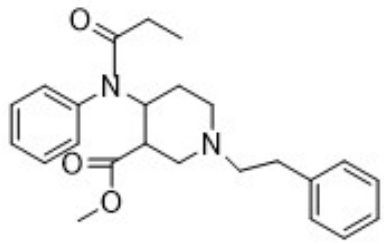
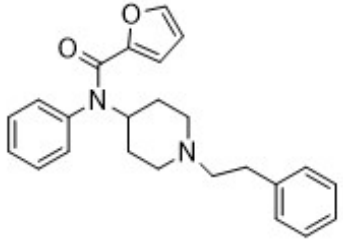
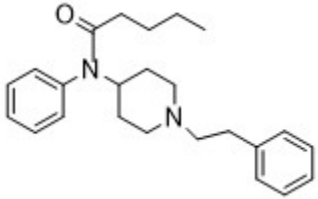
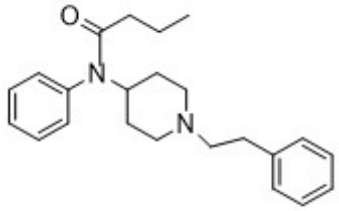
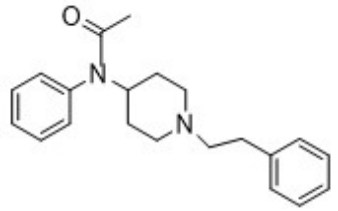
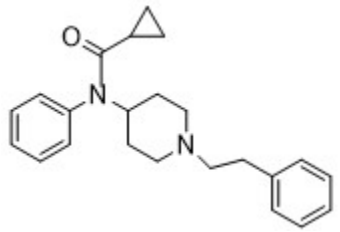
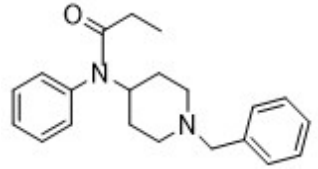
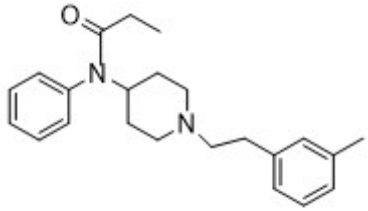
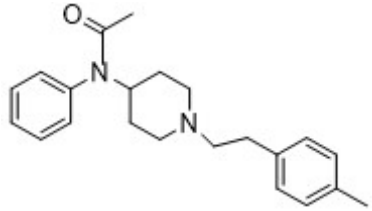
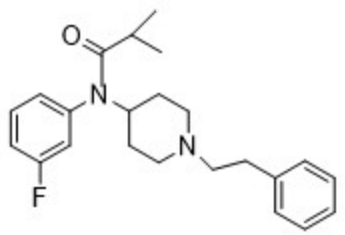
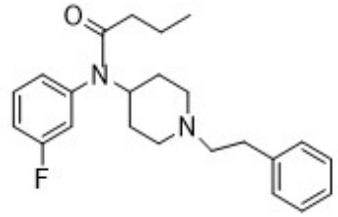
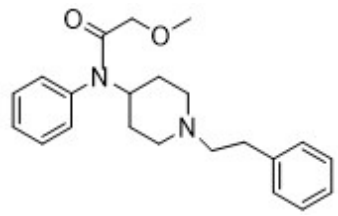
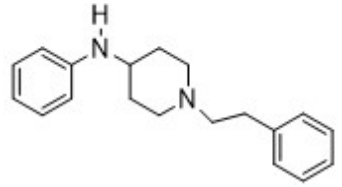
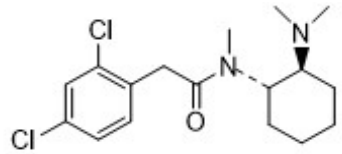
Table S1. HPLC mobile phase compositions and programming for sample analysis (Flow rate = 0.300 mL/min)

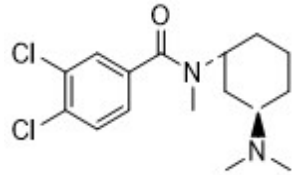
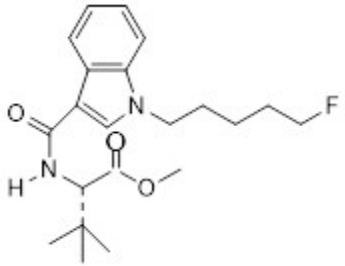
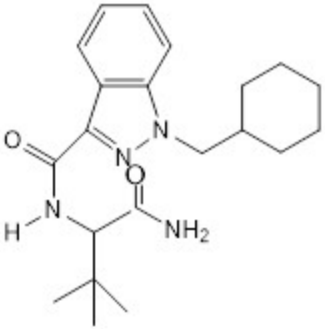
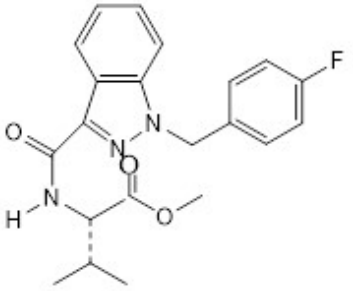
Time	0.1% Formic acid (aq.) (%)	Methanol (%)
0.00	95.0	5.0
2.00	95.0	5.0
3.00	90.0	10.0
5.50	70.0	30.0
6.00	60.0	40.0
7.50	50.0	50.0
9.00	50.0	50.0
9.30	48.0	52.0
10.00	48.0	52.0
10.30	45.0	55.0
11.50	45.0	55.0
12.00	40.0	60.0
12.50	40.0	60.0
13.00	35.0	65.0
16.00	20.0	80.0
18.00	20.0	80.0
23.00	10.0	90.0
25.00	5.0	95.0
26.00	95.0	5.0

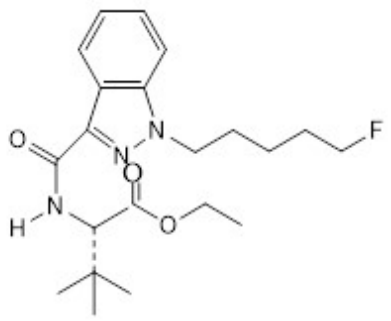
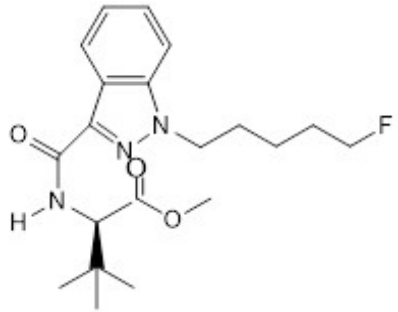
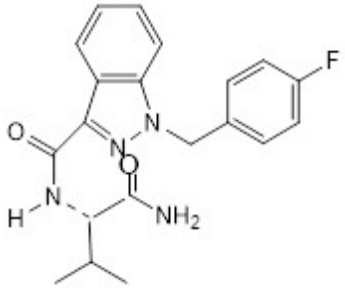
Table S2. Optimized MS/MS transitions and collision energies for all target drugs and their corresponding internal standards.

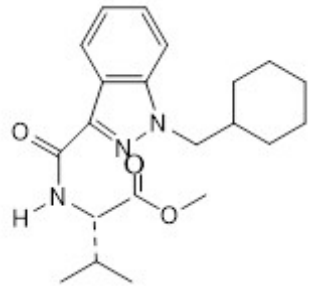
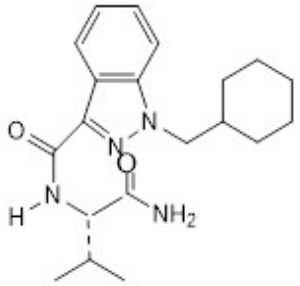
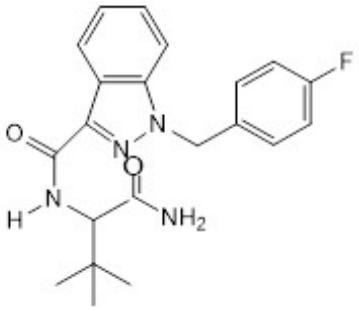
Analytes	MS/MS Transitions	Collision Energy	Internal Standards	MS/MS Transitions	Collision Energy	Chemical Structures
Synthetic Fentanyls						
Carfentanil	395.1>335,105	15,50	CAF-D_5	400.2>340.2,246	16	
Furanyl Fentanyl	375.2>188.1,105	26	FUF-D_5	380.2>188,105	20	
Valeryl Fentanyl	365.2>188.1,105	24	VAF-D_5	370.2>188.1,105	25	
Butyryl Fentanyl	351.2>188.1,105	25	BUF-D_5	356.2>188.1,105	22	

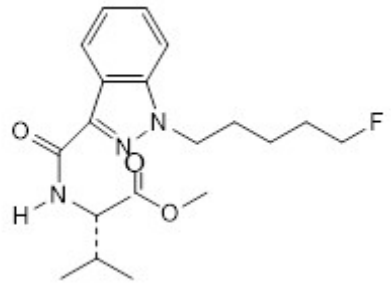
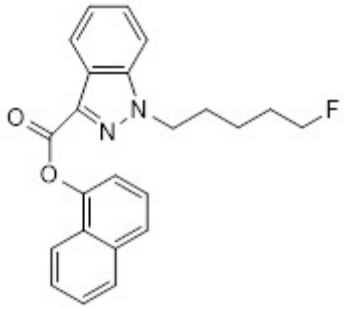
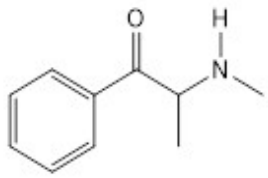
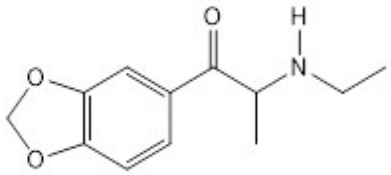
Acetyl Fentanyl	323.2>188,105	22	ACF-13C$_6$	329.2>188.1,105	20	
Cyclopropyl Fentanyl	349.2>188.1,105	26	CPF-D$_5$	354.2>188.1,105	25	
Benzyl Fentanyl	323.2>174.1,91	22				
3'-Methyl Fentanyl	351.2>202.1,119	24				
4'-Methyl Acetyl Fentanyl	337.2>202.1,119	22	MAF-D$_5$	342.2>202.1,119	24	

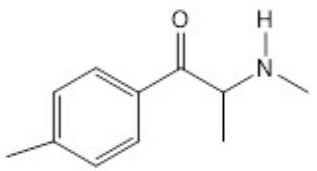
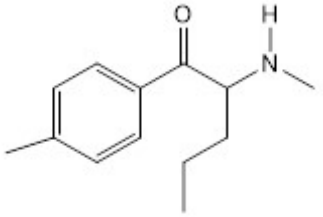
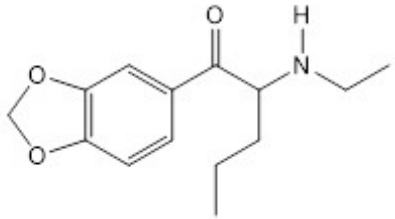
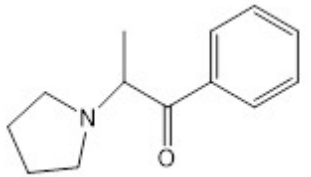
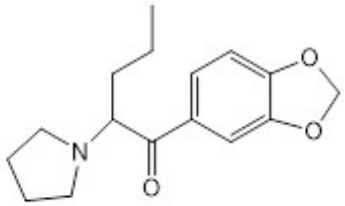
4-Fluro-Isobutyryl Fentanyl	369.2>188.1,105	26	FIBF-D_7	376.2>188.1,105	26	
para-Fluorobutyryl Fentanyl	369.2>188,104.9	26				
Methoxyacetyl Fentanyl	353.2>188.1,104.9	22	MOAF-D_5	358.2>188.1,105	22	
4-ANPP	281.1>188.1,105	14	ANPP-D_5	286.2>188,105.1	15	
U-48800	343>298,217.8	15	U488-D_3	346.1>298,217.9	15	

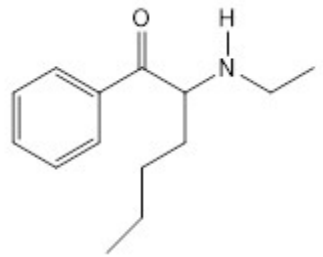
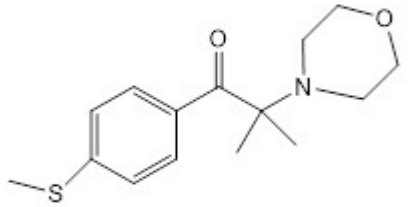
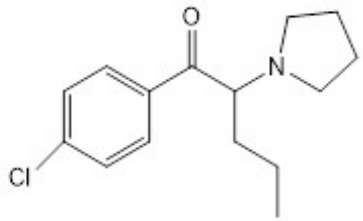
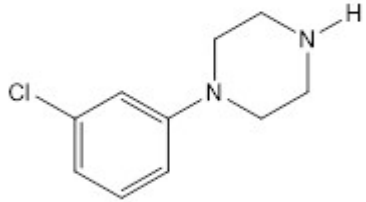
U-47700	329.1>284,203.9	18	U477-D_3	332.1>287,176	15	
Synthetic Cannabinoids						
5-Fluoro MDMB-PICA	377.1>232,144	10				
MAB-CHMINACA	371.1>354,326	13,50	MABC-D_4	375.2>358.2,330.1	7	
MMB-FUBINACA	384.1>324.1,252.9	11				

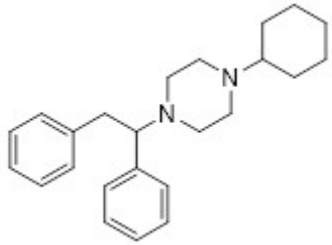
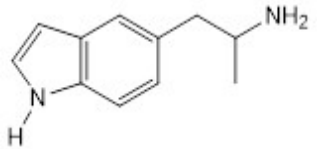
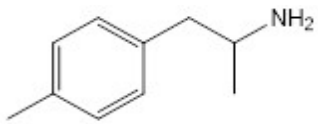
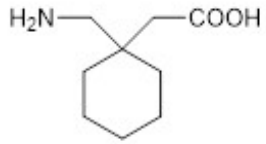
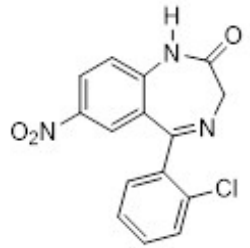
5-Fluoro EDMB-PINACA	392.1>318.2,346.1	9				
(R)-5-Fluoro ADB	378.2>318.1,233	16				
AB-FUBINACA	369.1>352,324.1	4	ABF-D_4	373.1>328.1,356.1	7	

MMB-CHMICA	371.1>240,143.9	14	
AB-CHMINACA	357.1>312.1,241	14	
ADB-FUBINACA	383.1>338.1,253	15	

5-Fluoro AMB	364.1>233,304.1	22				
NM2201	376.1>232,143.9	5,43				
Synthetic Cathinones						
Methcathinone	164.1>146.1,131.1	12	MC-D_3	167.1>149.1,130.9	10	
Ethylone	222.1>174.1,203.9	16	ETO-D_5	227.1>179.1,209.1	15	

4-MMC/ Mephedrone	178.1>160.1,145.1	11	MMC-D_3	181.1>163.1,148	10	
4-Methyl Pentedrone	206.1>188,175	5				
N-Ethylpentylone	250.1>232.1,202.1	12	NEP-D_5	255.1>207.1,237.1	20	
α-Pyrrolidinopropiophenone	204.1>105.1,98.1	28				
3,4-Methylenedioxypyrovalerone	276.1>126.1,135	27	MDPV-D_8	284.2>134.2,175	30	

α-Ethylaminohexanophenone	220.1>202.1,146	10				
2-Methyl-4'-(methylthio)-2-Morpholinopropiophenone	280.1>165,88	18				
4-Chloro-α-PVP	266.1>125,195	23				
Piperazines						
1-(3-Chlorophenyl) Piperazine (mCPP)	197.1>153.9,119.1	21	CPP-D_8	205.1>158,122.9	23	

MT-45	349.2>181.1,169.1	22	MT45-D_{11}	360.3>181,166	22	
Indole						
5-IT	175.1>158,130	10				
Amphetamine						
4-methylamphetamine	150.1>105,133.1	17				
Anticonvulsants						
Gabapentin	172.1>154.1,137.1	13	GBP-D_{10}	182.2>164,147.2	12	
Clonazepam	315.9>269.9,241	27	CZP-D_4	320>274,217.8	24	

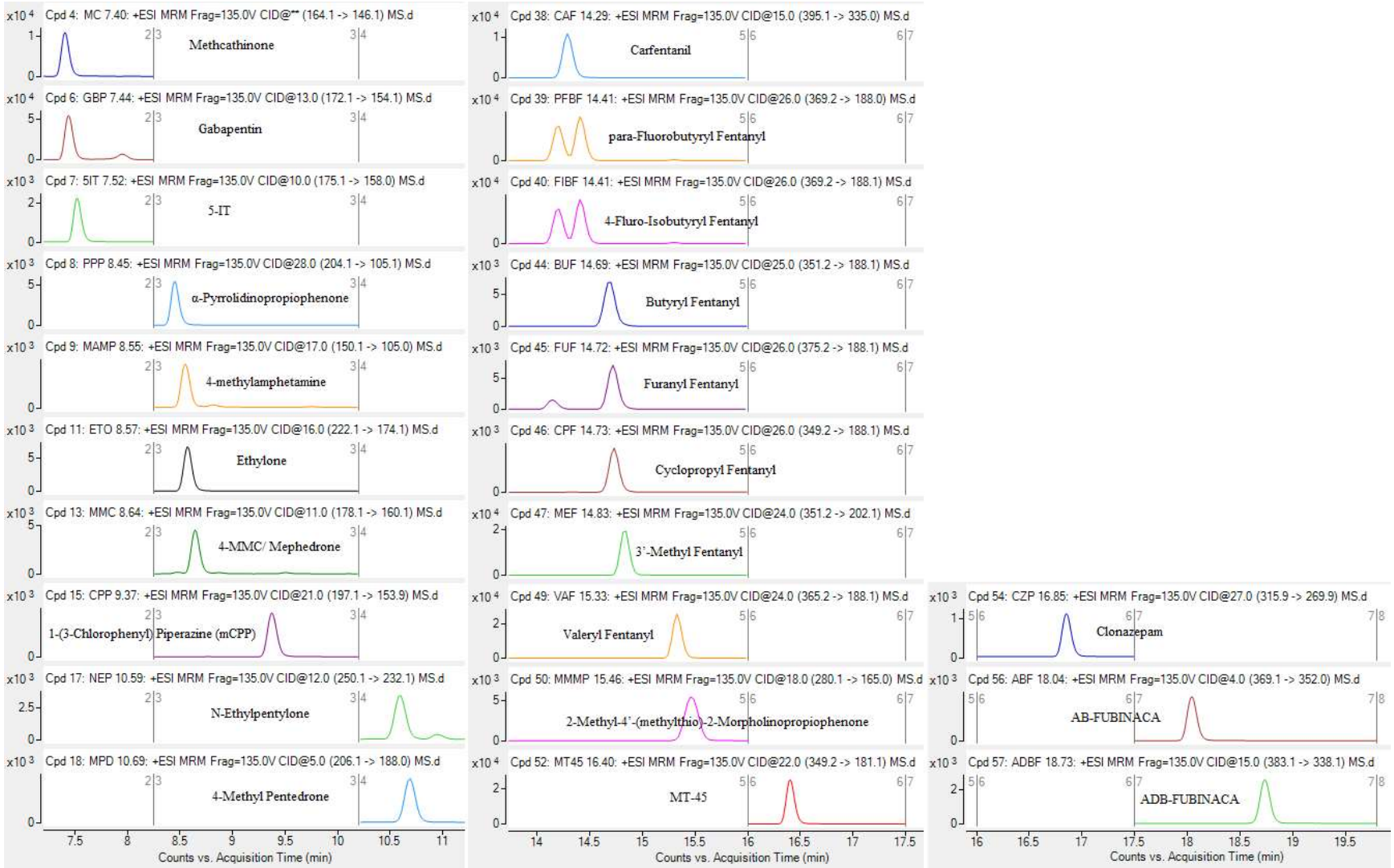


Figure S1. Schematic diagram showing the chromatographic separation of select drugs in a drug-spiked (50 or 100 ng) wastewater extract. All other target drug's chromatograms are presented in Figure **1**.

Optimization of Extraction Efficiency. For the simultaneous extraction of cannabinoids, cathinones, and synthetic opioids, the three most frequently used extraction cartridges (HLB, MCX, and WCX) were examined for their optimum extraction efficiency. It is important to note that the recoveries reported herein are absolute recoveries. In real sample analysis, deuterated analogs (internal standards) were spiked prior to extraction that corrected the loss of target NPSs. At 2 or 4 ppb spiking level, the spiking recoveries of target NPSs using HLB and HLB-A ranged from 50.6 ± 1.5 % (MAMP) to 115 ± 3.4 % (MABC) and 50.6 ± 4.0 % (MAMP) to 132 ± 2.5 % (CPF), respectively (Figure 2A). Similarly, spiking recoveries of target NPSs using MCX and WCX ranged from 51.8 ± 0.34 % (MAMP) to 132 ± 3.2 % (MABC) and 48.0 ± 1.5 % (MAMP) to 128 ± 0.90 % (CPF), respectively. However, the spiking recoveries of some NPSs including BUF, FUF, and MEF were >150% with all cartridges whereas the spiking recoveries of U477 could not be determined using HLB and HLB-A cartridges.

At 50 or 100 ppb spiking level, spiking recoveries of target NPSs using HLB and HLB-A ranged from 39.9 ± 1.9 % (PVP) to 174.0 ± 12.1 % (MAMP) and 50.3 ± 3.98 % (PPP) to 128.7 ± 3.0 % (ADBF), respectively (Figure 2B). However, the average percentage recoveries were exceptionally high (>200%) for 5-IT and MAMP while exceptionally low (<3%) for NM2201 with HLB and/or HLB-A. Similarly, the spiking recoveries of target NPSs using MCX and WCX at 50 or 100 ppb spiking level ranged from 54.2 ± 5.3 % (NM2201) to 127.2 ± 5.8 % (FUF), and 41.3 ± 2.4 % (5-IT) to 133.7 ± 2.5 % (BUF), respectively. Bade et al.[21] found MCX-extraction more efficient than HLB-extraction for cathinones whereas Fontanals et al.[1] preferred WCX over MCX for cathinones. To the authors' knowledge, there was no report of the optimized extraction of synthetic fentanyls from wastewater. Salguero et al.[2] used both HLB and MCX cartridges to cover the wide range of physio-chemical properties of NPSs including cathinones and cannabinoids.

However, the simultaneous extraction of diverse NPSs using a unique SPE cartridge is always a method of choice owing to the cost, time, and labor associated with the extraction through multiple cartridges. As the triplicate spiking recoveries of target NPSs using MCX extraction cartridge found an optimum; MCX was considered for the extraction of NPSs from the collected wastewater samples to determine their prevalence in communities. Overall, the absolute spiking recoveries of target NPS were satisfactory not only at the mid-point calibration-level but also at the environmentally relevant concentration level.

Prevalence of Gabapentin. Gabapentin is a gamma-aminobutyric acid (GABA)-mediated potential drug of abuse. The rate of on- and off-label prescriptions of gabapentin as an alternative to opioids for pain management has been increased recently due to the associated lower cost and its classification as a non-controlled substance in the U.S.[3] The co-administration of gabapentin and opioids causes the drug-induced respiratory depression and increase the opioid-related deaths.[4] The gabapentin overdose cases are alarming along with opioid epidemic in the Midwest and Southeast regions including Illinois and Kentucky. Kentucky designated gabapentin as a schedule V drug while Illinois initiated prescription monitoring program to track gabapentin prescriptions. In this study, Gabapentin was quantified in all wastewater samples and the average concentrations in communities C1, C2, C3, and C4 were 131 ± 7.6 µg/L, 58.6 ± 5.1 µg/L, 25.5 ± 1.2 µg/L, and 12.1 ± 1.4 µg/L, respectively. The average concentration of gabapentin in wastewater influent from five WWTP in Germany was ranged from 3.67 to 11.88 µg/L.[5] In the U.S., gabapentin was not only quantified in wastewater (up to 3.3 µg/L) in Minnesota[6] but also detected in wastewater infiltrated surface water collected from four national parks in the Midwest U.S.A. including Mississippi National River and Recreation Area (up to 1.08 µg/L) and Indiana Dunes National Lakeshore (up to 2.79 µg/L),

respectively.[7] The prevalence of gabapentin in target four rural communities in Illinois was also determined based on the residual gabapentin in wastewater, 90% excretion rate,[8] 10% loss (in-sewer degradation, the degradation in wastewater during sample collection, and the degradation prior sample extraction),[9] and a single daily dose of 1050 mg by an individual in the WWTP catchment. The estimated daily doses of gabapentin in C1, C2, C3, and C4 communities were 40.6 ± 1.1, 21.0 ± 2.2, 18.5 ± 0.58, and 8.32 ± 0.87 per 1000 people, respectively.

References

1. Fontanals, N.; Marcé, R. M.; Borrull, F. Solid-phase extraction followed by liquid chromatography-high resolution mass spectrometry to determine synthetic cathinones indifferent types of environmental water samples. *J. Chromatogr. A* **2017**, *1524*, 66–73.
2. Salgueiro-González, N.; Castiglioni, S.; Gracia-Lor, E.; Bijlsma, L.; Celma, A.; Bagnati, R.; Hernández, F.; Zuccato, E. Flexible high resolution-mass spectrometry approach for screening new psychoactive substances in urban wastewater. *Sci. Total Environ.* **2019**, *689*, 679–690.
3. Evoy, K. E.; Covvey, J. R.; Peckham, A. M.; Ochs, L.; Hultgren, K. E. Reports of gabapentin and pregabalin abuse, misuse, dependence, or overdose: An analysis of the food and drug administration adverse events reporting system (FAERS). *Res. Social Admin. Pharm.* **2019**, *15*, 953-958.
4. Gomes, T.; Juurlink, D. N.; Antoniou, T.; Mamdani, M. M.; Paterson, J. M.; van den Brink, W. Gabapentin, opioids, and the risk of opioid related death: A population-based nested case – control study. *PLoS Med.* **2017**, *14*, e1002396.
5. Oertel, R.; Baldauf, J.; Rossmann, J. Development and validation of a hydrophilic interaction liquid chromatography-tandem mass spectrometry method for the quantification of the antidiabetic drug metformin and six others pharmaceuticals in wastewater. *J. Chromatogr. A* **2018**, *1556*, 73-80.
6. Writer, J. H.; Ferrer, E.; Barber, L. B.; Thurman, E. M. Widespread occurrence of neuro-active pharmaceuticals and metabolites in 24 Minnesota rivers and wastewaters. *Sci. Total Environ.* **2013**, *461-462*, 519–527.
7. Elliott, S. M.; VanderMeulen, D. D. A regional assessment of chemicals of concern in surface waters of four Midwestern United States national parks. *Sci. Total Environ.* **2017**, *579*, 1726–1735.
8. Baselt, R.C. Disposition of Toxic Drugs and Chemicals in Man. Eleventh Edition, **2017.**
9. O'Brian, J. W.; Banks, A. P. W.; Novic, A. J.; Mueller, J. F.; Jiang, G.; Ort, C.; Eagelsham, G.; Yuan, Z.; Thai, P. K. Impact of in-Sewer Degradation of Pharmaceutical and Personal Care Products (PPCPs) Population Markers on a Population Model. *Environ. Sci. Technol.* **2017**, *51*, 3816-3823.