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Eliminating Solid Phase Extraction with Large-Volume Injection LC/MS/MS: Analysis of Illicit and Legal Drugs and Human Urine Indicators in US Wastewaters

AUREA C. CHIAIA,[†]
CALEB BANTA-GREEN,[‡] AND
JENNIFER FIELD*[,†,§]

Department of Chemistry and Department of Environmental Toxicology, Oregon State University, Corvallis, Oregon 97331, and Alcohol and Drug Abuse Institute, University of Washington, Seattle, Washington 98105

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Large-volume (1800 μL) injection (LVI) followed by liquid chromatography/tandem mass spectrometry was developed and optimized to eliminate the need for off- and on-line solid phase extraction as a sample preparation step. Centrifugation of raw municipal influent followed by LVI was optimized for the routine determination of illicit drugs and related substances in municipal wastewaters. The accuracy of the method is demonstrated by standard addition for analytes with concentrations ranging from 4 to 3 500 000 ng/L. Precision, as indicated by relative standard deviation is <12% within a day and ≤20% for between-days for analytes with corresponding stable-isotope-labeled internal standards. Instrumental detection limits range from 0.5 to 4 ng/L while lower limits of quantification range from 2.5 to 10 ng/L. The method is demonstrated on wastewater treatment plant influents (24 h, flow-normalized) collected from seven municipalities located in the US. Methamphetamine concentrations and loads are the greatest yet reported while cocaine concentrations and index loads are similar to European locations. Creatinine is introduced as human urine indicator that can be potentially used as an alternative to population estimates for indexing illicit drug loads for different municipalities.

Introduction

Current analytical methods for quantifying low concentrations of analytes in wastewater including pharmaceuticals (*1*) and illicit drugs (*2*−*5*) commonly include sample concentration steps such as solid-phase extraction (SPE). Off-line SPE for illicit drugs typically involves the concentration of samples ranging in volume typically from 50 to 200 mL (*2*−*4*, *6*), while only a small fraction of the final extract is actually injected (e.g., 10−20 μL). Despite the perceived advantages of off-line SPE for sample concentration and clean up, limitations include low and variable analyte recovery, contamination of SPE materials in the case of fluorochemicals (*7*, *8*), and the time and cost involved. On-line SPE is described as a time and cost-saving alternative due to its fully automated nature (*9*, *10*). However, this approach requires the use of a standalone additional instrumentation so that the costs associated with SPE are not eliminated.

Large-volume injection (LVI) is a relatively little-known technique that dates back to the early 1980s (*11*, *12*) and involves the direct injection of sample volumes that range from 100 μL (*13*, *14*) to 5000 μL (*15*) versus the more conventionally injected volumes of 10−20 μL. LVI offers several advantages, such as an increase in sensitivity and accuracy, since there is minimal sample handling, and no SPE materials are utilized. LVI increases sample throughput at minimal cost and handling compared to both off- and on-line SPE, because no SPE cartridges are needed. In addition, for LVI the total sample volume required is smaller than for off-line SPE, since the entire sample can be injected.

Existing LVI applications include the determination of amino acids (*16*), biogenic amines (*17*), and pesticides, herbicides and fungicides in vegetables (*18*), soils (*19*), and water (*20*). However, the potential of LVI is not yet fully recognized, since previous reports describe cumbersome manual injectors and the use of only external standard calibration, and to the best of our knowledge, only a few demonstrations of LVI exist that use internal standard calibration and address matrix effects for complex environmental samples (*7*, *8*).

Examination of the current literature on the analysis of illegal drugs indicated an opportunity to improve upon existing SPE-based sample preparation methodology. Therefore, the objective of this research was to develop and validate large-volume injection (1800 μL) as a fully automated (no SPE) approach for the analysis of illicit and legal drugs and urinary indicators in municipal wastewater. Drugs of abuse, such as methamphetamine and cocaine, were selected for study due to the current interest in wastewater measurements of illicit drugs as quantitative indicators of population-level drug use within communities (*21*). Prescription opiods were selected for their utility as positive controls, as these substances are widely used and data on their sales is available (*22*). In addition, creatinine is introduced as a substance known to occur in human urine as an alternative index to population estimates that are known to fluctuate. Matrix effects for all analytes were explicitly addressed through standard-addition experiments. Finally, the analytical method is demonstrated on a small number (seven) of 24 h flow-normalized composite of raw influent samples collected from wastewater treatment plants (WWTPs) within the United States.

Experimental Methods

Standards and Reagents. Details on the purchase, preparation, and storage of standards and reagents are provided in Supporting Information.

Sample Collection and Preservation. Polypropylene bottles (VWR International, West Chester, PA) including trip blanks and duplicates were sent to seven municipal WWTPs around the US. Single 24 h flow-normalized composite of raw influent was collected under refrigerated conditions (4 °C), frozen, and then shipped frozen on ice overnight. The samples were stored at −20 °C upon receipt and analyzed within 3 weeks of collection.

Large-Volume Injection Liquid Chromatography/Tandem Mass Spectrometry. For the analysis of all analytes (except creatinine), frozen samples were brought to room temperature and 7 mL of aliquot volume was centrifuged in a IEC clinical centrifuge (Thermo IEC, Nutley, NJ) for 30 min

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* Corresponding author. E-mail: jennifer.field@oregonstate.edu.

[†] Department of Chemistry, Oregon State University.

[‡] University of Washington.

[§] Department of Environmental Toxicology, Oregon State University.

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at a maximum speed of 7100 rpm (5125 g). After centrifugation, supernatant was transferred into a 6 mL autosampler glass vial and spiked with stable-isotope-labeled internal standards including 380 pg of (±)-MDMA-d_5, (±)-MDA-d_5, (±)-amphetamine-d_6, (±)-methamphetamine-d_5, cocaine-d_3, benzoylecgonine-d_3, oxycodone-d_3, hydrocodone-d_6, (±)-methadone-d_9, PCP-d_5, LSD-d_3, and flunitrazepam-d_7 and 1100 pg of caffeine-$^{13}C_3$, (±)-cotinine-d_3, and (1*S*,2*R*)-(+)-ephedrine-d_3. All samples were analyzed within 24 h of preparation.

For the analysis of creatinine, samples were brought to room temperature and a 2 mL of aliquot was centrifuged in an Eppendorf centrifuge (5415 C) for 30 min at a maximum speed of 14 000 rpm (10 000*g*). After centrifugation, 1.2 mL of supernatant was transferred to a 2 mL glass autosampler vial and spiked with 900 ng of the creatinine-D_3 internal standard. After preparation, the samples were analyzed within 24 h.

Large-volume injections and separations were performed on an Agilent 1100 HPLC system (Santa Clara, CA) that was modified by adding the commercially available option of a 900 μL injection upgrade kit (Agilent part no. G1363A) that consisted of a 900 μL analytical head, a 900 μL stainless steel sample loop extension, and a 900 μL needle. To reach a capacity of 1800 μL, a commercially available 1400 μL stainless steel seat extension loop (Agilent part no. G13G13-87308) was installed between the seat capillary fitting and port 5 (injection valve) of the analytical head.

For all analytes separation except creatinine, a 2.0 × 4.0 mm C18 security guard cartridge (Phenomenex, Torrance, CA) was connected to a 150 × 4.6 mm × 5 μm particle size Atlantis T3 C18 column (Waters Corp., Milford, MA). The column temperature was 35 °C and the flow rate was held at 500 μL/min. Initially, the injection valve is in the “bypass” mode such that the mobile phase does not pass through the injection hardware but is diverted directly to the analytical column. The injection program is then initiated with a needle wash followed by withdrawal of 900 μL of sample that is then injected into the 1400 μL seat capillary. A second 900 μL of sample is withdrawn and added to give a total volume of 1800 μL in the injector assembly. At this point, the injection valve is changed automatically to the “main-pass” position so that the sample is transferred to the analytical column and the injection valve remains in this position for 9 min. During this 9 min, the divert valve that is located between the analytical column and the ESI interface is set to direct flow to waste. After 9 min, the injection valve is programmed to switch back to the ‘bypass’ position.

The mobile phase consists of 0.1% acetic acid in 5% methanol (A) and acetonitrile (B). The gradient starts by holding A (90%) for 8 min, then increasing B to 25% in 6 min followed by a ramp of B to 100% in 10 min, after which 100% B is maintained for 2 min. The gradient is brought to initial conditions (90% A) and is held for 9 min for the recalibration of the column, giving a total run time of 35 min.

Creatinine analyses were performed on the same Agilent 1100 HPLC system equipped with the injection upgrade kit. However, the 1400 μL seat-extension loop was not necessary for the analysis of creatinine since an injection volume of 100 μL was used. The 100 μL sample volume was directly injected into a 2.0 × 4.0 mm C18 security guard cartridge that was attached to a 150 × 4.6 mm 5 μm particle size Luna C18 column (Phenomenex, Torrance, CA). Isocratic conditions with a mobile phase of 10 mM ammonium acetate in 5% methanol were used at a column temperature of 35 °C and a flow rate of 500 μL/min.

Detection and quantification was performed on a Waters Quattro Micro tandem mass spectrometer (Milford, MA) operated in positive mode with an electrospray ionization (ESI) interface. The source and desolvation temperature were set to 150 and 450 °C, respectively. A total of 43 transitions were acquired to quantify analytes (Table S1, Supporting Information) and internal standards (Table S2, Supporting Information) in multiple reaction monitoring (MRM) mode. An interchannel and scan delay of 0.03 s between groups of transitions was used to enhance sensitivity. Analytes were monitored using a single transition with the exception of methamphetamine, amphetamine, benzoylecgonine, and norcocaine that were monitored using two transitions with the second transition used as a visual check. Creatinine was quantified in MRM mode using two transitions, where the second transition was used as a visual check. For details on the preparation of calibration curves and quality control, see the Supporting Information.

Accuracy and Matrix Effects. Standard addition was performed for all analytes in raw municipal wastewater in order to determine if matrix effects could be compensated for through the use of stable-isotope internal standards. The initial concentrations of analytes present in a single sample of raw influent were deduced using the solvent-based calibration curve for $n = 4$ replicates of a single wastewater sample. Each analyte was then spiked into four additional ($n = 1$) aliquots of the same wastewater sample in order to increase the background signal 1.5 to 3 times that of the original signal. An example of the masses added and data treatment is given in the Supporting Information. The mass ±95% CI of each analyte in the unspiked ($n = 4$) replicates was determined by standard addition and compared at the 95% CI with the mass calculated from the solvent-based calibration curves in the unspiked samples.

Precision. Precision both within-day and between-days was determined by subdividing a single sample of raw influent into 16 aliquots and analyzing $n = 4$ on each of four consecutive days during a single week. All the aliquots were spiked to give a final concentration of 30 ng/L or higher to ensure that, at the onset of the analysis, all analytes were present. Between-day precision as indicated by %RSD was estimated as the sum of the between-day and within-day variation (see eq S1, Supporting Information, for details) and represents the maximum uncertainty around measurements made on different days.

Detection and Quantification Limits. The instrumental detection limits (IDL) for illicit and legal drugs, metabolites, caffeine, and cotinine defined as that concentration needed to achieve a signal/noise (S/N) ≥ 3 were calculated by spiking low level standards in distilled water with concentrations ranging from 0.5 to 7 ng/L. To determine the IDL for the human urine indicator creatinine, DI water was spiked to give a concentration range of 50–500 ng/L.

The instrumental lower limits of quantification (LLOQ) were defined as the lowest point on the calibration curve and that gave a $S/N \geq 10$. However, in the case of caffeine, cotinine, and creatinine, which occur in wastewater at concentrations in the range of thousands of nanograms/liter, the lowest concentrations selected for use in the calibration curve gave S/N significantly greater than 10:1. Therefore, the reporting limits used for this study do not represent the lowest achievable limits of quantification.

Storage Stability. Due to the potential instability of the unpreserved raw influent samples, a storage stability study was conducted prior to sample collection to determine the hold times for −20 °C storage. The details of the storage/stability study are provided in the Supporting Information.

Results and Discussion

Large-Volume Injection (LVI). Chromatograms (Figure 1 and Figures S1 and S2 of the Supporting Information) obtained from actual samples of raw municipal influent under LVI-LC/MS/MS conditions indicate satisfactory separation and detection of all analytes in influent wastewater. The injection

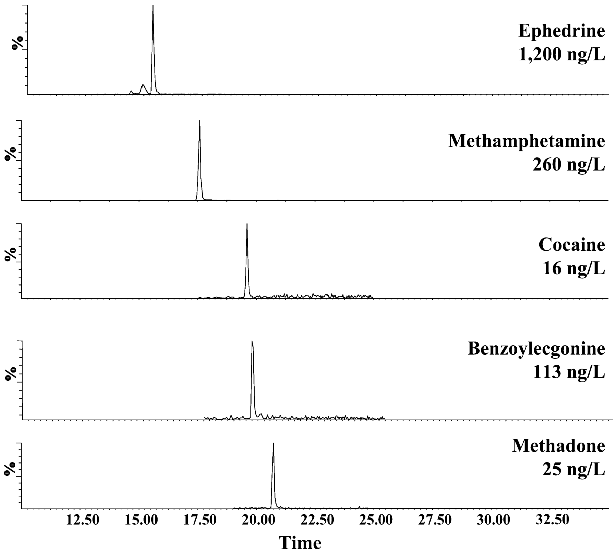

FIGURE 1. Example of a typical chromatogram for selected analytes including ephedrine, methamphetamine, cocaine, benzoylecgonine, and methadone acquired by large-volume (1,800 μL) injection-LC/MS/MS conditions. Time line: 8 min "extraction", 1 min wash, 17 min separation, 9 min re-equilibration.

of a large volume (1800 μL) onto an analytical column is analogous to the sample application step of SPE and to frontal chromatography (*23*). The analytes of interest give large apparent retention factors (k) (*23*) and the absence of peak broadening indicate effective retention by the functionalized C18 groups in the analytical column (Figure 1 and Figures S1 and S2 of the Supporting Information). Narrow, symmetrical peaks obtained under LVI conditions are consistent with other demonstrations of LVI (*14, 24*). In contrast, salts with low k values breakthrough and are run to waste instead of to the ESI interface.

A critical function for optimum control of LVI is optimizing the timing/rotation of the injection valve (main-pass/bypass) for control over the gradient and for minimizing total run times. For this study, 8 min was deemed sufficient to transfer the sample to the column, as determined by experiments with an unretained solute. However, experiments with only an 8 min hold time under initial solvent conditions gave unacceptable RSDs of >30% (e.g., $n = 5$ replicate injections of a raw wastewater sample). The addition of a 1 min "wash step" that consisted of allowing an additional 500 μL of the initial mobile phase to flow through the sample loops and analytical column reduced RSDs to 8%. The wash step ensures that the injection system is cleared (e.g., no carryover) and is analogous to wash steps employed during SPE (*3, 6*).

Once the 1800 μL volume of sample is transferred (first 9 min of analysis time), the injection valve is programed to returned to the "bypass" position so that the mobile phase/gradient bypasses the large volume injection loops. The use of the bypass function effectively decreases the overall run time of the analysis by decreasing the time required for the gradient to reach the analytical column (dwell time). To further minimize run times, the flow rate was increased to 500 μL/min and the source and desolvation temperatures were increased to 150 and 450 °C, respectively.

In contrast to LVI-LC/MS/MS, off-line SPE typically generates extracts that require additional concentration, typically under a stream of N_2, and only a fraction of the total final SPE extract is injected. Analytes in SPE extracts undergo separation and detection by LC/MS/MS as a separate step, whereas elution and separation are linked simultaneously in a single step in LVI-LC/MS/MS. Because many of the processes that occur during LVI are chemically redundant with steps taken during SPE, SPE is eliminated as a costly and time-consuming sample preparation step without causing deleterious effects on the chromatography even for a sample matrix as complex as raw municipal wastewater.

TABLE 1. Instrumental Detection Limits (IDL), Lower Limits of Quantification (LLOQ), and within-Day and between-Days Method Precision

compounds	IDL (ng/L)	LLOQ (ng/L)	within-day precision (%RSD)	between-day precision (%RSD)
methamphetamine	1.5	10.0	4	8
amphetamine	1.5	10.0	8	14
ephedrine	2.5	10.0	4	12
cocaine	2.0	2.5	5	10
benzoylecgonine	1.0	10.0	6	13
norcocaine	2.0	2.5	8	28
norbenzoylecgonine	2.5	5.0	6	15
hydrocodone	2.0	2.5	8	20
oxycodone	2.0	2.5	12	13
methadone	2.0	2.5	4	8
MDA	2.0	2.5	12	20
MDMA	1.0	2.5	8	14
MDEA	3.5	5.0	16	28
MBDB	4.0	5.0	14	28
ketamine	4.0	5.0	11	19
norketamine	3.5	5.0	12	17
2-oxo-3-hydroxy-LSD	2.5	5.0	12	42
LSD	0.5	2.5	6	6
PCP	2.5	5.0	9	16
flunitrazepam	1.5	2.5	5	5
cotinine	4.5	250	7	10
caffeine	6.0	250	6	6
creatinine	250	50000	8	9

Due to the expected high concentrations of creatinine in wastewater and high water solubility, initial experiments to measure creatinine in raw wastewater began with injection volumes <900 μL. Injection volumes > 100 μL gave poor peak shapes, which is indicative of breakthrough and consistent with an expected low-k value (Figure S1, Supporting Information).

Method Accuracy. Initially, potential analyte loss during centrifugation was investigated by spiking all analytes before ($n = 4$) and after ($n = 4$) centrifuging replicate aliquots of a single raw influent sample. Average analyte concentrations were compared using the Student's t test, and no statistical difference at the 95% CI was found, indicating that no significant loss occurred during centrifugation, which is consistent with their low K_{ow} values (*4, 25, 26*). For this reason, all wastewater samples were centrifuged prior to analysis and spiked with internal standards after the centrifugation step.

Concentrations of analytes in raw wastewater samples were determined by standard addition and were statistically equivalent at the 95% CI to those determined from solvent-based calibration curves (Figure S3, Supporting Information). Statistically equivalent values were obtained for individual analytes that varied in concentration from 4 to 3 500 000 ng/L (Table S3, Supporting Information). Others report that stable-isotope-labeled internal standards compensate for matrix effects when analyzing for illicit drugs in wastewater (*4, 10*). All subsequent quantification was performed using calibration standards prepared in DI water.

Method Precision. Within-day precision, as indicated by %RSD, ranged from 4−16% with an average of 8% (Table 1). The %RSDs are similar to those reported for the analysis of illicit drugs in wastewater using on-line SPE (*10*) and off-line SPE (*2, 3*). Between-day precision with the exception of 2-oxohydroxy-LSD ranged from 6 to 28% with an average of 15% (Table 1). Others report higher between-day precision than within-day precision for illicit drugs determined using

SPE-based methodology (*2, 3, 6*). The highest %RSDs (≥28% for both within-day and between-day) correspond to analytes such as norcocaine, MDEA, MBDB, and 2-oxohydroxy-LSD for which stable-isotope-labeled internal standards are not available (Table 1). Therefore, data for these analytes should be treated as semiquantitative until suitable internal standards become available.

Detection and Quantification Limits. Instrument detection limits (IDL) (Table 1) ranged from 0.5 to 6 ng/L for the illicit and legal drugs, metabolites, caffeine, and cotinine, and the values are similar in range to those obtained for illicit drugs by off-line (*2–4, 6*) and on-line SPE with LC/MS/MS detection (*10*). The high apparent IDL value of creatinine (250 ng/L) is due to the 100 μL volume injected, which is 18 times smaller than the volume used for the other analytes. The lower limits of quantification (LLOQ) for illegal and legal drugs and metabolites ranged from 2.5 to 10 ng/L (Table 1). LLOQs for human-urine indicators range from 250 ng/L (caffeine and cotinine) to 50 000 ng/L (creatinine).

Demonstration for Raw Wastewater Influent. Concentrations of individual analytes were used to calculate index loads (mg/person/day) (eq S2, Supporting Information). Index loads are calculated in a manner equivalent to that of "per capita loads" (also reported in units of mg/person/day) as described by others (*5, 27–29*). However, the term "index load" is introduced as an alternative term in order to stress the fact that, in contrast to the measured drug concentrations and wastewater flows, the population served by WWTPs is an estimate. Current approaches do not take into account movement of individuals (e.g. commuting) so that terms such as "per capita" suggest a level of accuracy about population estimates that is potentially misleading.

The uncertainty about the average influent concentrations (Table 2) and their associated index loads (Table 3) are reported as the relative standard deviations computed using the between-day precision (Table 1 and Supporting Information). These error estimates are only partial errors, since they derive only from estimate of analytical error and do not include sampling error or flow errors (Table 3).

Amphetamine and Related Compounds. Methamphetamine occurred in five of the seven participating WWTPs with concentrations ranging from below detection to 2000 ng/L (Table 2). The highest index loads correspond to WWTPs 1, 2, 5, and 6, which are located in the western and southern regions of the US (Table 3). WWTPs located in the Northeast gave index loads of 0.005 mg/person/day (WWTP 4) or values that could not be reported due to concentrations below detection (WWTP 3). WWTP 7 located in the Midwest also gave methamphetamine concentrations below detection (Table 2) so that no index load can be reported (Table 3). The methamphetamine concentrations and index loads are significantly greater than those reported in other studies that report methamphetamine concentrations up to 300 ng/L (*2, 10, 29, 30*).

Amphetamine was quantified in all WWTPs (Table 2) at concentrations greater than those reported by others (*2, 3, 10, 29, 30*) and similar to concentrations found in the northeastern Spain (*31*). Lower index loads (<0.1 mg/person/day) for amphetamine correspond to WWTPs 3, 4, and 7, for which methamphetamine index loads are also low or below reporting limits (Table 3), which potentially indicates that amphetamine occurs in these municipalities due to its prescription use (*32*). When methamphetamine index loads are higher, we hypothesize that the higher index loads of amphetamine may reflect both prescription use as well as its excretion as a methamphetamine metabolite (*32*).

Ephedrine, which is a prescription drug, was found for all WWTPs at concentrations up to 6900 ng/L (Table 2). The index loads for ephedrine likely reflect prescription use; however, ephedrine also is a precursor compound use in the manufacture of methamphetamine (*33*).

Cocaine. Cocaine and its main metabolite, benzoylecgonine, were quantified in each of the seven WWTPs that participated in the study with cocaine index loads ranging from 0.009 to 0.40 mg/person/day. Higher concentrations (Table 2) and index loads from 0.10 to 1.50 mg/person/day (Table 3) were expected for benzoylecgonine because benzoylecgonine is excreted in urine at higher concentrations than cocaine (*34*). The concentrations and index loads for cocaine and benzoylecgonine for this limited data set are similar to those reported for European cities (*2, 3, 10, 28–30*). Lower concentrations and a frequency of detection were observed for the more minor metabolites of cocaine, including norbenzoylecgonine and norcocaine (Table 2).

Other Illicit Drugs. MDMA, also known as ecstasy, occurred in five out of seven WWTPs, while MDA was detected only in three of the seven WWTPs (Tables 2 and 3). The measured MDMA and MDA concentrations are consistent with other studies (*2, 3, 10, 29, 30*). Other illicit drugs including MDEA, MBDB, LSD, 2-oxohydroxy-LSD, and flunitrazepam were not detected in any of the samples. Ketamine, which has legal and illegal uses (*32*), was detected in only two WWTPs, and PCP was found in only one WWTP (Tables 2 and 3).

Prescription Opiods. Hydrocodone, which is the most frequently prescribed drug in the US and the most frequently prescribed opiod, ranged in index loads from 0.007 to 0.090 mg/person/day, while oxycodone index loads ranged from 0.008 to 017 (Tables 2 and 3). Hummel et al. (*4*) reported similar concentrations of hydrocodone and oxycodone up to 95 and 70 ng/L, respectively, for influent wastewaters collected in Germany. Methadone, a prescription opiod used as a painkiller and in the treatment of addiction to heroin, was found at index loads ranging from 0.003 to 0.048 mg/person/day, which is a range consistent with those reported by others (*6, 35*).

Human Urine Indicators. Three analytes that are likely human urine indicators including cotinine, caffeine, and creatinine were quantified in all WWTPs sampled. Caffeine concentrations ranged from 11 500 to 120 000 ng/L (Table 2), while cotinine, the main metabolite of nicotine, ranged from 130 to 2700 ng/L (Table 2). High concentrations and detection in all samples is consistent with the notion that these analytes are indicators of municipal wastewater (*3, 36, 37*). Concentrations of creatinine ranged from 220 000 to 1 500 000 ng/L and were significantly higher than that for either caffeine or cotinine (Table 2). To the best of our knowledge, this is the first attempt to quantify creatinine in wastewater. In contrast, creatinine analysis is routine for purposes of drug testing in human urine (*38, 39*). Due to creatinine's ubiquitous occurrence in human urine and wastewater, we hypothesize that the mass loads of illicit drugs can be indexed against creatinine. The analytical methodology presented here is a requisite first step toward testing this hypothesis.

Insights and Future Research. The observed ranges in index loads for illicit drugs including methamphetamine and cocaine and the regions of the US in which they occur are generally reflective of known drug use patterns in the United States (*40, 41*). The finding that methamphetamine concentrations for several municipalities are much higher than those reported in previous literature, all of which is from Europe, is reflective of known international drug use patterns (*42*). Attempts have been made to compare measured values for raw wastewater samples with estimated values (*28*); however, these have been rudimentary, based upon compounds with complex sociological and pharmacological phenomenon, and have not incorporated components of error surrounding sampling and flow measurements. Ad-

TABLE 2. Average Concentrations ± Relative Standard Deviation (ng/L) of Illicit Drugs, Prescription Opiods, and Human-Urine Indicators in Seven Different Municipal Wastewater Treatment Plants Collected from Regions within the US[a]

Municipality Information							
wastewater treatment plant	plant 1	plant 2	plant 3	plant 4	plant 5	plant 6	plant 7
region located	South	West	North East	North East	South	West	Midwest
population	350 000	841 000	27 300	39 800	48 953	54 890	650 000
average flow (L/d)	1.87×10^{8}	7.08×10^{14}	1.70×10^{7}	2.15×10^{7}	3.78×10^{7}	4.96×10^{7}	1.87×10^{14}
analyte	**average concentration (ng/L)**						
methamphetamine	920 ± 70	2000 ± 200	ND[c]	10 ± 1	920 ± 70	150 ± 10	<LLOQ
amphetamine	220 ± 30	550 ± 80	80 ± 10	120 ± 20	250 ± 40	90 ± 10	130 ± 20
ephedrine	3400 ± 400	6900 ± 800	1300 ± 200	1400 ± 200	2700 ± 300	580 ± 70	1300 ± 200
cocaine	350 ± 40	230 ± 20	56 ± 6	66 ± 7	72 ± 7	10 ± 1	860 ± 90
benzoylecgonine	2800 ± 400	2100 ± 300	370 ± 50	620 ± 80	420 ± 60	110 ± 20	1500 ± 200
norcocaine	15 ± 4	9 ± 2	<LLOQ	<LLOQ	4 ± 1	ND	50 ± 10
norbenzoylecgonine	170 ± 30	170 ± 30	14 ± 2	20 ± 3	17 ± 3	ND	60 ± 10
hydrocodone	130 ± 30	210 ± 40	13 ± 3	13 ± 3	80 ± 20	14 ± 3	30 ± 6
oxycodone	80 ± 10	210 ± 20	58 ± 8	15 ± 2	220 ± 30	29 ± 4	80 ± 10
methadone	33 ± 3	59 ± 5	4.9 ± 0.4	16 ± 1	62 ± 5	14 ± 1	47 ± 4
MDA	6 ± 1	2 ± 1	7 ± 1	ND	ND	ND	ND
MDMA	70 ± 10	30 ± 4	2.7 ± 0.4	ND	10 ± 1	ND	3.5 ± 0.4
MDEA	ND	ND	ND	ND	ND	ND	ND
MBDB	ND	ND	ND	ND	ND	ND	ND
ketamine	ND	16 ± 3	ND	ND	ND	<LLOQ	ND
norketamine	ND	ND	ND	ND	ND	ND	ND
2-oxo-3-hydroxy-LSD	ND	ND	ND	ND	ND	ND	ND
LSD	ND	ND	ND	ND	ND	ND	ND
PCP	5 ± 1	ND	ND	ND	ND	ND	ND
flunitrazepam	ND	ND	ND	ND	ND	ND	ND
cotinine	2000 ± 200	2700 ± 300	130 ± 10	400 ± 40	2300 ± 230	180 ± 20	1200 ± 100
caffeine	57000 ± 3000	120000 ± 7000[b]	40000 ± 2400	41000 ± 3000	38000 ± 2000	11500 ± 700	30000 ± 2000
creatinine	220000 ± 20000	1500000 ± 100000	740000 ± 70000	1100000 ± 100000	240000 ± 20000	373000 ± 34000	490000 ± 40000

[a] Regions as defined on a Website maintained by the United States Drug Enforcement Administration (*43*). [b] Calibration curve extended to 200 000 ng/L for samples from this plant. [c] ND = not detected; for instrumental detection limits, see Table 1. <LLOQ = below the detection limits of quantification (see Table 1).

TABLE 3. Index Loads ± Relative Standard Deviation (mg/person/day) of Illicit Drugs, Prescription Opiods, and Human-Urine Indicators in Seven Different Municipal Wastewater Plants Collected from Regions within the US[a]

	municipality information						
wastewater treatment plant	plant 1	plant 2	plant 3	plant 4	plant 5	plant 6	plant 7
region located	South	West	North East	North East	South	West	Midwest
population	350 000	841 000	27 300	39 800	48 953	54 890	650 000
average flow (L/d)	1.87×10^{8}	7.08×10^{14}	1.70×10^{7}	2.15×10^{7}	3.78×10^{7}	4.96×10^{7}	1.87×10^{14}
analyte	**index load**						
methamphetamine	0.49 ± 0.04	0.80 ± 0.10	NR[b]	0.0050 ± 0.0004	0.71 ± 0.06	0.13 ± 0.01	NR
amphetamine	0.12 ± 0.02	0.20 ± 0.03	0.050 ± 0.01	0.06 ± 0.01	0.19 ± 0.03	0.08 ± 0.01	0.06 ± 0.01
ephedrine	1.80 ± 0.20	2.90 ± 0.40	0.80 ± 0.10	0.80 ± 0.1	2.08 ± 0.25	0.52 ± 0.06	0.60 ± 0.08
cocaine	0.19 ± 0.02	0.10 ± 0.01	0.040 ± 0.004	0.040 ± 0.004	0.06 ± 0.01	0.009 ± 0.001	0.40 ± 0.04
benzoylecgonine	1.50 ± 0.20	0.90 ± 0.10	0.20 ± 0.03	0.30 ± 0.04	0.32 ± 0.04	0.10 ± 0.013	0.80 ± 0.10
norcocaine	0.008 ± 0.002	0.004 ± 0.001	NR	NR	0.003 ± 0.001	NR	0.026 ± 0.007
norbenzoylecgonine	0.09 ± 0.01	0.07 ± 0.01	0.008 ± 0.001	0.010 ± 0.002	0.013 ± 0.002	NR	0.028 ± 0.004
hydrocodone	0.070 ± 0.010	0.090 ± 0.020	0.008 ± 0.002	0.007 ± 0.001	0.057 ± 0.01	0.012 ± 0.002	0.015 ± 0.003
oxycodone	0.040 ± 0.010	0.090 ± 0.010	0.040 ± 0.005	0.008 ± 0.001	0.17 ± 0.02	0.026 ± 0.003	0.040 ± 0.005
methadone	0.018 ± 0.001	0.030 ± 0.002	0.0030 ± 0.0002	0.0090 ± 0.0007	0.048 ± 0.004	0.012 ± 0.001	0.023 ± 0.002
MDA	0.003 ± 0.001	0.0010 ± 0.0002	0.005 ± 0.001	NR	NR	NR	NR
MDMA	0.040 ± 0.010	0.012 ± 0.002	0.0020 ± 0.0002	NR	0.007 ± 0.001	ND	0.0017 ± 0.0002
MDEA	NR	NR	NR	NR	NR	NR	NR
MBDB	NR	NR	NR	NR	NR	NR	NR
ketamine	NR	0.007 ± 0.001	NR	NR	NR	NR	NR
norketamine	NR	NR	NR	NR	NR	NR	NR
2-oxo-3-hydroxy-LSD	NR	NR	NR	NR	NR	NR	NR
LSD	NR	NR	NR	NR	NR	NR	NR
PCP	0.0020 ± 0.0004	NR	NR	NR	NR	NR	NR
flunitrazepam	NR	NR	NR	NR	NR	NR	NR
cotinine	1.03 ± 0.10	1.10 ± 0.10	0.08 ± 0.01	0.20 ± 0.02	1.7 ± 0.17	0.16 ± 0.02	0.60 ± 0.06
caffeine	31 ± 2	50 ± 3	24 ± 2	22 ± 1	29 ± 1.74	10 ± 1	15 ± 1
creatinine	120 ± 11	620 ± 30	460 ± 40	590 ± 50	180 ± 16.2	337 ± 30	240 ± 20

[a] Regions as defined on a Website maintained by the United States Drug Enforcement Administration (*43*). [b] NR = not reported because either not detected or concentrations less than the lower limits of quantification (see Table 2).

ditional tools, such as the use of indicator compounds, like creatinine, are needed to enhance our capabilities for comparing the index loads for different municipalities.

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Supporting Information Available

Details on the standards, reagents, and practices used. This material is available free of charge via the Internet at http://pubs.acs.org.

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