LC-MSMS Method for the Detection and Quantification of Pharmaceuticals and Drugs of Abuse in New York City Waterways

Natalie Fernandez, Viviana Chavez, Teeshavi Acosta, Marta Concheiro-Guisan
Department of Sciences, John Jay College of Criminal Justice, City University of New York, NY

Abstract

- The presence of pharmacologically active compounds in this environment can be extremely harmful to aquatic life as they are continuously exposed to these substances.
- The goal of this study was to identify and quantify drugs found in New York City waterways over the summer of 2021 and determine if there was a correlation between drug concentrations and enterococcus bacteria found in the water.
- An analytical method was developed and validated for 28 different drugs including cocaine, amphetamines, opioids, cannabis, and prescription medicines. River water samples were extracted by solid phase extraction and analyzed via liquid chromatography tandem mass spectrometry. The linearity ranged between 5 to 1000 ng/L, and the method showed acceptable bias and imprecision. A total of 231 samples were analyzed from 18 locations collected weekly for 13 weeks (May-August).
- The most common substances detected were metoprolol, benzoylecgonine and atenolol, followed by methamphetamine, cocaine, EDDP, and norfentanyl. No correlation was found between bacterial content and drug concentration. We developed a sensitive and specific method for the determination of licit and illicit drugs in river water samples. Common drugs of abuse and prescription medicines were detected in NYC waterways at ng/L levels.

Introduction

- Due to the increase of substance abuse, drugs are appearing as emerging contaminants in surface and ground water which can be harmful to aquatic life.
- Wastewater samples and river water samples around the world are being analyzed to determine the concentrations of licit and illicit drugs.
- There is limited information on the presence of drugs in the New York City river waters and the source of these emerging contaminants.
- Samples for this study were collected at various points from the Hudson River near combined sewage overflows (CSOs) and wastewater treatment plants.
- These samples were analyzed for their bacterial content and drug concentrations to determine if there was a correlation between the two. Bacterial content can be attributed to untreated wastewater pollution (CSOs).

Materials

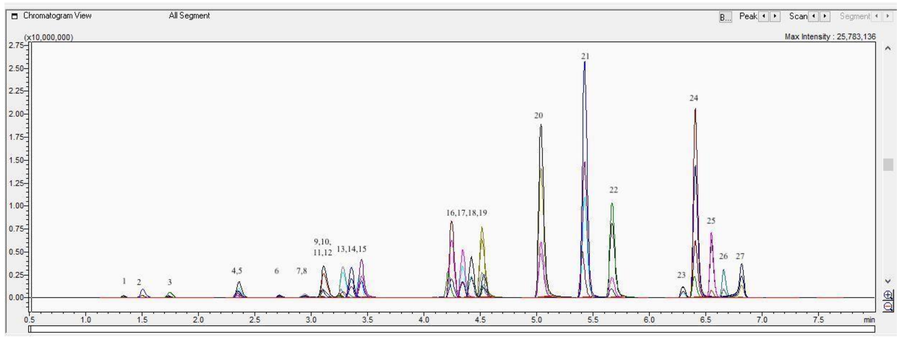
- Strata-XC cation exchange SPE cartridges 33 µm, 3 mL, 60 mg and 6mL/ 200 mg cartridges (Phenomenex)
- UPLC-Triple Quadrupole LCMS-8030 (Shimadzu)
- Chromatographic column Kinetex C18 100 x 2.1 mm with particle size 1.7 µm (Phenomenex)

Results

Table 1. Intra and inter-day precision (CV%) and bias (%) at the limit of quantification (LOQ), the low QC (LQC 20 ng/L) and HQC (200 ng/L).

LOQ		LQC			HQC		
Inter-day Precision	Bias	Inter-day Precision	Intra-day Precision	Bias	Inter-day Precision	Intra-day Precision	Bias
1.14 to 12.63	-7.37 to 7.03	7.62 to 39.27	8.3 to 70.4	-6.37 to 27.20	7.53 to 23.11	5.1 to 38.1	-13.46 to 6.54

Bias and Precision out of Range:
- ✔ Bias: Clonidine 27.20%; Methadone 21.01%
- ✔ Precision: THC-COOH 22.00%; Ranitidine 22.43 and 23.11%; Atenolol 20.63% ; Methamphetamine 30.7%; Sulfamethoxazole 24.1%; EDDP 37.1%; Methadone 70.4%



- **Linearity from LOQ (5 ng/L) to 1000 ng/L** ; linear model; $1/x^2$ weighing
- **Fig 1.** Total ion chromatogram (TIC) of all multiple reaction monitoring (MRM) transitions for analytes included in this study at 500 ng/L. 1, Morphine; 2, Oxymorphone; 3, Hydromorphone; 4, Ranitidine; 5, Atenolol; 6, Codeine; 7, Oxycodone; 8, Clonidine; 9, Hydrocodone; 10, Amphetamine; 11, 6-acetylmorphine; 12, Methamphetamine; 13, MDA; 14, MDMA; 15, Norfentanyl; 16, Benzoylecgonine; 17, Sulfamethoxazole; 18, Cocaine; 19, Metoprolol; 20, Cocaethylene; 21, Fentanyl; 22, EDDP; 23, Paroxetine; 24, Methadone; 25, Fluoxetine; 26, Sertraline; 27, Alprazolam.

Table 2. Matrix effect (%) of river water from different water sources at 50 ng/L. (n=10)

Ion Suppression (20 out of 28 analytes)	**No Matrix Effect** (8 analytes)
-54.83 to -27.80	-23.77 to 4.24

- ✔ The eight analytes that had no matrix effect: oxycodone, clonidine, 6-acetylmorphine, sulfamethoxazole, cocaine, metoprolol, cocaethylene, and methadone

Table 3. Summary of positive cases with their range of concentrations.

Analyte	# of Positive Cases	Range of Concentrations (ng/L)
Metoprolol	196	LOD-25.128
BE	142	LOD-103.219
Atenolol	134	LOD-10
Cocaine	109	LOD-9.873
Methamphetamine	109	LOD-10
EDDP	89	LOD-12.661
Norfentantyl	22	LOD-LOQ
Methadone	5	LOD-LOQ
Sulfamethoxazole	5	LOD-LOQ
Amphetamine	5	LOD
Cocaethylene	2	LOD
Fentanyl	1	LOD

- ✔ The most present analytes were metoprolol, benzoylecgonine, atenolol, cocaine and methamphetamine.
- ✔ Benzoylecgonine was the analyte with the highest concentration at 103.219 ng/L.

Methods

Sample Preparation (Strata-XC SPE Cartridges):
- Conditioning: 6mL methanol & 6mL water
- Load Sample: 45mL of river water, 500 µL of formic acid & 100 µL of 0.1µg/mL internal standard mixture
- Wash: 4mL 1% formic acid in water
- Elution 1: 6mL dichloromethane:isopropanol (50:50) for the cannabinoids
- Elution 2: 6mL dichloromethane:isopropanol: ammonium hydroxide (78:20:2) for the basic drugs
- Add 100 µL of 1% HCl in methanol (1:99) before evaporation to the basic drugs
- Reconstitute the cannabinoids in 200 µL of 0.1% formic acid in water: methanol (60:40)
- Reconstitute the basic drugs in 200 µL of 0.1% formic acid in water

LCMS Method
- Mobile Phase A: 0.1% formic acid in water
- Mobile Phase B: methanol
- Gradient for the basic drugs: 10 to 60 MPB% at 5 min, increased to 90 MPB% from 6 to 9 min, back to initial conditions at 9.5 min; total chromatographic run time of 15 min
- Gradient for the cannabinoid: 40 to to 95 MPB% from 0 to 5 min and returned to initial conditions at 5.5 min; total run time of 7 min
- Flow 0.3mL/min; 35°C column temperature
- ESI +; 2 MRM transitions per compound

Conclusions

- A sensitive method was developed and validated for the identification and quantification of licit and illicit drugs in river water samples by LC-MSMS.
- The limit of quantification was determined to be 5 ng/L and the limit of detection was determined to be 10 ng/L.
- The most reoccurring analytes were metoprolol, benzoylecgonine, atenolol and methamphetamine.

References

- Hamrud, E. M. (2021, May 23). *Do Pharmaceuticals Pose a Threat to Aquatic Life? Experts Weigh In*. ScienceAlert. https://www.sciencealert.com/do-pharmaceuticals-pose-a-threat-to-aquatic-life-here-s-what-experts-think
- Jacox, A., Wetzel, J., Cheng, S. Y., & Concheiro, M. (2017). Quantitative analysis of opioids and cannabinoids in wastewater samples. *Forensic Sciences Research*, *2*(1), 18–25.
- Nicole Centazzo, Bonnie-Marie Frederick, Alethea Jacox, Shu-Yuan Cheng & Marta Concheiro-Guisan (2019) Wastewater analysis for nicotine, cocaine, amphetamines, opioids and cannabis in New York City, Forensic Sciences Research, 4:2, 152-167.
- Cantwell, M. G., Katz, D. R., Sullivan, J. C., Shapley, D., Lipscomb, J., Epstein, J., Juhl, A. R., Knudson, C., & O'Mullan, G. D. (2018). Spatial patterns of pharmaceuticals and wastewater tracers in the Hudson River Estuary. *Water Research*, *137*, 335–343.
- Kay, P., Hughes, S. R., Ault, J. R., Ashcroft, A. E., & Brown, L. E. (2017). Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. *Environmental Pollution*, *220*, 1447–1455.
- Pochodylo, A., & Helbling, D. E. (2016). *Target Screening for Micropollutants in the Hudson River Estuary during the 2015 Recreational Season*. New York State Department of Environmental Conservation.