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TECHNICAL NOTE

TOXICOLOGY

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Temporal Analysis of the Cocaine Metabolite Benzoylecgonine in Wastewater to Estimate Community Drug Use*

ABSTRACT: Indirect estimation methods of cocaine consumption may not reflect the real extent of cocaine use. Another approach is sewage epidemiology. This direct approach is based on analysis of a stable cocaine metabolite, benzoylecgonine (BE), in wastewater. Influent to the Lubbock (Texas) Water Reclamation Plant was sampled twice a week to assess weekly variations in estimates of cocaine consumption over a 5-month period. BE was extracted from influent wastewater samples using solid phase extraction and analyzed using gas chromatography/mass spectrometry. Measured concentrations of BE were converted to cocaine equivalents; the estimated average daily consumption of cocaine during the study period was 1152 ± 147 g. Based on BE concentrations and sewage epidemiology, higher cocaine consumption was observed on weekends compared to weekdays ($p < 0.0003$). This method was effective in monitoring BE in wastewater and could be used to complement survey data in estimating cocaine use at a local level.

KEYWORDS: forensic science, cocaine, benzoylecgonine, sewage epidemiology, wastewater, drug use, gas chromatography/mass spectrometry

Abuse of illicit drugs is a major problem in society; it leads to high morbidity and mortality and is also responsible for many socioeconomic problems (1). Trends in drug use are estimated indirectly from consumer interviews, population surveys, individual medical records, and crime statistics (2). These indicators typically provide frequency information on drug use but may not realistically reflect actual drug use at a local level (3,4); human behavior is unpredictable and unreliable, thus use estimates obtained by interviewing known or potential users may bias these indirect approaches (4). New direct approaches could complement survey data and perhaps provide more realistic estimates of illicit drug use as well as detect changes in abuse trends (3–6). Zuccato et al. (4) proposed a new approach based on measuring urinary excreted cocaine and its metabolites in local wastewater. This approach, termed ''Sewage Epidemiology,'' has been applied in several studies investigating the occurrence of pharmaceuticals and illicit drugs and their metabolites in the aquatic environment (3,4,6,7).

Recent issues regarding water shortages (especially in arid areas) have provided the impetus for exploring wastewater recycling. Some concerns have been raised about the quality of recycled water, particularly the presence of pharmaceuticals and personal care products. The presence of pharmaceuticals (legal and illegal) in treated wastewater and/or surface water is potentially an important human and environmental health issue (8,9). Verification of the presence and form of illicit drugs in sewage and aqueous environments is also of interest from a forensic perspective. Comprehensive information on the concentrations of illicit drugs in raw sewage provides a complementary approach to survey data for estimating trends in illegal drug usage (3–5). Such information can be useful to social scientists and authorities for responding with prevention campaigns in regions of abuse. Regular monitoring can also be used to quickly evaluate the effectiveness of a drug prevention campaign (3). Recent studies (4,5) show the occurrence of several drugs (cocaine, methamphetamines, and marijuana) and their metabolites in wastewater. According to the authors, these studies have revealed short- and long-term variations in cocaine use and provided some proof that official national figures based on surveys underestimate the extent of cocaine use (4,10).

In humans, cocaine is extensively metabolized to benzoylecgonine (BE) by chemical hydrolysis and to ecgonine methyl ester (EME) by enzymatic hydrolysis (11). BE is the major metabolite of cocaine; its presence in urine confirms cocaine use (12). Only a small fraction of cocaine is excreted as parent compound, whereas *c.* 45% of a cocaine dose (on average) is excreted as BE (13,14). In urine, cocaine can only be detected up to 8 h after use, while BE and EME can be identified for more than 96 h after cocaine use (15).

In the present study, we tested the occurrence and concentrations of the cocaine metabolite BE in wastewater influent entering the Lubbock (Texas) Water Reclamation Plant (LWRP). This monitoring experiment offers insight into weekly variation of the cocaine metabolite BE in wastewater. The concentrations of BE derived from our analysis were also used to calculate cocaine equivalents deposited in the sewer system through excretion by users. The

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cocaine equivalents and wastewater daily volumes and flow rates were then used to estimate cocaine use by the local population similar to studies in Europe (3,4,6).

Materials and Methods

Reagents

All reagents and solvents used were of analytical grade. BE, deuterated benzoylecgonine (BE-d_3), and *N*-Methyl-*N*-trifluoroacetamide (MSTFA) were obtained from Sigma Aldrich (St. Louis, MO), Isotec (Miamisburg, OH), and Pierce Biotechnology (Rockford, IL), respectively. Hydrochloric acid, ammonium hydroxide, acetonitrile, and methanol were all obtained from Fisher Scientific (Fair Lawn, NJ). The Oasis® SPE MCX 60-mg cartridges were obtained from Waters (Milford, MA).

Sample Site and Collection

The LWRP was chosen as the study site. It serves the entire city of Lubbock, a population of *c.* 269,140 inhabitants (16). Wastewater is delivered to the plant through 900 miles of collection lines and 21 lift stations. Lubbock's water consumers can be characterized as residential (85%), small commercial (10%), municipal (4%), and other user classes (1%) including industrial, schools, wholesale, and irrigation. The LWRP treats *c.* 21 million gallons of wastewater per day and has an average daily flow design capacity of 31.5 million gallons. On a typical Monday, the flow rate of wastewater into the plant is 23.62 million gallons; on a typical Friday, the flow rate of wastewater into the plant is 19.71 million gallons.

Two influent water samples (1 L) were collected on Mondays and Fridays to evaluate potential differences in BE concentrations during the week. The Monday sample represented wastewater inflow from the previous weekend; the Friday sample represented wastewater inflow from the previous weekdays. All samples were collected in the morning before 10 am (CST) every other week. Samples were collected in amber glass bottles, adjusted to pH = 2 with 37% hydrochloric acid, and stored in the dark at 4°C until analysis. The pH adjustment was previously found to be necessary to prevent degradation of BE during storage (3,17). Sample collection took place from February 8, 2010 to June 4, 2010.

Sample Preparation and Extraction

Prior to extraction, all samples were filtered under vacuum with three layers of P5 filters (Whatman, Clifton, NJ) and divided into 500-mL glass bottles (preliminary laboratory experiments indicated only minimal loss of BE during the filtration process). Filtered 500 mL water samples were spiked at a constant concentration of BE-d_3 surrogate standard (5 μL of the stock). BE-d_3 was used to test the extraction efficiency from wastewater and possible ionization suppression (matrix effects) during gas chromatography/mass spectrometry (GC/MS) analysis. Water sample extraction was carried out on a Burdick & Jackson solid phase extraction (SPE) vacuum manifold (Morristown, NJ) using Oasis® MCX 60-mg SPE cartridges. The cartridges were conditioned with 3 mL of methanol and 3 mL Milli-Q (Millipore, Billerica, MA) water (>18 MΩ) at a rate of 1 mL/min. Water samples were passed through cartridges at a rate of 10 mL/min.

After SPE extraction of samples, the cartridges were washed sequentially with 2 mL Milli-Q water, 1 mL of 0.1 M hydrochloric acid, and 1 mL methanol. After washing the cartridge, the column was dried under high vacuum for 5 min. Final extracts were eluted using 5% ammonium hydroxide in methanol, collected in 6-mL glass tubes, and evaporated to dryness under a gentle stream of nitrogen.

Derivatization of BE

To determine BE (and BE-d_3) in sample extracts using GC/MS, the extracts were first derivatized to enhance the volatility of the target analytes. MSTFA was used as the derivatization reagent, forming the trimethylsilyl (TMS) derivative of BE or BE-d_3. The extracted and evaporated samples were reconstituted in 0.25 mL acetonitrile and 0.25 mL MSTFA, and then sonicated for 30 min. Derivatized samples were filtered through a 0.2 μm Millipore disk filter and transferred to amber GC vials.

Gas Chromatography/Mass Spectrometry

GC/MS analyses were performed using an Agilent (Santa Clara, CA) 6890 series GC and 5973 Mass Selective Detector equipped with a spectral library. A DB-5MS column (30 m × 0.25 mm I.D.) was used for separation. The oven temperature program was initially set at 150°C with a 3 min hold, and then increased at a rate of 15°C/min to 300°C, followed by a 10 min hold. Two microliters of sample were injected in the splitless mode. Helium was used as the carrier gas at 28.4 mL/min. The MS was set to operate under selected ion monitoring mode targeting ion masses of 82, 240, and 361 for BE and 243 and 364 for BE-d_3. MS Chemstation® software (Agilent) was used to control the GC system and for data processing. Using this analytical method, BE-TMS was easily separated from other interferences in wastewater extracts and accurately quantified using the responses of the target ions.

Quantitative Analysis and Recovery Experiments

Quantification of BE and BE-d_3 was performed with the aid of standards and five-point calibration curves (ranging from 5000 to 50,000 μg/L) for both analytes in methanol. Recovery experiments were also conducted to determine whether the target compound was consistently recovered from spiked Milli-Q water samples using the SPE extraction procedure. Five microliters of a 1 μg/μL BE-d_3 standard (in methanol) were spiked into replicated 500-mL Milli-Q water samples and extracted. Although average BE-d_3 recoveries from spiked water were 89 ± 13% (coefficient of variation = 14%), we did not adjust sample concentrations based on recovery of the BE-d_3 surrogate. All calibration curves met predetermined performance-based quality assurance criteria for accuracy, precision, and linearity. Validation of each calibration curve was determined by calculating the concentration of the analyte from each of the analyses used to generate the calibration curve. Each calibration point was 80–120% of its true value. Check standards used to evaluate the calibration curves and continuing calibration check standards were also part of each analytical batch. The calculated amount for these check standards was ±20% of the true value. Based on the procedure described above, the reporting limit for BE in wastewater was 5 μg/L.

Statistical analyses were performed using R® software version 2.10.1 (R Foundation, Vienna Austria). A paired *t*-test was conducted at the 95% confidence interval ($\alpha = 0.05$, df = 6) on mean cocaine equivalents for samples collected on Fridays (weekday) and samples collected on Mondays (weekend) to determine if the differences in cocaine equivalents were statistically significant.

Calculations and Assumptions

Although cocaine metabolism and excretion vary from person to person, on average *c.* 45% of a cocaine dose is excreted in urine as BE (4,13,14). We used this conversion and the concentration of BE in wastewater to estimate the amount of cocaine consumed. Cocaine loads (g/day) were calculated from the BE concentration in wastewater (μg/L), the wastewater influent flow rate (89.52×10^6 or 74.7×10^6 L/day for Monday or Friday, respectively), and multiplying by a factor of 2.3 (accounts for the cocaine that is excreted as BE [45%] and the molar mass ratio [0.954] between BE and cocaine) (4,14). The cocaine loads obtained were then related to the number of people served by the treatment plant (269,140). Demographic information obtained from the City of Lubbock website (16) indicated that 32.6% and 24.6% of the population was in the "young adult" age group (15–34 years old) and "middle-aged" age group (35–54 years old), respectively. Those percentages were used to estimate cocaine doses used by the particular age groups per day at the time of sampling. These analyses were for illustration purposes only and do not imply use of cocaine by a particular age group.

Calculation of Cocaine Equivalents

An example calculation of cocaine equivalents is presented below. In the example, the concentration of BE in an extracted wastewater sample (as determined by GC/MS) collected on a Monday was 5560 μg/L. The first step was to multiply the BE concentration by the volume of sample that was analyzed: $(5560\ \mu g/L) \times (5 \times 10^{-4}\ L) = 2.78\ \mu g$ of BE. The next step was to divide the mass of BE by the volume of wastewater sample that was extracted: 2.78 μg ÷ 0.5 L = 5.56 μg/L (this represents the concentration of BE entering the LWRP). Next, we multiplied the concentration of BE in 1 L of wastewater by the volume of wastewater entering the LWRP on the day the sample was collected: $5.56\ \mu g/L \times (89.5 \times 10^6\ L/day) = 4.98 \times 10^8\ \mu g/day$. In the final step, the mass of BE was converted to cocaine equivalents: $(4.98 \times 10^8\ \mu g/day) \times 2.3 = 1145$ g/day.

Calculation of Cocaine Doses

Cocaine equivalent data were normalized based on population as well as cocaine doses. Example calculations are presented below using the mass of cocaine equivalents calculated above (1145 g/day). The first step was to normalize the cocaine equivalents data based on 269,140 people served by the treatment plant: 1145 g/day ÷ 269,140 = 4.25 g/day per 1000 people. The next step was to convert mass of cocaine equivalents into doses using the conversion of 1 dose = 100 mg (18): 4.25 g/day per 1000 people ÷ 100 mg/dose = 42.5 doses/day. A similar set of calculations was performed for the "young adult" and "middle-aged" age groups.

Results

BE and Cocaine Equivalents in Water Samples

BE was detected in all wastewater influent samples collected biweekly from February 8, 2010 to June 4, 2010. BE concentrations varied in Monday samples and Friday samples (Table 1). Overall, Monday samples appeared to have slightly higher concentrations of BE in wastewater, with an average of 6.20 ± 0.43 μg/L compared to Friday samples whose average was 5.99 ± 0.35 μg/L. The overall average concentration of BE recovered from all the wastewater samples during the 5-month sampling period was 6.2 ± 0.4 μg/L.

Calculated cocaine equivalents (using the calculations and assumptions described above) are shown in Table 1. During the study period, an overall average of 1152 ± 147 g of cocaine was estimated to be consumed per day in Lubbock. The average weekly variations in cocaine use estimates are illustrated in Fig. 1. Estimated cocaine use was consistently higher on weekends (Monday

TABLE 1—*Estimates of cocaine use as determined from benzoylecgonine (BE) levels in wastewater influent.*

				Estimated Local Cocaine Use					
				Per 1000 People		Per 1000 YA[‡]		Per 1000 MA[§]	
Week	Day	BE*	Cocaine Equivalent[†]	g/day	Doses/day[¶]	g/day	Doses/day[¶]	g/day	Doses/day[¶]
1	Monday	5.56	1145	4.25	42.5	13.05	130.5	17.29	172.9
	Friday	5.54	952	3.54	35.4	10.85	108.5	14.38	143.8
2	Monday	6.49	1336	4.96	49.6	15.23	152.3	20.18	201.8
	Friday	6.71	1153	4.28	42.8	13.14	131.4	17.41	174.1
3	Monday	6.47	1332	4.95	49.5	15.18	151.8	20.12	201.2
	Friday	6.16	1058	3.93	39.3	12.06	120.6	15.98	159.8
4	Monday	6.41	1321	4.91	49.1	15.06	150.6	19.95	199.5
	Friday	5.94	1020	3.79	37.9	11.63	116.3	15.41	154.1
5	Monday	6.88	1416	5.26	52.6	16.14	161.4	21.39	213.9
	Friday	5.79	994	3.69	36.9	11.33	113.3	15.01	150.1
6	Monday	5.96	1227	4.55	45.5	13.98	139.8	18.53	185.3
	Friday	5.79	994	3.69	36.9	11.33	113.3	15.01	150.1
7	Monday	6.02	1239	4.6	46	14.12	141.2	18.71	187.1
	Friday	6.09	1046	3.88	38.8	11.92	119.2	15.80	158.0
8	Monday	5.83	1200	4.46	44.6	13.68	136.8	18.12	181.2
	Friday	5.88	1010	3.75	37.5	11.51	115.1	15.25	152.5
	Mean ± SD	6.1 ± 0.4	1152 ± 147	4.3 ± 0.5	42.8 ± 5.4	13.1 ± 1.7	131 ± 17	17.4 ± 2.2	174 ± 22

*Concentration of BE entering the Lubbock Water Reclamation Plant (wastewater influent) in μg/L.
[†]Cocaine equivalent in g/day.
[‡]Young adults (15–34 years) = 32.6% of Lubbock population.
[§]Middle-aged adults (35–54 years) = 24.6% of Lubbock population.
[¶]1 dose = 100 mg (18).

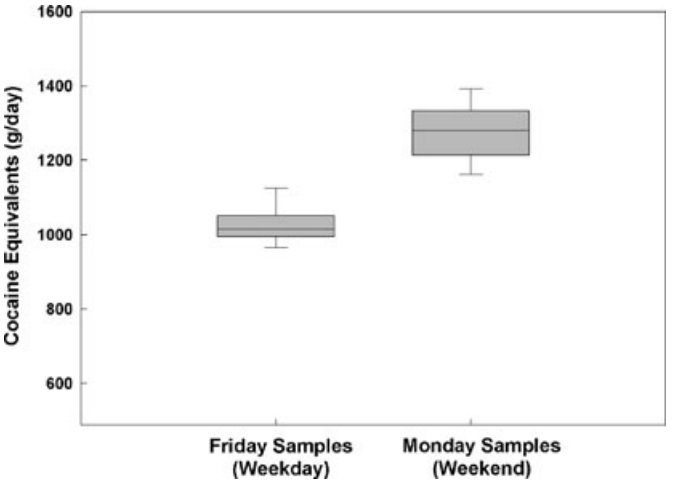

FIG. 1—*Box and whisker plot of cocaine equivalents during the study period. The box represents the upper (15th) and lower (25th) quartiles of the data. The line within the box is the median. The upper and lower ranges of the data are indicated by the error bars.*

samples) than weekdays (Friday samples) during the study period. The results of the paired *t*-test indicated that at a 95% confidence level there was evidence that the means of the two samples (Monday and Friday) were significantly different ($p < 0.0003$).

Lubbock wastewater was found to contain slightly >1 kg of cocaine equivalents per day. Using those data, we were able to estimate consumption among population groups (Table 1). The data revealed that on average, the estimated consumption per 1000 people from the general population was 4.3 ± 0.5 g/day or 42.8 ± 5.4 doses/day. Estimated cocaine use per 1000 young adults (15–34 years) and middle-aged adults (35–54 years) was 13.1 ± 1.7 g/day or 131 ± 17 doses/day and 17.4 ± 2.2 g/day or 174 ± 22 doses/day, respectively.

Discussion

Results of this study indicated that there was a statistically significant difference in estimated cocaine consumption between Monday and Friday. This suggested a higher consumption of cocaine on weekends since wastewater sampled on Monday was representative of the weekend and wastewater sampled on Friday was representative of weekdays. The daily amount of wastewater flow into the treatment plant varied during the week; Monday has a consistently higher influent volume than Friday. This may be influenced by several factors including human activities on weekends which in turn influence the amount of BE in wastewater. It is important to note that the cocaine estimation method assumes a BE/cocaine ratio produced through metabolism of cocaine. It is possible that some BE in wastewater is from the disposal of cocaine into the sewer system; therefore, our method may overestimate the amount of cocaine present in the wastewater as it assumes that the cocaine present only comes from excretion.

Some aspects of the method need to be refined before it can be used as a general monitoring tool, such as additional knowledge of the chemical and biological stability of cocaine and BE and how they partition in sewage. In addition, to gather more insight on areas within a community where cocaine consumption is higher, it would be helpful to have the ability to sample wastewater at different points within the system. This would provide more information regarding areas with high cocaine use.

Population surveys may be useful to describe patterns of drug use, but they have limitations in assessing the extent of cocaine consumption owing to the variability of human behavior. In that respect, the method described herein may provide a more realistic picture of actual cocaine use or at least provide supplementary information to survey data. The present study showed that sewage epidemiology was a useful tool for detecting BE and (based on literature values) subsequently estimating cocaine consumption. The method described could be an efficient tool for investigating temporal variations (daily, weekly, and seasonal) at a local level. In addition, this method along with the ability to sample wastewater at the neighborhood level could provide a valuable forensic tool.

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