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Determination of Anxiolytic and Antidepressant Medicines in New York City Wastewater Samples

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Abstract

Wastewater-based epidemiology (WBE) provides information about a population's exposure to certain chemical agents, such as drugs of abuse and medicines, by the analysis of human biomarkers, also known as excretion products, in wastewater samples. Although this is a growing field worldwide, mainly in Europe, Oceania and Asia, limited data from the US are currently available. We developed and validated an analytical method to quantitatively and qualitatively determine the presence of commonly prescribed drugs to treat anxiety (alprazolam, buspirone, clonazepam, lorazepam, and propranolol) and depression (bupropion, citalopram, clomipramine, duloxetine, fluoxetine, imipramine, paroxetine, sertraline, and venlafaxine) in wastewater using liquid chromatography tandem mass spectrometry (LC-MSMS). We applied this method in the analysis of 48 authentic wastewater samples, collected from six different wastewater plants in New York City through one year. Ion suppression (n=10) was detected for all the analytes in the method as the matrix effects ranged from -30.6 to -99.6%. All the drugs were detected in at least one location with the exceptions of lorazepam and duloxetine, which were not detected in any plant. The antidepressant venlafaxine was the most commonly detected drug (n=31), with concentrations from 46.5 to 298.5 ng/L. Hunts Point wastewater plant had 10 out of 14 analytes present in the wastewater samples examined compared to the other 5 sites which had 8 analytes or less present in the wastewater samples. The amount of samples positive for antidepressants increased before every holiday except New Year's Day (Memorial Day, Independence Day, and Labor Day) whereas the amount of samples positive for anxiolytic drugs relatively remained the same or changed by one value throughout the year. We developed and validated a sensitive and specific method for the detection of 14 anxiolytic and antidepressant drugs in wastewater. Wastewater analysis is a valuable tool which can be used to observe drug usage in large and small populations.

Introduction

Wastewater-based epidemiology (WBE) is a novel and promising discipline that analyzes specific human metabolic excretion products (biomarkers) in wastewater as indicators of consumption or exposure of the population served by the sewer network under investigation to different substances (Gracia-Lor, et al. 2017). The main advantages of using wastewater analysis, compared to other approaches to estimate drug exposure in a certain population, include low costs, obtaining "real-time" results and the ability to choose specific locations and populations to analyze (Jacox, et al. 2017). The disadvantages of wastewater analysis include a variation in the population due to visitors and tourists which leads to data that is not truly representing the desired community, and changes in sewage flow (rainfall), which may affect the analytical results found (Jacox, et al. 2017).

Prescription anxiolytic and antidepressant medications have become drugs of interest in the scientific community due to their potential to be hazardous to humans as well as the environment (Jurado, et al. 2012), and their potential to be abused (Racamonde, et al. 2014). Researchers from Columbia University defined non-medical drug usage as the utilization of psychotropic medication without a prescription, in greater amounts, more often, or longer than prescribed, or for a reason other than the originally prescribed (Blanco, et al. 2013). According to the Drug Abuse Warning Network (DAWN), as reported by the Substance Abuse and Mental Health Services Administration (SAMHSA) the national amount of emergency department visits for antidepressants and anxiolytic drugs have increased by 47.4%, combined, between the years of 2004 and 2011 (SAMHSA, 2012a). In New York City, the amount of emergency department visits for antidepressants have slightly increased by 26.7% between the years of 2004 and 2011 from 91,604 to 124,903 visits, while for anxiolytic drugs, the amount of emergency department

visits more than doubled (increased by 58%) from 233,605 to 561,235 visits (SAMHSA, 2012b). Nonmedical use of psychotherapeutic drugs is ranked second after marijuana in the US (Evans, E.A. and Sullivan, M., 2014).

The most common antidepressants employed to treat depression includes the tricyclic antidepressants, atypical antipsychotics, selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs) (Moffat, et al. 2011). Antidepressants work by targeting neurotransmitters in the brain such as serotonin, norepinephrine and/or dopamine, which regulate one's mood (New York State Office of Mental Health , 2018). Serotonin and norepinephrine work specifically to "influence mental behavior patterns", while dopamine "influences movement;" together, these neurotransmitters help to regulate normal brain function (Blows, W., 2000). Anxiolytic drugs are used to reduce anxiety; some benzodiazepines as well as minor tranquillizers are normally used as anxiolytic drugs (Moffat, et al. 2011). Many antidepressants and anxiolytic drugs are reported as providing a "euphoric" and "high" sensation along with other stimulant-like effects; these sensations may be a reason these drugs tend to be misused (Evans and Sullivan, 2014).

Antidepressant and anxiolytic medications are excreted in urine, and therefore they end up in the wastewater. Majority of the wastewater studies have taken place in cities in Europe, Oceania or Asia with a wide range in population size from less than 1000 people to more than one million (Al Aukidy, et al. 2012; Gurke, R., et al. 2015; Loos, et al. 2013; Mastroianni, et al. 2016; Paíga, et al, 2016; Papageorgiou, et al. 2016; Pereira, et al. ,2015; Pereira, et al, 2016; Racamonde, et al. 2014; Subedi, et al. 2013; Wu, et al. 2015; Van Der Ven, et al.2004; Yuan, et al. 2013). However, very few studies about wastewater analysis were developed in the US (Ferrey, et al. 2015; Ferrer & Thurman, 2012). Most of the aforementioned studies researched a combination of

prescribed anxiolytic and antidepressant medications along with other licit and illicit drugs in wastewater, river water, drinking water and/or sludge. To analyze these wastewater samples, many researchers have turned to liquid chromatography tandem mass spectrometry (LC-MSMS). LC-MSMS is a highly selective, robust and quick instrument that can detect analytes to the ng/L level (Van Der Ven, et al. 2004). In the review by Cunha, et al. (Cunha, et al. 2017), 99% of studies utilize some form of mass spectrometry to detect drugs in wastewater. Solid phase extraction (SPE) has also been a well-known extraction method for drugs in wastewater samples; however, various researchers have used different types cartridges and reagents to analyze the same types of drugs (Asimakopoulos, et al. 2017; Ferrer & Thurman, 2012).

The goals of this project were (1) to develop and validate an analytical method for the determination of anxiolytic and antidepressant medications in wastewater samples at the ng/L level, and (2) to apply this method to investigate anxiolytic and antidepressant drug use in the different boroughs of New York City. In this study, there were 14 target analytes; 9 were antidepressants (bupropion, citalopram, clomipramine, duloxetine, fluoxetine, imipramine, paroxetine, sertraline and venlafaxine) and the other 5 were anxiolytic drugs (alprazolam, buspirone, clonazepam, lorazepam and propranolol). These drugs were chosen since they are the most commonly prescribed anxiolytic and antidepressant medication in New York State. The method was applied to 48 authentic wastewater samples collected from various wastewater treatment plants in the Bronx, Brooklyn, Queens and Manhattan before and after major holidays (New Year's Day, Memorial Day, Independence Day, and Labor Day) in 2016.

Materials and Methods

Reagents

The analyte standards alprazolam, bupropion, buspirone, citalopram, clomipramine, clonazepam, duloxetine, fluoxetine, imipramine, lorazepam, paroxetine, propranolol, sertraline, and venlafaxine were purchased from Cerilliant (Round Rock, TX) at 1 mg/mL in methanol. The internal standards alprazolam-d_5, bupropion-d_9, buspirone-d_8, citalopram-d_6, clomipramine-d_3, clonazepam-d_4, duloxetine-d_3, imipramine-d_5, lorazepam-d_4, paroxetine-d_6, propranolol-d_7, sertraline-d_3, and venlafaxine-d_6 were also purchased from Cerilliant, at 100 µg/mL in methanol. ACS grade methanol and HPLC grade 2-propanol were from Fisher Scientific (Fair Lawn, NJ). LC/MS grade acetonitrile and LC/MS grade formic acid were also from Fisher Scientific. Reagent grade ethyl acetate and ammonium hydroxide (28-30%) were from Pharmco-Aaper (Brookfield, CT).

Materials

The wastewater samples were collected and stored in Nalgene™ certified wide-mouth amber HDPE 250 mL bottles from Fisher Scientific. The filtration step was performed using a Whatman glass microfiber filter (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK) along with a Millipore glass filter apparatus from Fisher Scientific. The analytes were extracted from the wastewater samples using polymeric mixed mode cation-exchange and reversed phase cartridges Strata XC of 3 mL/60 mg and 6 mL/200 mg from Phenomenex (Torrance, CA).

Method optimization

The ionization and fragmentation process by MSMS, the chromatographic separation, the sample preparation and the extraction of the analytes from wastewater were optimized. To optimize the multiple reaction monitoring (MRM) transitions in the MSMS 1 or 2 µL of 0.1 or 1 µg/ml of each standard and internal standard were directly injected into the MSMS. The quantifier transition for a compound was the product ion that had the highest abundance and qualifier transition had the second highest abundance value. The nebulizing gas flow, drying gas flow, and mobile phase gradient were optimized to improve the sensitivity of the instrument. The nebulizing gas flow rates of 1 and 3 L/min were examined. Various drying gas flow rates of 5, 10 and 18 L/min were used to determine the best setting that would increase analyte intensity. The type of ionization sources examined included electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and dual ionization sources (DUIS), which is the combination of ESI and APCI.

The mobile phase gradient was optimized by changing the organic mobile phase, the composition of the mobile phases during the run and the initial mobile phase mixture. The organic mobile phases tested were methanol and acetonitrile. The best organic mobile phase was selected based on the one that allowed the greatest yield for the target analytes and the internal standards. The gradient was optimized by observing the peaks in the chromatogram for each drug; ideal peaks are narrow, separated from other analytes and pointed. To optimize the composition of the reconstitution solvent, 10, 20 and 30% of 0.1% formic acid with acetonitrile were examined. The optimal reconstitution solvent was chosen based on the largest peak area produced for the analytes.

The filtration process was optimized to determine the best procedure regarding the acidification of the authentic samples to ensure analyte stability. These samples were prepared in 50 mL of UHP water. This study was done using a blank sample (0 ng/L), a sample at 10 ng/L, and another sample at 100 ng/L. Each sample was spiked with 50 µL internal standard mixture and their respective standard volumes (50 µL of 0.01 µg/mL for the 10 ng/L samples and 50 µL of 0.1 µg/mL for the 100 ng/L samples). Three sets were compared: no filtration, adding acid prior to filtration and adding acid after the filtration step. A volume of 250 µL of HCl was added to each sample at a specified time according to each set. The optimal method was determined based on the overall peak areas of the target analytes after analysis.

Different solid phase extraction (SPE) cartridges and procedures were evaluated for method optimization. Reversed phase (Strata X) and mixed-mode cartridges (Strata XC) were tested; the cartridge type, which yielded the most favorable results, produced the largest peak areas for the analytes and was selected for the remainder of the study. The washing step was optimized by changing the amount of wash steps involved and by using different solvents such as 0.1N hydrochloric acid (HCl) in UHP water and 0.1% formic acid in UHP Water. The elution step was optimized by varying the solvents such as dichloromethane, ethyl acetate, isopropanol, and ammonium hydroxide, and the ratio of the solvents used. The elution step was selected based on the mechanism of the cartridge and the method that had a higher yield of the target analytes.

Instrumentation

The mass spectrometer was a triple quadrupole LCMS 8030 from Shimadzu (Kyoto, Japan). The nebulizing gas flow was 2 L/min and the drying gas flow was 15 L/min. The heat block temperature was 400℃ and the desolvation line (DL) temperature was 250 °C. The instrument

operated in DUIS mode with an interface electrospray current of 4.5 µA and corona needle current of 4.5 µA. Two multiple reaction monitoring (MRM) transitions were monitored for each compound (Table 1), with one used as a quantifier and the other as a qualifier. For the internal standards, one MRM was monitored. The transitions and collision energies for each internal standard are listed in Table 2.

Table 1. MRM transitions of the antidepressant and anxiolytic drugs along with the retention time (RT) and collision energies (CE).

Drug	RT (min)	Precursor ion *m/z*	Quantifier Product ion *m/z*	CE (eV)	Qualifier Product ion *m/z*	CE (eV)
Bupropion	3.7	240.1	184.0	-12	131.0	-26
Venlafaxine	3.9	277.7	58.1	-21	260.1	-12
Buspirone	4.2	385.8	122.0	-32	265.1	-30
Propranolol	4.4	259.7	116.1	-18	183.0	-17
Citalopram	5.1	324.9	109.0	-29	262.1	-20
Paroxetine	5.7	329.7	192.0	-21	70.1	-31
Imipramine	5.9	280.7	86.1	-18	58.0	-36
Duloxetine	6.0	297.9	122.9	-50	44.1	-13
Clonazepam	6.2	316.1	270.1	-25	214.0	-36
Lorazepam	6.2	321.8	276.1	-23	304.0	-16
Fluoxetine	6.4	310.0	148.0	-8	44.1	-14
Alprazolam	6.5	309.1	205.1	-42	281.0	-27
Sertraline	6.5	307.1	276.0	-12	158.9	-25
Clomipramine	6.8	314.9	86.1	-18	58.1	-39

Table 2. MRM transitions for internal standards (ISTD) in the study along with the retention time (RT) and collision energies (CE).

ISTD	RT (min)	Precursor ion *m/z*	Product ion *m/z*	CE (eV)
Bupropion-d_9	3.6	249.2	131.0	-28
Venlafaxine-d_6	3.9	284.3	64.2	-23
Buspirone-d_8	4.2	394.3	122.0	-33
Propranolol-d_7	4.4	267.2	189.1	-18
Citalopram-d_6	5.1	331.3	109.0	-25
Paroxetine-d_6	5.7	336.2	76.1	-32
Imipramine-d_5	5.9	284.2	89.1	-18
Duloxetine-d_3	6.0	301.2	47.1	-12
Clonazepam-d_4	6.1	320.1	274.0	-26
Lorazepam-d_4	6.2	326.1	280.0	-21
Fluoxetine-d_6	6.4	316.2	44.1	-13
Alprazolam-d_5	6.4	314.9	287.1	-30
Sertraline-d_3	6.5	309.1	158.8	-30
Clomipramine-d_3	6.8	318.2	89.1	-21

The chromatographic separation was carried out on an ultra-high performance liquid chromatography (UHPLC) Nexera instrument from Shimadzu. The Nexera UHPLC system consisted of a binary LC-30AD high-performance liquid chromatography pump, online degassing unit (DGU-20A) and cooled autosampler (SIL-30AC). The chromatographic column utilized was a reversed phase Kinetex C18 column 1.7 um, 2.1x100 mm from Phenomenex. The oven temperature was 40℃, and the flow was 0.4 mL/min. Under initial conditions, the pressure of the pumps was approximately 6,300 to 6,800 psi. Mobile phase A was 0.1% formic acid in UHP water and mobile phase B was 0.1% formic acid in acetonitrile. The gradient had an initial

composition of 10% mobile phase B, which gradually increased to 40% mobile phase B in 6 min. Then there was an increase to 90% mobile phase B between 6 to 8 min. Between 8 to 9 min, the amount of mobile phase B decreased to 10%, and was held for 1 min after which the gradient program ended at 10 min. Figure 1 shows a summary of the gradient.

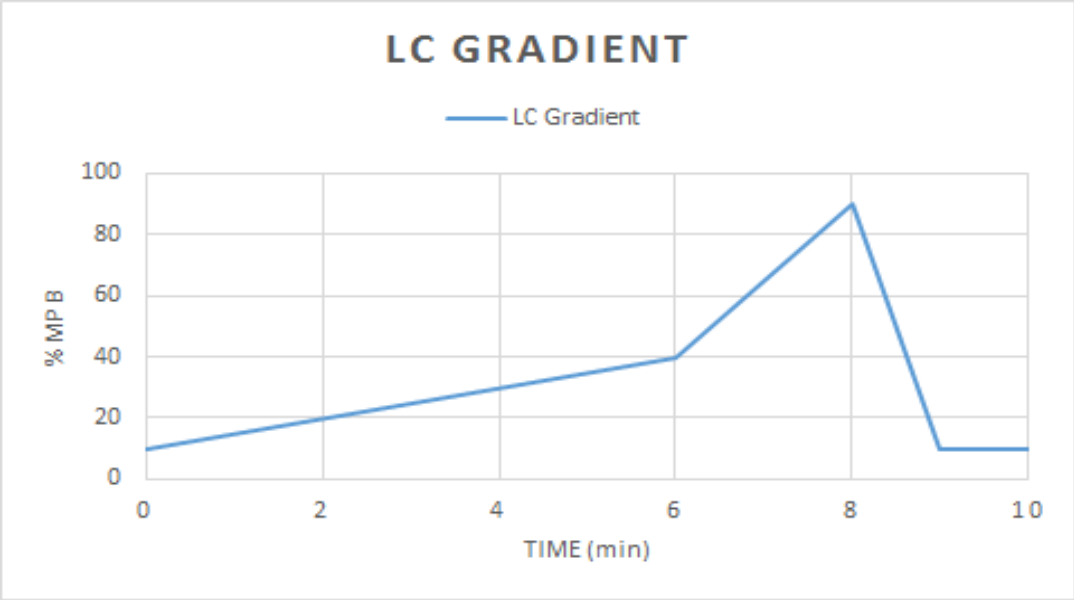

Figure 1. The liquid chromatography gradient for the antidepressants and anxiolytic drugs in the study. MPB: mobile phase B.

Sample preparation

A volume of 50 mL wastewater sample was measured in a graduated cylinder and spiked with 50 µL of 0.1 µg/mL internal standard mixture. The sample was filtered using the Millipore glass microfiber filter. Next, 250 µL of hydrochloric acid was added, and the sample was vortexed for 10 s.

Solid phase extraction

Strata XC cartridges 6 mL/ 200 mg for the wastewater samples were used. The cartridge was conditioned with 6 mL methanol and 6 mL UHP water. Next, the wastewater sample was subjected to the column. The cartridge was washed with 4 mL 0.1% formic acid in UHP water, followed by 4 mL of 30% methanol in UHP water. The cartridge was dried for 15 min under vacuum. To elute the target analytes, 2 aliquots of 4 mL elution solvent ($V_{ethyl\ acetate}$:$V_{isopropanol}$: $V_{ammonium\ hydroxide}$ = 70:20:10) were added. The eluent was evaporated in a Biotage Turbovap (Uppsala, Sweden) at 60℃. The initial flow of N_2 gas was 7 psi and was gradually increased to 20 psi, the maximum pressure, in increments of 3 psi. After evaporation, the samples were reconstituted with 100 µL of the initial mobile phase composition of 10% of 0.1% formic acid in acetonitrile (mobile phase B) with 0.1% formic acid in UHP water (mobile phase A). The samples were vortexed for 10 s and centrifuged for 10 min at 7,830 rpm. The supernatant was removed and transferred to the LC-MS vials for analysis.

Calibrator, quality control solutions and samples

Each analyte standard stock solutions were prepared from an ampoule having an initial concentration of 1000 µg/mL to 100 µg/mL via serial dilution with 10 mL of LC-MS grade methanol. These standards were combined into a working solution at 1 µg/mL by adding 100 µL of each analyte to methanol in a 10 mL volumetric flask. This stock solution was transferred to a 10 mL amber vial and was used for the remainder of the study. Other stock solutions of 0.1 µg/mL and 0.01 µg/mL were prepared by 1:10 serial dilutions in methanol.

The calibrators used in the study had the following concentrations: 5, 10, 50, 100, 500 and 1000 ng/L. Each calibrator was prepared by spiking the appropriate volume of the corresponding

working solution to 3 mL of UHP water. Fifty of 0.1 µg/mL internal standard and 15 µL of hydrochloric acid were also added. The Strata XC 3 mL/60 mg cartridges were used for the calibrators' extraction procedure. Three mL of methanol was used to condition the cartridges followed by 3 mL of UHP water. The calibrators were loaded into their respective cartridges and flowed through the columns via gravity. For the wash step, 2 mL of 0.1% formic acid in UHP water followed by 2 mL of 30% methanol in UHP water were added to the cartridges. The cartridges were dried for 15 min under vacuum. For the elution step, 4 mL of the elution solvent containing ethyl acetate, isopropanol and ammonium hydroxide ($V_{\text{ethyl acetate}}$:$V_{\text{isopropanol}}$: $V_{\text{ammonium hydroxide}}$ = 70:20:10) were added to the cartridges. The eluents were evaporated using N_2 gas from the Turbovap instrument for 45 min at 20 psi. Upon evaporation, the samples were reconstituted with 100 µL of the initial mobile phase composition of 10% of 0.1% formic acid in acetonitrile (mobile phase B) with 0.1% formic acid in UHP water (mobile phase A). The samples were then vortexed for 10 s and transferred to the LC-MS vials for analysis.

The quality control (QC) samples were prepared in duplicates at 15 ng/L (75 µL of 0.01 µg/mL QC working solution) and 800 ng/L (40 µL of 1 µg/mL QC working solution) in 50 mL of UHP water. An aliquot of 50 µL of internal standard was added to each quality control sample after which the samples were filtered. After the filtration process, 250 µL of hydrochloric acid were added. The QC samples were vortexed for 10 s and followed the same SPE procedure as the one previously described for the wastewater samples.

Authentic wastewater samples

Authentic wastewater samples were collected from wastewater treatment plants (WWTP) from four municipal boroughs of New York City (Figure 2), namely Manhattan (North River and

Newtown Creek-Manhattan pool), The Bronx (Hunts Point), Brooklyn/Queens (Newtown Creek -Brooklyn/Queens pool) and Queens (Tallman Island and Jamaica). The characteristics of each WWTP are summarized in Table 3.

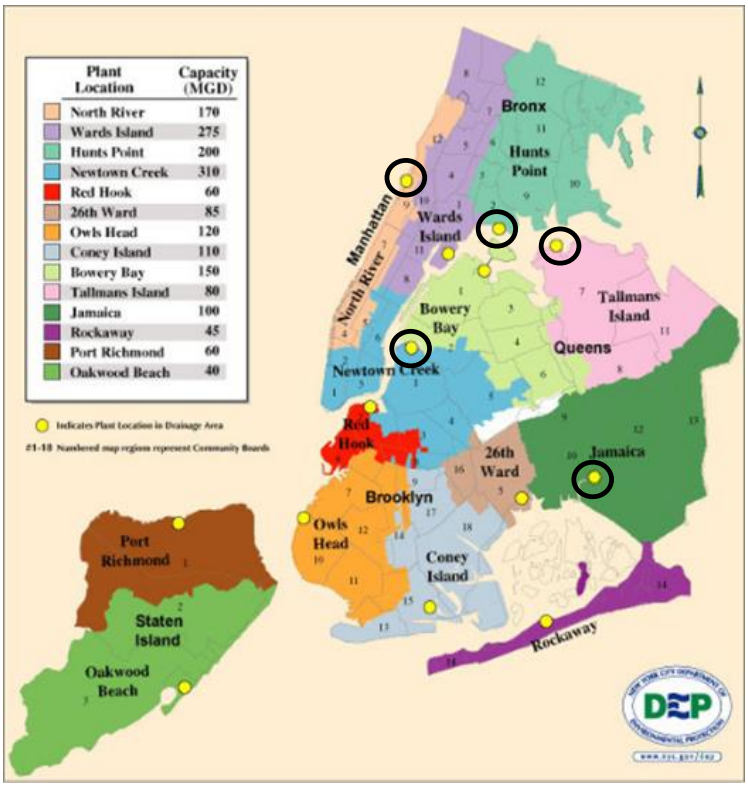

Figure 2. A map of the various wastewater plant locations and their capacity in New York City. Circled in black are the wastewater plants included in this study. Information provided by the New York City Department of Environmental Protection (DEP) website (www.nyc.gov/dep).

Table 3. The capacities, populations and areas covered by the 6 wastewater treatment plants (WWTPs) in the study. MGD represents million gallons per day.

WWTPs	Capacity (MGD)	Population	Area Covered
North River	170	588,772	Manhattan
Newtown Creek	210 (120 in Manhattan, 90 in Brooklyn/Queens	1,068,012	Manhattan and Brooklyn/Queens
Hunts Point	200	684,569	Bronx
Jamaica	80	728,123	South section of Queens
Tallman	100	410,812	Northeast section of Queens

The type of influent in all these plants is primarily urban residential. These data were retrieved from the New York City Department of Environmental Protection (DEP) website (www.nyc.gov/dep). The choice of these wastewater treatment plants was based on the size of the population they serve and study logistics.

According to 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. The collection of 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^{TM} Wide-mouth HDPE 250 mL bottles between 8 am to 11 am on the collection days. This collection window was based on the DEP personnel's availability and operating schedule. Sampling was done on days before and

after major holidays in 2016 including Memorial Day (May 27th, May 31st), 4th of July (July 1st, July 5th), Labor Day (September 2nd, September 6th) and New Year's (December 30th and 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℃ until the day of analysis.

Method validation

To validate the method, standard practices recommended by the Scientific Working Group for Forensic Toxicology (SWGTOX) were used as a reference (SWGTOX 2013). The parameters evaluated were: linearity, limit of detection (LOD) and quantification (LOQ), carryover, imprecision, accuracy, matrix effect, extraction and process efficiency.

The linearity of the study was assessed over five days. The linearity was considered as desirable if the coefficient of determination was ≥ 0.99 and the residuals were within ±20%. LOD and LOQ were determined by analyzing the lowest concentration of the analyte that could be identified and quantified by the analytical method. The LOD and LOQ were selected based on ideal peak shape, a retention time within ± 0.2 min the average of the calibrators, the ideal ion ratio being within ±20% the average of the calibrators, and a signal to noise ratio which was greater than 3 for LOD and greater than 10 for LOQ.

For the carryover procedure, a blank sample was directly injected after the highest calibrator at 1000 ng/L was analyzed. Carryover did not occur if the concentration of the analyte in the blank was lower than the LOD.

The intra-day imprecision and accuracy study involved the analysis of 10 QC samples which were all analyzed in the same day; 5 had a concentration of 15 ng/L and the other 5 had a concentration of 800 ng/L. For the inter-day imprecision and accuracy study, the QC samples at

concentrations 15 ng/L and 800 ng/L were analyzed on five different days. The intra- and inter-day imprecision was determined using the coefficient of variation (CV) of the measured values and expected to be less than 20%. The accuracy was calculated as a percentage of the target concentration and was required to be within 80 –120%. The intra- and inter- day accuracy was calculated as the mean QC concentrations x 100/QC target concentration.

The matrix effect, extraction efficiency, and process efficiency were measured by comparing three sets of samples using the deuterated analogs as surrogate analytes. Set 1 had 3 neat samples containing 50 µL of the internal standard mixture at 0.1 µg/mL concentration in mobile phase; Set 2 had 5 authentic wastewater samples, which were spiked with 50 µL of internal standard mixture at 0.1 µg/mL concentration prior to the SPE procedure; and Set 3 had 10 authentic wastewater samples, which were spiked with 50 µL internal standard mixture at 0.1 µg/mL concentration added after the SPE procedure and prior to evaporation. The matrix effect was determined by comparing the average peak areas of Set 1 and Set 2. The matrix effect for the different analytes was calculated by the following formula:

*Matrix Effect= [100 * (Average Peak Area Set 1/ Average Peak Area Set 2)] - 100*

The matrix effect (ion enhancement or ion suppression) had to be within 25%, and the variation among different sources had to be less than 20%. The extraction efficiency was determined by comparing Set 2 and Set 3 and the process efficiency was determined by comparing Set 1 and Set 2. Below are the formulas for the calculations of extraction efficiency and process efficiency.

*Extraction Efficiency= (Average Peak Areas Set 2/ Average Peak Areas Set 3) * 100*

*Process Efficiency= (Average Peak Areas Set 2/ Average Peak Areas Set 1) * 100*

Results

Method validation

The linear range for the calibrators was from 5 or 10 to 1000 ng/L. Figure 3 shows the total ion chromatogram of the calibrator 50 ng/L. The LOD was 1 ng/L for all analytes except lorazepam, which had a LOD of 5 ng/L. The LOQ was 5 ng/L for all analytes except lorazepam, which had a LOQ of 10 ng/L.

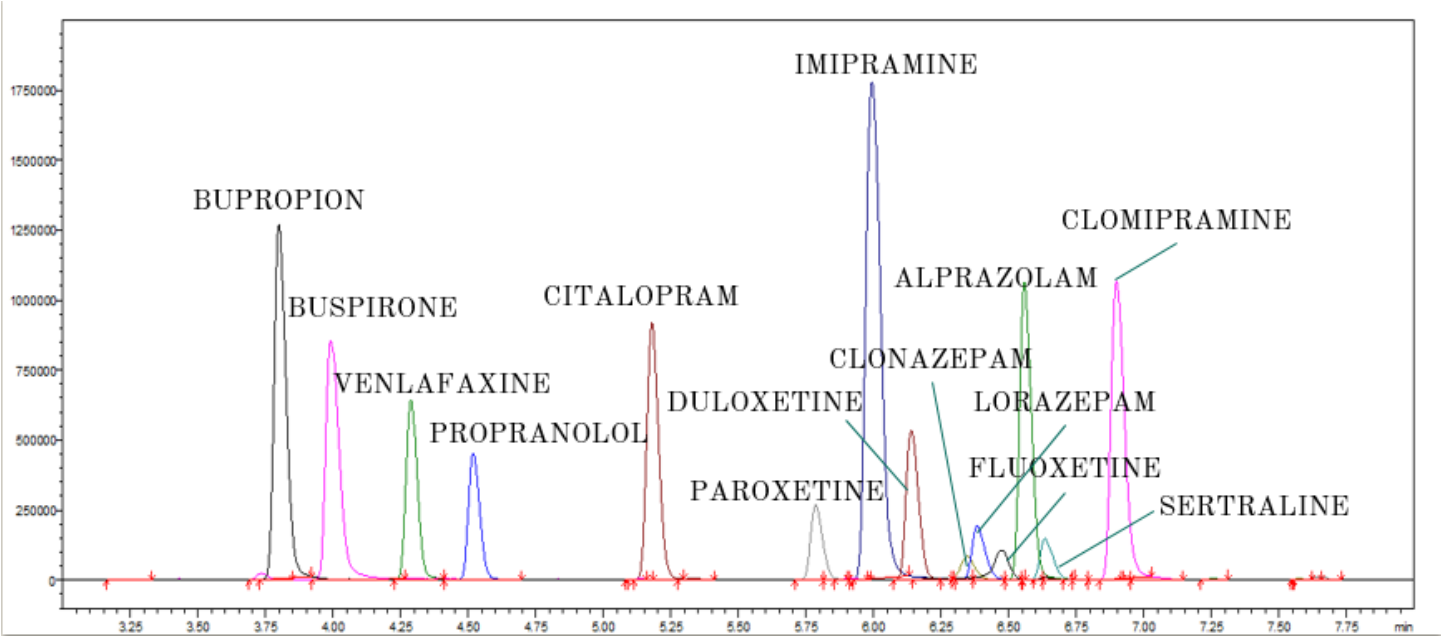

Figure 3. Total Ion Chromatogram (TIC) of the MRM transitions of all the analytes included in the study from a 50 ng/L extracted calibrator.

The carryover validation parameter was evaluated by reinjecting the blank after the highest calibrator at 1000 ng/L was injected into the LC-MSMS instrument. The concentrations for the carryover samples were all lower than the LOD values, meaning no carryover was present.

The intra-day QC samples at a concentration of 15 ng/L had an imprecision ranging from 1.7 % to 35.6% and an accuracy ranging from 86.8% to 118.8%. The intra-day QC samples which had a concentration of 800 ng/L had an imprecision ranging from 2.8% to 14.5% and an accuracy ranging from 86.8% to 102.7%. The inter-day QC samples at a concentration of 15 ng/L had an imprecision ranging from 7.8% to 42.8% and an accuracy ranging from 94.0% to 118.8%. The inter-day QC samples at a concentration of 800 ng/L had an imprecision ranging from 2.9% to 33.2% and an accuracy ranging from 76.91% to 107.4%. These results are summarized in Tables 4 and 5. Bupropion, fluoxetine, lorazepam and sertraline, showed imprecision values outside the recommended range (28-42.8%).

Table 4. Summary of the results for the imprecision in the intra-day and inter-day study at QC 15 ng/L and QC 800 ng/L (n=5).

Analyte	Intra-day		Inter-day	
	QC 15 ng/L	QC 800 ng/L	QC 15 ng/L	QC 800 ng/L
Bupropion	3.3	5.3	9.3	33.2
Buspirone	3.0	2.9	9.3	6.8
Venlafaxine	4.0	4.1	9.9	10.5
Propranolol	4.8	4.8	9.3	6.4
Citalopram	6.9	2.8	21.1	8.6
Paroxetine	9.6	4.8	10.1	9.0
Imipramine	4.7	4.4	14.1	7.6
Clonazepam	3.9	5.4	7.8	4.1
Duloxetine	6.0	4.8	10.8	9.4
Fluoxetine	3.5	4.9	37.7	2.9
Lorazepam	35.6	6.8	42.8	9.2

Alprazolam	12.9	14.5	17.7	7.8
Sertraline	15.9	4.5	28.0	6.4
Clomipramine	1.7	5.1	9.9	5.1

Table 5. Summary of the results for accuracy in the intra-day and inter-day study at QC 15 ng/L and QC 800 ng/L (n=5).

Analyte	Intra-day		Inter-day	
	QC 15 ng/L	QC 800 ng/L	QC 15 ng/L	QC 800 ng/L
Bupropion	96.9	102.5	118.8	107.4
Buspirone	102.1	97.5	106.0	99.9
Venlafaxine	99.4	102.7	106.3	104.0
Propranolol	97.4	90.1	104.6	99.9
Citalopram	92.5	91.6	102.2	95.5
Paroxetine	91.0	97.4	100.6	102.3
Imipramine	96.1	100.9	105.9	103.7
Clonazepam	86.8	88.2	104.5	95.0
Duloxetine	99.2	96.6	99.4	100.5
Fluoxetine	89.3	93.8	104.8	97.8
Lorazepam	110.3	86.8	94.0	76.9
Alprazolam	109.8	94.7	103.1	103.2
Sertraline	91.3	98.5	117.2	98.5
Clomipramine	97.6	97.1	104.2	100.13

Ion suppression was detected for all the analytes in the method, and these matrix effects ranged from -30.6 to -99.57%. The CV among the different sources (n=10) was within the desired range

for the following drugs: bupropion-d_9, buspirone-d_8, venlafaxine-d_6, paroxetine-d_6, imipramine-d_5, clonazepam-d_4, duloxetine-d_3, fluoxetine-d_6, and lorazepam-d_4. However, propranolol-d_7 (45.1%), alprazolam-d_5 (31.4%), sertraline-d_3 (39.2%), clomipramine-d_3 (37.3%), and specially citalopram-d_6 (96%), were outside of the desired range. Due to this issue, the determination of citalopram was considered "semi-quantitative". To compensate for these effects, the deuterated analogs were employed as internal standards for all compounds in the method. The extraction efficiency was above 50% for all compounds, except for paroxetine (35.9%) and duloxetine (36.4%). Due to the high ion suppression effects, the process efficiency ranged from 0.5% to 59.6%. These results are summarized in Table 6.

Table 6. Summary of the results for the matrix effect, extraction efficiency and process efficiency.

Internal Standard	Extraction Efficiency	Matrix Effect	CV (%, n=10)	Process Efficiency
Bupropion-d_9	112.5	-99.6	28.2	0.5
Buspirone-d_8	73.7	-89.7	29.6	7.6
Venlafaxine-d_6	77.7	-78.1	20	17
Propranolol-d_7	96.7	-94.1	45.1	5.7
Citalopram-d_6	53.3	-95.3	96	2.5
Paroxetine-d_6	35.8	-90.7	22.2	3.3
Imipramine-d_5	64.1	-91.2	21.3	5.6
Clonazepam-d_4	72.8	-43.2	9.8	41.4
Duloxetine-d_3	36.4	-91.8	22	3
Fluoxetine-d_6	56.1	-90.2	21	5.5
Lorazepam-d_4	85.9	-30.6	8.9	59.6

Alprazolam-d_5	79.1	-52.8	31.4	37.4
Sertraline-d_3	61.5	-93.3	39.2	4.1
Clomipramine-d_3	66.1	-94.9	37.3	3.4

Authentic wastewater samples

All the drugs were detected in at least one out of the six locations with the exceptions of lorazepam and duloxetine, which were not detected in any of the WWTP. Regarding the drugs employed in the treatment of anxiety, the most prevalent was propranolol, followed by buspirone and alprazolam. Propranolol was detected in 25 samples with a minimum concentration of 14.5 ng/L detected at Tallman (September 2nd) and a maximum concentration of 97.1 ng/L detected at the Newton Creek Brooklyn/Queens (January 3rd) site. Buspirone was detected in 3 samples with a minimum concentration of 5.4 ng/L detected at Hunts Point (September 6th) and a maximum of 19.4 ng/L also detected at Hunts Point (May 27th). Alprazolam was also detected in 3 samples with a minimum concentration of 5.1 ng/L at Hunts Point (May 27th), and a maximum of 6.1 ng/L at Hunts Point (September 6th) and Newtown Creek Manhattan (January 3rd). Clonazepam was detected in one sample with a concentration of 6 ng/L at the Newtown Creek Brooklyn/Queens (July 1st) location. Tallman and North River service the lowest amount of people having populations of 410,812 and 588,772, respectively. Newtown Creek Manhattan and Newtown Creek Brooklyn/Queens service a population of 1,068,012 combined. Hunts Point service a population of 684,589 and Jamaica service a population of 728,123.

With regard to the antidepressants, the most prevalent was venlafaxine, followed by citalopram, fluoxetine, paroxetine, bupropion and sertraline. Venlafaxine was detected in 31 out of the 48 samples. Venlafaxine had a minimum concentration of 46.5 ng/L detected at Hunts Point (July

5th) and a maximum concentration of 298.5 ng/L detected at North River (December 30th). Citalopram was detected in 26 samples with a minimum concentration of 31.4 ng/L detected at Tallman (July 5th) and a maximum concentration of 152.8 ng/L detected at the Newtown Creek Brooklyn/Queens (December 30th). Fluoxetine was detected in 11 samples with a minimum concentration of 7.4 ng/L detected at Hunts Point (July 5th) and a maximum concentration of 35.2 ng/L at Jamaica (July 1st). Paroxetine was detected in only 4 locations with a minimum concentration of 5.4 ng/L detected at Jamaica (July 5th) and a maximum concentration of 7.5 ng/L also at Jamaica (December 30th). Bupropion was found in 3 samples with a minimum concentration of 16.8 ng/L found at Hunts Point (January 3rd) and a maximum concentration of 149.8 ng/L found at Jamaica (December 30th). Sertraline was also detected in 3 samples with a minimum of 16 ng/L at Jamaica (May 27th) and a maximum of 55.6 ng/L at Hunts Point (May 27th). Imipramine and clomipramine were only detected in one sample at 7.4 ng/L in Hunts Point (September 2nd) and 20.8 ng/L in Jamaica (July 1st), respectively. Table 7 summarizes these results for the authentic samples.

Table 7. Range of anxiolytic and antidepressant drugs' concentrations in the authentic wastewater samples (n=48) in New York City in 2016.

ANALYTE	N	Min Concentration (ng/L)	Max Concentration (ng/L)
Venlafaxine	31	46.5	298.5
Citalopram	26	31.4	152.8
Propranolol	25	14.5	97.1
Fluoxetine	11	7.4	35.2
Paroxetine	4	5.4	7.5
Alprazolam	3	5.1	6.1
Bupropion	3	16.8	149.8
Buspirone	3	5.4	19.4
Sertraline	3	16.0	55.6
Clomipramine	1	20.8	
Clonazepam	1	6.0	
Imipramine	1	7.4	
Duloxetine	0	ND	
Lorazepam	0	ND	

A chromatogram from a wastewater sample collected at Hunts Point on July 1st can be seen in Figure 4, where 4 out of the 14 drugs were present. Venlafaxine had the highest concentration at 102.4 ng/L, followed by citalopram which had a concentration of 79.7 ng/L, propranolol with a concentration of 36.4 ng/L, and lastly fluoxetine with a concentration of 15.3 ng/L.

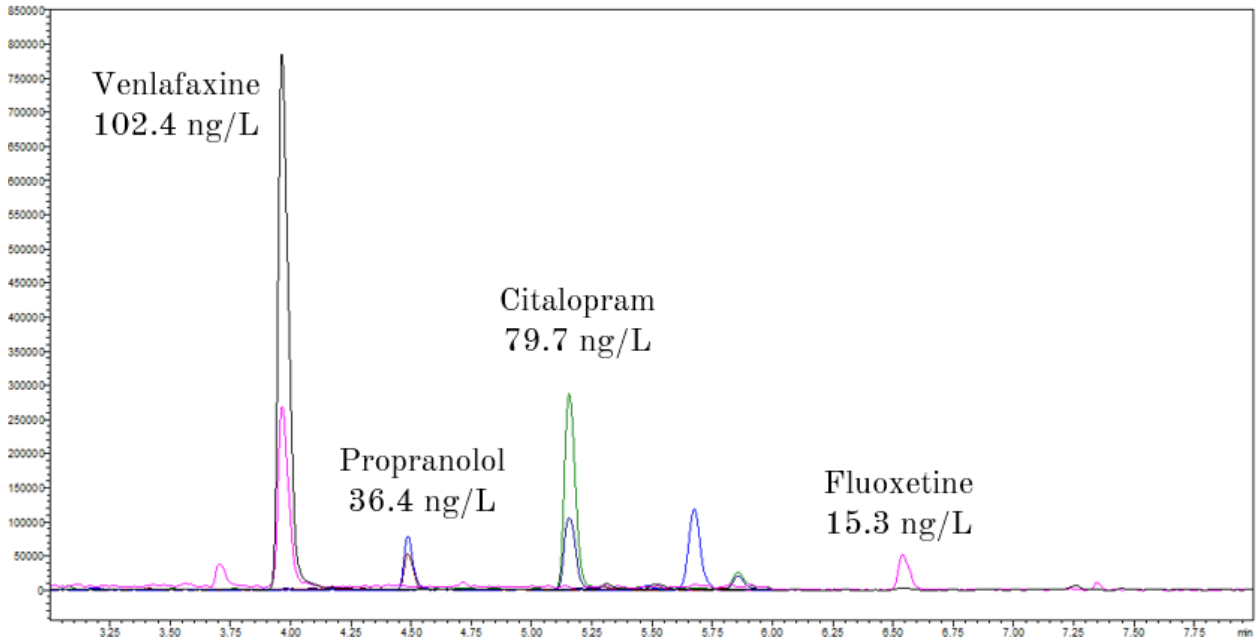

Figure 4. MRM chromatogram of the results from the Hunts Point July 1st sample containing venlafaxine, citalopram, propranolol and fluoxetine.

Antidepressants were found in more samples before and after New Years' Eve and prior to Independence Day (12 samples) and the least in samples after Labor Day (7 samples). Anxiolytic drugs were found in more samples prior to Memorial Day (5 samples) and the least in samples after Independence Day (3 samples). The location with the highest number of samples positive for antidepressants was Hunts Point (29 samples), whereas the location with the lowest number of samples positive for antidepressants was North River (3 samples). The location which had the highest number of samples positive for anxiolytic drugs was also Hunts Point (13 samples) and the location with the lowest number of samples positive for anxiolytic drugs was Newtown Creek-Manhattan (1 sample).

The dates which had the highest number of samples positive for antidepressants and anxiolytic drugs combined were 5/27 (prior to Memorial Day), 7/1 (prior to Independence Day), 12/30 (prior to New Year's Day) and 1/3 (after New Year's Day), where the total was 16 samples. The amount of samples positive for antidepressants tend to increase (by 4 samples, maximum) before every holiday (Memorial Day, Independence Day, and Labor Day) except New Year's Day (amount of samples were the same, n=12, before and after New Year's Day) whereas the amount of samples positive for anxiolytic drugs relatively remained the same or changed by one value (3-5 samples positive for anxiolytic drugs throughout the year). Table 8 and Figure 5 summarizes the results for antidepressants and anxiolytic drugs before and after the major holidays. The maximum and minimum concentrations for the antidepressants and the anxiolytic drugs are also shown in Table 8.

Table 8. Summary of the results based on drug type at different times of the year with the maximum and minimum concentrations.

Time of Year	Antidepressants	Max and Min (ng/L)	Anxiolytics	Max and Min (ng/L)
Prior to Memorial Day (5/27)	11	172.6 (Venlafaxine, NC-BKLYN/QNS)-6.7 (Paroxetine, Hunts Point)	5	19.4 (Buspirone, Hunts Point)-5.1 (Alprazolam, Hunts Point)
After Memorial Day (5/31)	8	142.9 (Venlafaxine, NC-BKLYN/QNS)-46.8 (Citalopram, Tallman)	4	43.9 (Propranolol, Jamaica)-23.9 (Propranolol, Tallman)
Prior To Independence	12	209.7 (Venlafaxine, North	4	46.5 (Propranolol,

Day (7/1)		River)- 15.3 (Fluoxetine, Hunts Point)		Jamaica)- 6 (Clonazepam, NC-BKLYN/QNS)
After Independence Day (7/5)	8	104.9 (Venlafaxine, Jamaica)- 5.4 (Paroxetine, Jamaica)	3	38.2 (Propranolol, Jamaica)- 14.7 (Propranolol, Tallman)
Prior to Labor Day (9/2)	8	119.5 (Venlafaxine, Tallman)- 7.4 (Imipramine, Hunts Point)	4	41.4 (Propranolol, Jamaica)- 13.8 (Buspirone, Hunts Point)-
After Labor Day (9/6)	7	146.7 (Venlafaxine, NC-Manhattan)- 7.3 (Paroxetine, Hunts Point)	4	56.4 (Propranolol, Jamaica)- 5.4 (Buspirone, Hunts Point)
Prior to New Year's Day (12/30)	12	194.2 (Venlafaxine, NC-Manhattan)- 7.5 (Paroxetine, Jamaica)	4	56.6 (Propranolol, Jamaica)- 15.4 (Propranolol, Tallman)
After New Year's Day (1 / 3)	12	220.5 (Venlafaxine, North River)- 12.5 (Fluoxetine, Hunts Point)	4	169.3 (Alprazolam, NC-Manhattan)- 28.3 (Propranolol, Jamaica)

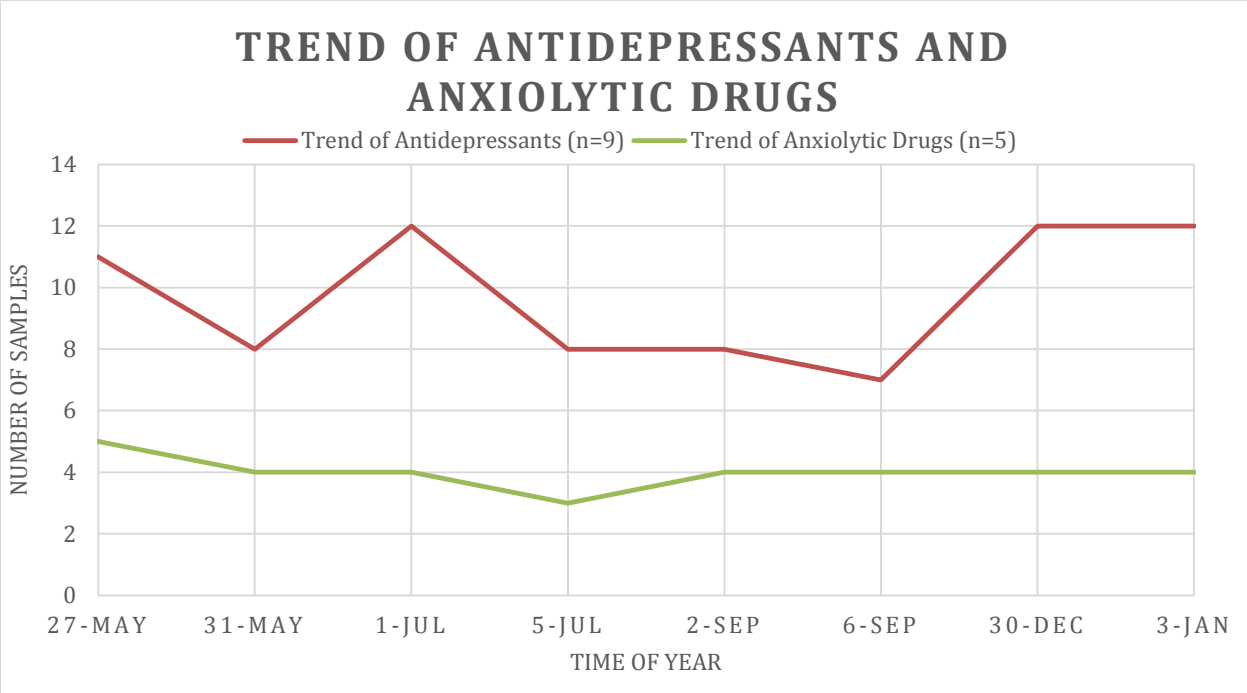

Figure 5. This chart shows the trends of the number of samples positive for antidepressant and anxiolytic drugs in wastewater in New York City over 2016. Over the course of the study we focused on 9 antidepressants and 5 anxiolytic drugs.

Discussion

A method was developed and validated to simultaneously quantitatively and qualitatively analyze 14 antidepressants and anxiolytic drugs in wastewater samples. An extensive literature review of the previously published methodologies for the determination of anxiolytics and antidepressants in wastewater was conducted (Asimakopoulos, et al. 2017; Ferrer & Thurman, 2012; Ferrey, et al. 2015; Gurke, et al. 2015; Loos, et al. 2013; Papageorgiou, et al. 2016; Subedi, et al. 2013; Vulliet, et al. 2011; Wu, et al. 2015; Yuan, et al. 2013). In these previous publications, the number of antidepressants and anxiolytic drugs determined were less than in the current methodology; 10 (Asimakopoulos, et al. 2017), 9 (Loos, et al. 2013), 7 (Subedi, et al.

2013), 6 (Ferrer & Thurman, 2012), 5 (Ferrey, et al. 2015; Gurke, et al. 2015; Yuan, et al. 2013), 4 (Papageorgiou, et al. 2016) and 2 (Wu, et al. 2013).

For the method developed in this study, 50 mL of wastewater sample was analyzed, achieving a LOQ of 5 ng/L for all analytes with the exception of lorazepam at 10 ng/L. This sensitivity is within the range of the previously published methodology or better. The study done by Asimakopoulos et al. also analyzes 50 mL of wastewater and had an LOQ range of 0.1 ng/L to 20 ng/L, where the LOQ for lorazepam was the highest at 20 ng/L (Asimakopoulos, et al. 2017). Researchers Ferrer & Thurman chose to use 100 mL of wastewater sample; their LOD had ranged from 5 to 500 ng/L (Ferrer & Thurman, 2012). Papageorgiou et al. employed 150 mL of wastewater sample, with LOQs from 4.9 to 532 ng/L (Papageorgiou, et al. 2016), and Wu et al. analyzed 200 mL of wastewater and achieved LOQs from 0.1 ng/L to 4.5 ng/L (Wu, et al. 2015). Loos et al. Vulliet et al. and Yuan et al. chose to analyze 500 mL of wastewater sample (Loos, et al. 2013; Vulliet, et al. 2011; Yuan, et al. 2013), while Ferrey et al. used 1 L of wastewater sample (Ferrey, et al. 2015). Loos et al. had LOQs ranging from 0.1- 10 ng/L, Vulliet et al. had LOQs ranging from 2- 85 ng/L (Vulliet, et al. 2011) and Yuan et al. had LOQs with a range of 20- 80 ng/L for lorazepam and paroxetine and a range of 1- 16 ng/L for alprazolam, clomipramine, fluoxetine and sertraline (Yuan, et al. 2013). In the Subedi et al. study, a mass of 1 g of freeze-dried sludge samples was used; the LOQs for antidepressant and anxiolytic drugs ranged from 0.1 ng/L to 5 ng/L, where venlafaxine had an LOQ of 0.1 ng/L and alprazolam had an LOQ of 5 ng/L in sludge (Subedi, et al. 2013).

In the current procedure, we acidified the wastewater samples with 250 µL of HCl after filtration to avoid losses in the filtration step. In the Ferrey et al. study, they acidified the wastewater with HCl after they filtrated the wastewater samples (Ferrey, et al. 2015). In the study done by Loos et

al. they acidified the samples with sulfuric acid after the filtration step (Loos, et al. 2013). The Papageorgiou et al. research group also added 5% Na_2EDTA acid after filtration of the wastewater samples (Papageorgiou, et al.2016). However, some researchers used a basic solution to increase the pH of the wastewater samples after filtration (Wu, et al. 2015; Yuan, et al. 2013) or did not filtrate the wastewater samples at all (Asimakopoulos, et al. 2017; Ferrer & Thurman, 2012; Subedi, et al. 2013). The Yuan et al. research group used an ammonia solution to increase the pH of the wastewater sample to 7 (Yuan, et al. 2013). The Wu et al. research group used a solution containing ammonia and methanol (1%, v/v) in order to increase the pH of the wastewater to 8 (Wu, et al. 2015).

Each study that examined both antidepressants and anxiolytic drugs utilized SPE to extract the analytes (Asimakopoulos, et al. 2017; Ferrer & Thurman, 2012; Ferrey, et al. 2015; Gurke, et al. 2015; Loos, et al. 2013; Papageorgiou, et al. 2016; Subedi, et al. 2013; Vulliet, et al. 2011; Wu, et al. 2015; Yuan, et al. 2013). In our study, Strata XC (cation- exchange) cartridges were used since most of the drugs in the study were basic and using a Strata X (reversed-phase) cartridge did not have peak intensities as high as the cation-exchange cartridge procedure. Several studies used reversed phase SPE cartridges to analyze pharmaceuticals in wastewater (Ferrer & Thurman, 2012; Ferrey, et al. 2015; Gurke, et al. 2015; Loos, et al. 2013; Papageorgiou, et al. 2016; Subedi, et al. 2013; Vulliet, et al. 2011; Wu, et al. 2015; Yuan, et al. 2013). Asimakopoulos et al. used both reversed phase and cation exchange cartridges (Asimakopoulos, et al. 2017).

Our method using liquid chromatography tandem mass spectrometry (triple quadrupole) is a common approach, as most researchers have found this analytical instrument to be successful in helping them obtain their findings in wastewater samples (Asimakopoulos, et al. 2017; Batt, et

al. 2008; Ferrey, et al. 2015; Gurke, et al. 2015; Jurado, et al. 2012; Loos, et al. 2013; Paíga, et Al. 2016; Papageorgiou, et al. 2016; Pereira, et al, 2016; Racamonde, et al. 2014; Vulliet, et al. 2011; Wu, et al. 2015; Yuan, et al. 2013). Some other researchers preferred to use liquid chromatography coupled to a Time of Flight (TOF) or Ion Trap (IT) instruments (Al Aukidy, et al. 2012, Ferrer & Thurman, 2012). In order to confirm the identity of the detected analytes, 2 MRM transitions have to be monitored, as we did in the present method as well as previous authors (Ferrey, et al, 2015; Gurke, et al. 2015; Subedi, et al, 2013; Vulliet et al. 2011; Yuan, et al. 2013). In the study done by Ferrer & Thurman, they monitored up to 4 MRM transitions for some of their analytes (Ferrer & Thurman, 2012). However, Asimakopoulos et al. along with Loos et al. monitored only one MRM transition to identify target analytes (Asimakopoulos, et al. 2017; Loos, et al. 2013).

Many challenges arose while developing the method, such as high ion suppression of the target analytes. To compensate for these effects, the deuterated analogs of the analytes were employed as internal standards. However, propranolol-d_7 (45.1%), citalopram-d_6 (96%), alprazolam-d_5 (31.4%), sertraline-d_3 (39.2%), and clomipramine-d_3 (37.3%) experienced unfavorable CV values during the matrix effect study, which may have compromised the quantitative results of the authentic specimens. Since wastewater samples are extremely complex, our method was optimized as much as possible to remove any unwanted interferences within the sample. For the future, it would be beneficial to minimize the ion suppression effects detected. Our SPE method and LC-MSMS settings may need further modification to help reduce ion suppression, especially since the ion suppression happens with ESI. The observed ion suppression in the matrix effect study means more research needs to be done to help reduce the matrix effects, and therefore improve the applicability of the current methodology.

The most common drugs detected during this study were venlafaxine followed by propranolol and citalopram. Venlafaxine was present at all six locations, while duloxetine and lorazepam were not detected at any of the sites. The lack of duloxetine and lorazepam in the wastewater samples may be due to extremely low yield or ion suppression from the matrix effect in the method. Throughout the optimization process of the study, duloxetine and lorazepam always had significantly lower peak areas than the other 12 drugs.

Hunts Point had the most drug presence in the wastewater as 10 out of the 14 drugs in the study were present at this site. The drugs present in the Hunts Point samples were bupropion, buspirone, venlafaxine, propranolol, citalopram, paroxetine, imipramine, fluoxetine, alprazolam and sertraline. Jamaica had the second highest number of positive samples as 8 out of the 14 drugs were present at this site. Jamaica had the following drugs in the wastewater samples: bupropion, venlafaxine, propranolol, citalopram, paroxetine, fluoxetine and clomipramine. Newtown Creek Brooklyn/Queens location only had 5 drugs present which were venlafaxine, propranolol, citalopram, clonazepam, and fluoxetine. Tallman had three analytes: venlafaxine, propranolol and citalopram present. Newtown Creek Manhattan location and the North River location both had only two drugs present. Newtown Creek Manhattan had venlafaxine and alprazolam present, while North River had venlafaxine and citalopram present. Tallman and North River serviced the lowest populations compared to the other plants, however significant drug concentrations were found in Tallman on 5 out of the 8 collection dates. For North River, significant drug concentrations were found on 2 out of the 8 collection dates. Hunts Point (population size 728,123) and Jamaica (population size 684,589) had significant drug concentrations found in samples collected on all 8 collection days. Newtown Creek Manhattan and Brooklyn/Queens plants combined had significant drug concentrations on 6 out of 8

collection days even though this plant services a population of 1,068,012. Population size did not play a role in the number and concentrations of antidepressants and anxiolytic drugs present in a sample. Some locations such as Hunts Point and Jamaica had a higher presence of antidepressant and anxiolytic drugs compared to Newtown Creek Manhattan and Brooklyn/Queens combined, even though Newtown Creek Manhattan and Brooklyn/Queens service a population size 31.8% larger than Hunts Point and 35.9% larger than Jamaica.

Our results showed more samples had antidepressants prior to the US holidays compared to after the holidays. In contrast, there was not much of a variation in the amount of samples which had anxiolytic drugs present over the course of the year. The amount of anxiolytic analytes in the wastewater samples ranged from 3 to 5 samples. Most analytes had a wide concentration range (venlafaxine, propranolol, citalopram, and bupropion), while alprazolam and paroxetine had a really small concentration range of 5.1-6.1 ng/L and 5.4-7.6 ng/L respectively. This could be due to the difference in the number of samples which had these analytes since only 3 samples had alprazolam, and 4 samples had paroxetine present in it out of the 48 samples. Bupropion was also found in 3 samples, but there was just a wider range of concentrations; bupropion was only found in Hunts Point and Jamaica.

In the Ferrey et al. study, they looked for illicit drugs and pharmaceuticals in lakes in Minnesota (Ferrey, et al. 2015). Out of the antidepressant and anxiolytic drugs relevant to our study, the Ferrey et al. research group only detected alprazolam and fluoxetine at low concentrations below 5 ng/L in the lakes (25 and 6%, respectively). In the study done by Ferrer & Thurman, drinking water, groundwater, surface water, and wastewater were collected from various locations in the US (Ferrer & Thurman, 2012). The following pharmaceuticals were found in the wastewater samples: bupropion, citalopram, fluoxetine, propranolol, and venlafaxine (Ferrer & Thurman,

2012). Propranolol was present in 88% of the wastewater samples with and average concentration of 53 ng/L; citalopram was detected in 79% of the wastewater samples with an average concentration of 85 ng/L; venlafaxine was found in 78% of the wastewater samples with an average concentration of 310 ng/L; bupropion was detected in 68% of the wastewater samples with an average concentration of 140 ng/L; and fluoxetine was detected in 25% of the wastewater samples with an average concentration of 65 ng/L (Ferrer & Thurman, 2012). The analytes detected in both of these studies were also present in the wastewater samples from our study; however, the amount of alprazolam and fluoxetine present in the wastewater from the study done by Ferrey et al. was a lot less than our LOQ value (5 ng/L). In the Ferrer and Thurman study, the average concentration for bupropion and venlafaxine were very close in value to our maximum concentrations for these analytes which were 149.8 ng/L and 298.5 ng/L respectively (Ferrer & Thurman, 2012). For citalopram and propranolol, the average concentrations detected by Ferrer & Thurman were within our minimum and maximum concentrations for those analytes, which were 31.4-152.8 ng/L and 14.5-97.1 ng/L, respectively (Ferrer & Thurman, 2012). Our fluoxetine concentration range was lower than the average detected by the Ferrer & Thurman research article, as our fluoxetine wastewater concentrations ranged from 7.4-35.2 ng/L (Ferrer & Thurman, 2012).

According to the New York State Office of Mental Health, fluoxetine (Prozac), sertraline (Zoloft), paroxetine (Paxil), and citalopram (Celexa) are some of the "most commonly prescribed" SSRIs for depression (New York State Office of Mental Health, 2018). Our results show that there is a wide range of antidepressant drug usage within the 4 boroughs and eight out of the 14 analytes were mentioned by the New York State Office of Mental Health (New York State Office of Mental Health, 2018). Tricyclic antidepressants have not been prescribed as much

lately due to many dangers and "potential side effects" they may cause (New York State Office of Mental Health, 2018). This was also seen in our results as imipramine was only found in 1 sample at a concentration of 7.4 ng/L.

Conclusions

Using SPE and LC-MSMS we successfully analyzed 14 antidepressants and anxiolytic drugs in wastewater samples in New York City. The LC-MSMS analytical instrument was selective and sensitive enough to detect target analytes that had concentrations as low as 5 ng/L. Even though most validation parameters were within the desired range (imprecision, accuracy, LOD, LOQ, linearity, process efficiency, extraction efficiency) ion suppression was an issue for a number of deuterated analytes in the study namely propranolol-d_7, alprazolam-d_5, $_{\text{sertraline}}$-d_3, clomipramine-d_3 and, especially, citalopram-d_6 (95.96%). The most common drugs detected during this study were venlafaxine followed by propranolol and citalopram. Hunts Point had the most drug presence in the wastewater as 10 out of the 14 drugs in the study were present at this site. Wastewater analysis was proven to be an effective tool to examine drug usage in a large population such as the one present in New York City. In this study, we were able to pinpoint the desired location and collect data about populations from 4 New York City boroughs.

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