Fast and Simple Creatinine Determination in Wastewater by Liquid Chromatography-Mass Spectrometry

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Background

- Wastewater-based epidemiology is a fast and cost-effective tool to assess illicit drug use in a community, complementing currently employed methods such as surveys.
- One of the main issues in determining drug use in a community through wastewater analysis is the drug/metabolite concentration fluctuations due to weather conditions and changes in the population.
- Creatinine is a product of the muscle metabolism, produced at a relatively constant rate throughout the day. This unique biological material is commonly employed in clinical and forensic toxicology as normalization factor for urine drug concentrations.
- Although several studies have investigated its utility as a normalization factor in wastewater analysis, the reported results have been contradictory.

Objectives

- To develop an analytical method for the determination of creatinine in wastewater samples,
- To apply this method to 48 authentic samples collected from 6 wastewater plants in New York City at 8 different time points throughout one year.

Methods

Authentic Samples Collection

Authentic wastewater samples were collected from wastewater treatment plants (WWTP) from four municipal boroughs of New York City, namely Manhattan (North River, Newtown Creek-Manhattan pool), The Bronx (Hunts Point), Brooklyn/Queens (Newtown Creek -Brooklyn/Queens pool) and Queens (Tallman Island, Jamaica). The type of influent in all these plants is primarily urban residential.

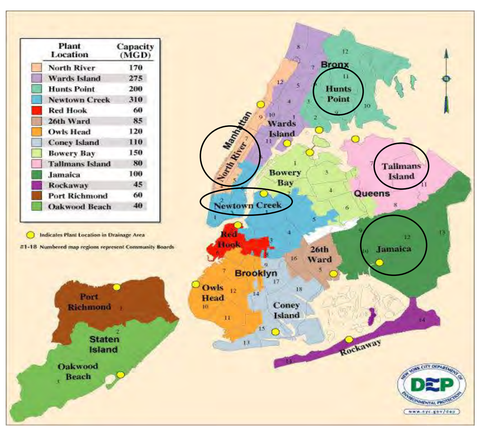
- North River WWTP serves a population of 588,772 from North River (northern Manhattan at westside of Manhattan above Bank Street)
- Newton Creek WWTP serves a population of 1,068,012 (south and eastern midtown section of Manhattan; northeast section of Brooklyn and western section of Queens)
- Hunts Point WWTP population served is 684,569 (eastern section of The Bronx)
- Tallman Island WWTP population served is 410,812 (northeast section of Queens)
- Jamaica WWTP serves a population of 728,123 (southern section of Queens)

According to DEP (www.nyc.gov/dep), after the preliminary treatment to remove large pieces of trash, the wastewater is pumped to the primary settling tanks for one to two hours. One-time grab samples (in triplicate) from the wastewater plant primary settling pool was performed by DEP authorized personnel. The samples were collected in Nalgene™ Wide-mouth HDPE 250 mL bottles between 8 am to 11 am on the collection days.

Sampling was done on days before and after major holidays in 2016:

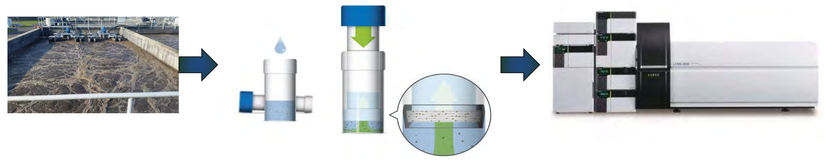
- Memorial Day (May 27th, May 31st)
- 4th of July (July 1st, July 5th)
- Labor Day (September 2nd, September 6th)
- New Year's (December 30th, January 3rd)

The samples were stored in coolers and shipped to the laboratory on the same day. Once in the laboratory, the samples were stored at -20°C until day of analysis.



Creatinine Method

Sample preparation procedure



- One milliliter of wastewater samples was centrifuged for 10 minutes at room temperature
- Two-hundred µL of the supernatant were transferred into the shell vial of the Filter Vial™ from Thomson Instrument Company, and fortified with 200 µL of IS at 0.1 mg/L. The plunger with filter was slightly inserted into the shell vial, vortexed and then inserted all the way.
- The filtered sample was directly injected into the LC-MSMS (Injection volume 20 µL).

Calibrators and QCs

- Calibrators were prepared at concentrations 0.01, 0.05, 0.1, 0.5, 1, 5 and 10 mg/L using 200 µL of the corresponding working solution and fortified with 200 µL of IS.
- QCs at 0.03 mg/L and 3 mg/L were prepared using 200 µL of QC stock solutions (0.03 mg/L and 3 mg/L, respectively) and fortified with 200 µL of IS at 0.1 mg/L.

LC-MS parameters

- The chromatographic separation was performed using a Luna C8 column (2x150mm ,3 µm) (Phenomenex).
- The mobile phase, 0.1 % formic acid in water (A) and in acetonitrile (B), was delivered at flow rate of 0.3 mL/min. The gradient increased from 2% to 15% B in 1.5 min and then to 95 % in 2 min, and it was held from 3.5 to 4 min. Then, it decreased to 2% from 4 to 4.5 min and was held until 6 min.
- The mass spectrometer was a triple quadrupole LCMS-8050 from Shimadzu. The heating gas and drying gas flows were both at 10 L/min, with a nebulizing gas flow at 2 L/min. The interface temperature was 300°C and the heat block temperature was 400°C.
- All compounds were analyzed using ESI +, and two transitions in multiple reaction monitoring (MRM) mode were acquired for each analyte .

Analyte	Precursor ion *m/z*	Product ion *m/z* (Quantifier)	CE (eV)	Product ion *m/z* (Qualifier)	CE (eV)
Creatinine	114	44	-18	86	-16
Creatinine- d_3	117	47	-21	89.1	-15

Results & Discussion

Creatinine Method Validation

The creatinine method was also validated following the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines (J Anal Toxicol, 2013, 37, 452–474);

- **Linearity** for creatinine was demonstrated using 7-point calibration curves (n=4) **from 0.01 to 10 mg/L**; r^2 ranged from 0.9997 to 0.9998 (0.99979±0.0009) and residuals were within ±20%.
- Triplicates of low (0.03 mg/L) and high (3 mg/L) QCs in 4 different days were used to measure the accuracy and imprecision (n= 12). **Accuracy** was **103%** for the low QC and **96%** for the high QC.
- **Imprecision** was calculated by considering the mean for all four days of analysis and were **7.2%** for low QC and **4%** for high QC.
- **Extraction efficiency** was evaluated at low and high QCs by analyzing triplicate water samples that were both diluted and filtered and those that were only diluted with IS, to evaluate the potential loss due to filtration. The extraction efficiencies were between **95.8%** and **98.2%**.
- Due to the presence of creatinine in all wastewater and river water samples tested, the matrix effects were done by comparing the creatinine-d_3 peak areas in QC samples (n=18) and in authentic wastewater samples (n=48). The analysis showed **ion suppression** of **-75.7%**. The CV was calculated for the authentic samples and it was 39.1% (n=48).
- The samples were **stable for at least 96 h at 4 °C in the filtration vials**.
- **No interferences** were detected by the presence of the other drugs and metabolites that were analyzed in this study.

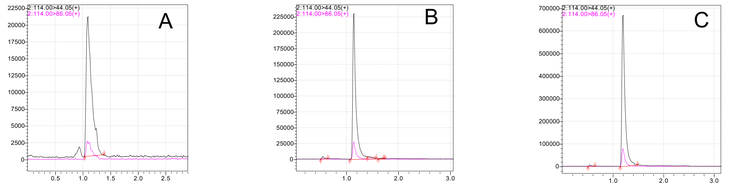


Figure. A: creatinine calibrator at 0.01 mg/L; B: authentic wastewater sample Tallman_12-30-16 at 0.22 mg/L; C: authentic wastewater sample North River_07-05-16 at 1.46 mg/L.

Authentic Wastewater Samples

- All samples were positive for creatinine in all WWTPs; the highest concentration was detected in Newtown Creek Brooklyn/Queens (2.68 mg/L) and the lowest concentration in Tallman (0.22 mg/L), with a median of 1.14 mg/L.
- The results (median, range) for each WWTP were:
  - ✓ Hunts Point (The Bronx) 0.81, 0.47-1.37 mg/L
  - ✓ Jamaica (Queens) 1.06, 0.68-1.66 mg/L
  - ✓ Newtown Creek-Manhattan pool 1.19, 0.9-1.61 mg/L
  - ✓ Newtown Creek-Brooklyn/Queens pool 1.64, 1.38-2.68 mg/L
  - ✓ Tallman (Queens) 0.59, 0.22-1.94 mg/L
  - ✓ North River (northern Manhattan) 1.34, 0.95-1.93 mg/L
- The concentrations reported in our study, 0.22 to 2.68 mg/L, were within the range of concentrations previously reported (0.06-10.7 mg/L) (Thai et al, Water Research, 2014, 55, 272-279). Although some studies reported stability issues of creatinine in wastewater and in the wastewater system, it has been successfully employed by other authors in the US (Burgard et al, Sci Total Environ, 2013, 450-451, 242-249), as a normalization factor to account for population variations among sampling periods. In the present study, the variability (CV) of creatinine concentrations within each wastewater plant at eight different time points was between 20 and 33.9%, except in the case of Tallman, which was 72.8%.

Conclusion

A fast and simple method was developed to determine creatinine in wastewater samples with high sensitivity (LOQ 0.01 µg/mL) and specificity (2 MRM transitions per analyte). The developed method could be applied to urine samples (dilution cut-off 20 mg/dL) as well. Creatinine was detected in all authentic wastewater samples with concentrations from 0.22 to 2.68 µg/mL, and it should be further investigated as a normalization factor in wastewater analysis.

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