SUPPLEMENTAL INFORMATION

Normalized Diurnal and Between-Day Trends in Illicit and Legal Drugs Loads that Account for Changes in Population

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EXPERIMENTAL METHODS

Chemical Analyses. All wastewater samples were analyzed for all substances (except creatinine) within one of two analytical sequences with sequence one containing days 1 and 3 while sequence two was comprised of samples from days 3 and 4. Samples analyzed for creatinine were run in a single run. The concentration range for standards used to construct the calibration curve for this study ranged 50,000-10,000,000 ng/L (creatinine); 2,000-200,000 ng/L (caffeine); 40-4,000 ng/L (methamphetamine); 5-500 ng/L (cocaine); and 40-4,000 ng/L (benzoylecgonine) in 5 mM ammonium acetate buffer. The lower limits of quantification for analytes were 50,000 ng/L (creatinine), 2,000 ng/L (caffeine), 40 ng/L (methamphetamine), 5 ng/L (cocaine), and 40 ng/L (BZE). In the case of creatinine and caffeine, the LOQs represent the lowest calibration standard needed to quantify the high concentrations of creatinine and caffeine and are well above instrumental detection limits for these analytes. All calibration curves had $R^2 > 0.98$ with 1/X weighting. Analytes were assigned a concentration if the signal-to-noise (S/N) met the criteria of $S/N \geq 10$ and was at or above the lowest calibration curve standard. For a given peak, if S/N was > 10 but below the lowest calibration standard, no concentration was assigned. The lower limit of quantification was determined as the lowest calibration standard. The ratio of quantifier to qualifier ion ratios were plotted against each other with R^2 values of > 0.89 for all analytes. For all analytical sequences, two quality control samples (standards) were analyzed after every eight samples and were considered acceptable with a percent agreement ≥70%.

Sampling Stability. Single 500 mL grab samples of raw influent were collected on three days and immediately transported to the laboratory. Prior to analysis, cocaine and benzoylecgonine were spiked to a concentration of ~100ng/L, each sample was shaken before removing three 15 mL aliquots for illicit drugs and caffeine and an additional three 1.5 mL aliquots for creatinine analysis and placed in a -20 °C freezer until analysis (these aliquots are time = 0). The remaining sample volume was then placed in a 4 ºC refrigerator and then subsampled according to the procedure describe above at hours 1, 2, 3, 4, 5, 6, 7, 12, and 24 for a total of ten samples per day. All samples were then analyzed as described above.

Accuracy and Precision. A single wastewater sample was picked at random within each analytical sequence and run four multiple times for precision, as indicated by % RSD (Table S2). For determining accuracy, a custom made, third party reference standard (human urine) was prepared by UTAK Laboratories Inc. (Valencia, CA). The reference urine was diluted by a factor of 1000 with 5 mM ammonium acetate buffer. The dilute solution was then spiked with internal standards for all substances and analyzed twice, once in the beginning of a sequence and again at the end (Table S4).

RESULTS AND DISCUSSION

Weighted Estimate of the Municipality's Urine Creatinine Concentration. The actual population in the municipality at any given time is unknown. As indicated, the most recent census found 54,462 residents and a separate study estimated 9,000 commuters for a total potential population of 63,462 during the study period. No demographic data was available for the commuters, it was assumed that they had the same ethnic/age distribution as that of the municipality. Because no independent measure of population was available, the measured creatinine loads were used to estimate the population utilizing the WWTP during the study period. The first step was to create a weighted estimate of the municipality's creatinine concentration in urine based on the municipality's demographics (e.g., ethnic and age distribution). The fraction of each ethnic group of the total population was determined (Table S2). Assuming that every ethnic group had the same age distribution, the fraction of each age group (Table S2) within each ethnic group was computed and then multiplied by the median, 10^{th} percentile, and 90^{th} percentile creatinine urine concentrations reported in Barr.[1] When an age bracket (e.g., 31-45) of the municipality spanned more than one age bracket (e.g., 30-39 and 40-49) in the published creatinine in urine dataset,[1] an average of the median, 10^{th} percentile, and 90^{th} percentile creatinine concentrations for the two age groups was used. This value was then used with Equation S3 to estimate the population utilizing the WWTP on each of the four sampling days.

Literature Cited

(1) Barr, D. B.; Wilder, L. C.; Caudill, S. P.; Gonzalez, A. J.; Needham, L. L.; Pirkle, J. L. Urinary creatinine concentrations in the U.S population: implications for urinary biologic monitoring measurements. *Environ. Health Perspect.* **2005,** *113*, 192-200.

(2) Population Research Center, P. S. U. Certified population estimate within city/town. In 2010.

(3) Murakami, K.; Sasaki, S.; Takahashi, Y.; Uenishi, K.; Watanabe, T.; Kohri, T.; Yamasaki, M.; Watanabe, R.; Baba, K.; Shibata, K.; Takahashi, T.; Hayabuchi, H.; Ohki, K.; Suzuki, J. Sensitivity and specificity of published strategies using urinary creatinine to identify incomplete 24-h urine collection. *Nutrition* **2008,** *24*, 16-22.

Table S1. Race and age distribution for the municipality studied.[2]

Group	Numbers of People	%
Asian	4610	0.07
Black	674	0.01
Hispanic	4709	0.07
American Indian	330	0.01
White	50469	0.80
Other	2424	0.04

Age distribution	%
Under 15	0.13
18 to 24	0.36
25 to 30	0.09
31 to 45	0.15
46 to 60	0.16
61 and over	0.15

Table S2. Intra-hour variation in wastewater flow as indicated by the percent relative standard deviation for the four sampling days.

Hour	Day 1	Day 2	Day 3	Day 4
8 am-9 am	11%	6%	28%	10%
9am-10 am	11%	9%	13%	24%
10 am-11am	11%	7%	21%	7%
11 am-12 pm	14%	20%	9%	10%
12 pm-1 pm	13%	18%	9%	11%
1 pm-2 pm	9%	10%	13%	11%
2 pm-3 pm	11%	10%	12%	12%
3pm-4pm	20%	14%	11%	11%
4 pm-5pm	8%	23%	12%	13%
5pm-6pm	14%	10%	8%	8%
6pm-7pm	12%	11%	10%	10%
7pm-8pm	10%	10%	12%	14%
8pm-9pm	12%	14%	10%	9%
9pm-10pm	14%	12%	12%	12%
10-pm-11pm	15%	11%	12%	14%
11pm-12pm	12%	11%	23%	9%
12pm-1am	17%	16%	17%	17%
1am-2am	18%	21%	16%	19%
2am-3am	7%	9%	27%	10%
3am-4am	17%	23%	16%	20%
4am-5am	31%	16%	22%	22%
5am-6am	11%	16%	26%	21%
6am-7am	34%	9%	15%	20%
7am-8am	5%	5%	10%	14%

Table S3. Precursor and product ion (m/z), cone (V), and collision voltages (eV).

	Precursor Ion (m/z)	Product Ion (m/z)	Cone (V)	Collision (eV)
Creatinine	113.9	43.8	10	15
	113.9	86.0	25	10
Creatinine D3	116.9	46.9	20	10
	116.9	89.1	20	10
Caffeine	195.2	110.2	30	25
	195.2	138.3	30	20
Caffeine D3	198.1	140.3	35	20
Methamphetamine	150.0	91.1	25	15
	150.0	119.2	20	10
Methamphetamine D5	155.1	91.7	20	20
Benzoylecgonine	290.2	105.1	30	20
	290.2	168.4	20	20
Benzoylecgonine D3	293.2	151.0	30	25
	293.2	171.4	30	20
Cocaine	304.1	105.1	35	25
	304.1	182.3	30	20
Cocaine D3	307.3	185.5	30	20

Table S4. Precision (% RSD) of the whole method for wastewater for each sequence.

Substance	Sequence 1[b]	Sequence 2[c]
Creatinine	3.2%	
Caffeine	1.2 %	9.3 %
Methamphetamine	1.3 %	13 %
Benzoylecgonine	16 %	25 %
Cocaine	16 %	19 %

[a] The separate analysis for creatinine was conducted in a single sequence that included all the samples from the four sampling days.
[b] Sequence 1 contained samples from days 1 and 3 (March 17th and 19th).
[c] Sequence 2 contained samples from days 2 and 4 (March 18th and 20th).

Table S5. Percent agreement between the average measured (n=2) and certified UTAK values.

Substance	Certified UTAK concentration (ng/L)	% Agreement Sequence 1[b]	Sequence 2[c]
Caffeine	60,000	77%	91%
Methamphetamine	750	84%	87%
Benzoylecgonine	350	68%	76%
Cocaine	100	70%	81%

NA = not available

[a] The separate analysis for creatinine was conducted in a single sequence that included all the samples from the four sampling days.

[b] Sequence 1 contained samples from days 1 and 3 (March 17th and 19th)

[c] Sequence 2 contained samples from days 2 and 4 (March 18th and 20th)

Table S6. Estimated total uncertainty expressed as % RSD for loads and analyte to creatinine ratios (See Equations S1 and S2).

	Load (days 1 and 3)	Ratio (days 1 and 3)	Load (days 2 and 4)	Ratio (days 2 and 4)
Creatinine		7.8[a]		
Caffeine	16	8	15	15
Methamphetamine	16	17	21	21
Benzoylecgonine	22	23	24	24
Cocaine	22	22	17	17

[a] The separate analysis for creatinine was conducted in a single sequence that included all the samples from the four sampling days.

Table S7. Average rate constants ± 95% confidence interval and the maximum estimated percent loss for analytes in samples stored at 4ºC in the autosampler for 24 h.

Substance	Average first-order rate constant ± 95% CI (h^{-1})	Estimated maximum loss at 24h (%)
Creatinine	-6.08E-03 ± -2.06E-3	13
Caffeine*	-1.18E-03 ± -1.95E-3	5
Methamphetamine*	-2.89E-04 ± -5.42E-3	8
Benzoylecgonine	-1.57E-02 ± -4.90E-3	32
Cocaine*	-2.03E-03 ± -1.13E-2	16

*Rate constant not statistically different from zero at the 95% CI.

Table S8. Estimated total urine volume for the municipality, the estimated population for a range of urine volumes from the literature,[b] and the % of the maximum (63,000) population (residents +commuters) as computed from Equation S3.

	Total Measured Mass Creatinine (kg)[a]	Estimated Total Volume Urine of the Municipality (L)	Population urine (L) per person per day 0.60 L	1.1 L	1.7 L	% of Maximum Population[c]
Wednesday	36	28,000	17,000	18,000	31,000	26-49%
Thursday	27	21,000	12,000	14,000	23,000	20-37%
Friday	22	17,000	10,000	11,000	19,000	16-30%
Saturday	19	15,000	9,000	10,000	16,000	14-30%

[a] Taken from Table 1.
[b] To estimate population from Equation S3, average urine volumes ± one standard deviation (L/d) as reported by Murakami et al. for n=654 individuals were used.[3]
[c] A total population of 63,462 is estimated for weekdays and 54,462 for Saturday.[2]

Equation S1. Calculation of total uncertainty from analysis, sampling, and flow.

$$\text{Total uncertainty} = \sqrt{(\text{RSD analytical})^2 + (\text{RSD sampling})^2 + (\text{RSD flow})^2}$$

Equation S2. Calculation of total uncertainty for analytes normalized to creatinine from analyses of analytes (A) and creatinine (Creat) and the sampling error for each analyte and creatinine.

$$\text{Total uncertainty} = \sqrt{(\text{RSD analytical}_A)^2 + (\text{RSD sampling}_A)^2 + (\text{RSD analytical}_{Creat})^2 + (\text{RSD sampling}_{Creat})^2}$$

Equation S3. Calculation to estimate total volume of urine for the municipality and the corresponding estimated number of people (population). The concentration of creatinine in urine (1,300 mg/L) was estimated from the known ethnic/age demographics of the community and the corresponding urine creatinine concentrations as reported by Barr et al.[1]

$$\boldsymbol{Creat_{total}^{municipality}\,(kg) * \frac{10^6 mg}{kg} * \frac{L_{urine}}{1{,}300\,mg} = L_{urine}^{municipality} * \frac{person}{L_{urine}} = population^{municipality}}$$

Equation S4. Calculation of caffeine doses assuming a 100 mg dose.

$$\frac{\text{Caffeine excreted (mg)}}{\text{day}} * \frac{\text{100 mg ingested}}{\text{1 mg excreted}} * \frac{\text{1 dose}}{\text{150 mg}} = \frac{\text{dose}}{\text{day}}$$

Equation S5. Calculation of methamphetamine doses assuming a 100 mg dose.

$$\frac{\text{Methamphetamine excreted (mg)}}{\text{day}} * \frac{\text{100 mg ingested}}{\text{40 mg excreted}} * \frac{\text{1 dose}}{\text{100 mg}} = \frac{\text{dose}}{\text{day}}$$

Equation S6. Calculation of cocaine doses from mass of benzoylecgonine (BZE) assuming a 100 mg dose.

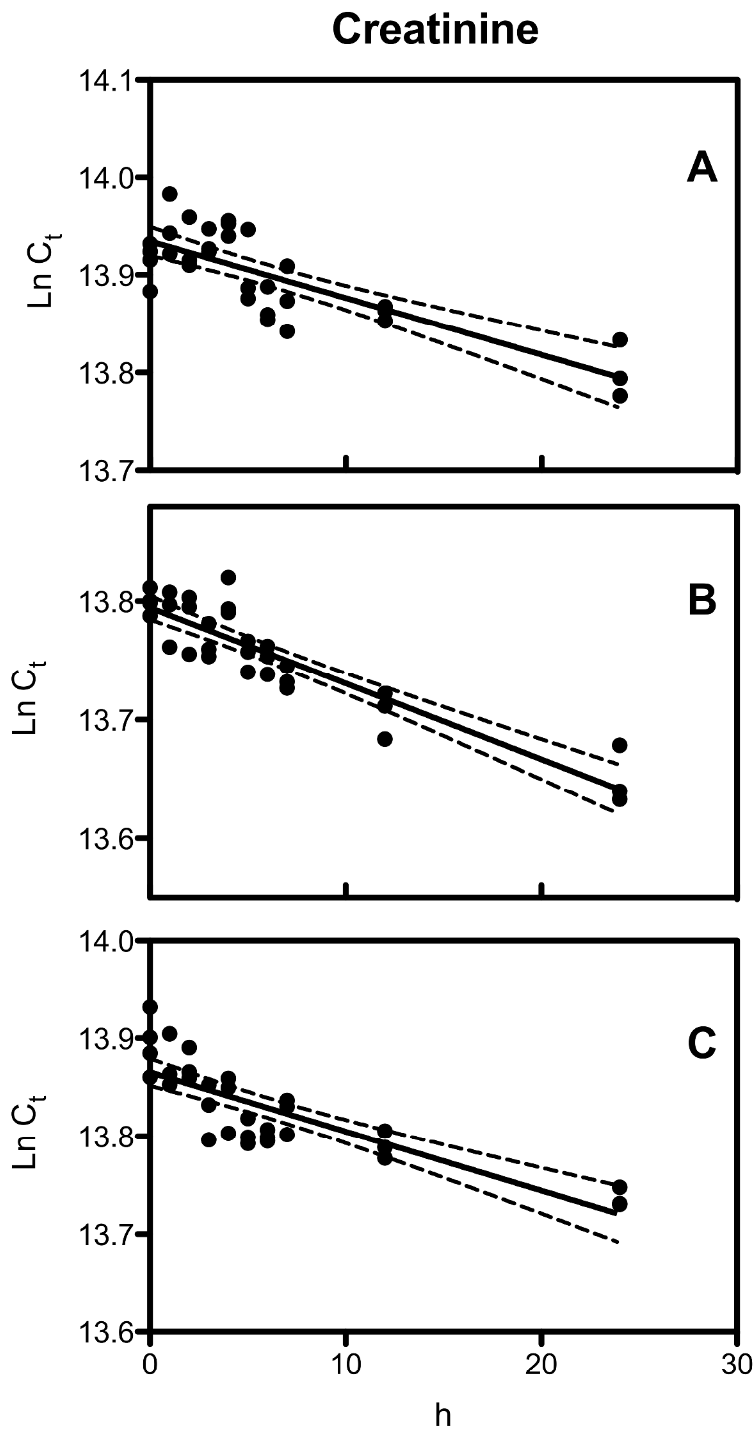
$$\frac{\text{BZE excreted (mg)}}{\text{day}} * \frac{\text{1 mM cocaine}}{\text{0.45 mM BZE excreted}} * \frac{\text{303E-4 mg cocaine}}{\text{1 mM cocaine}} * \frac{\text{1mM BZE}}{\text{289E-4 mg BZE}} * \frac{\text{dose}}{\text{100 mg}} = \frac{\text{dose}}{\text{day}}$$

Equation S7. Calculation of cocaine doses assuming a 100 mg dose.

$$\frac{\text{Cocaine excreted (mg)}}{\text{day}} * \frac{\text{100 mg ingested}}{\text{1 mg excreted}} * \frac{\text{1 dose}}{\text{100 mg}} = \frac{\text{dose}}{\text{day}}$$

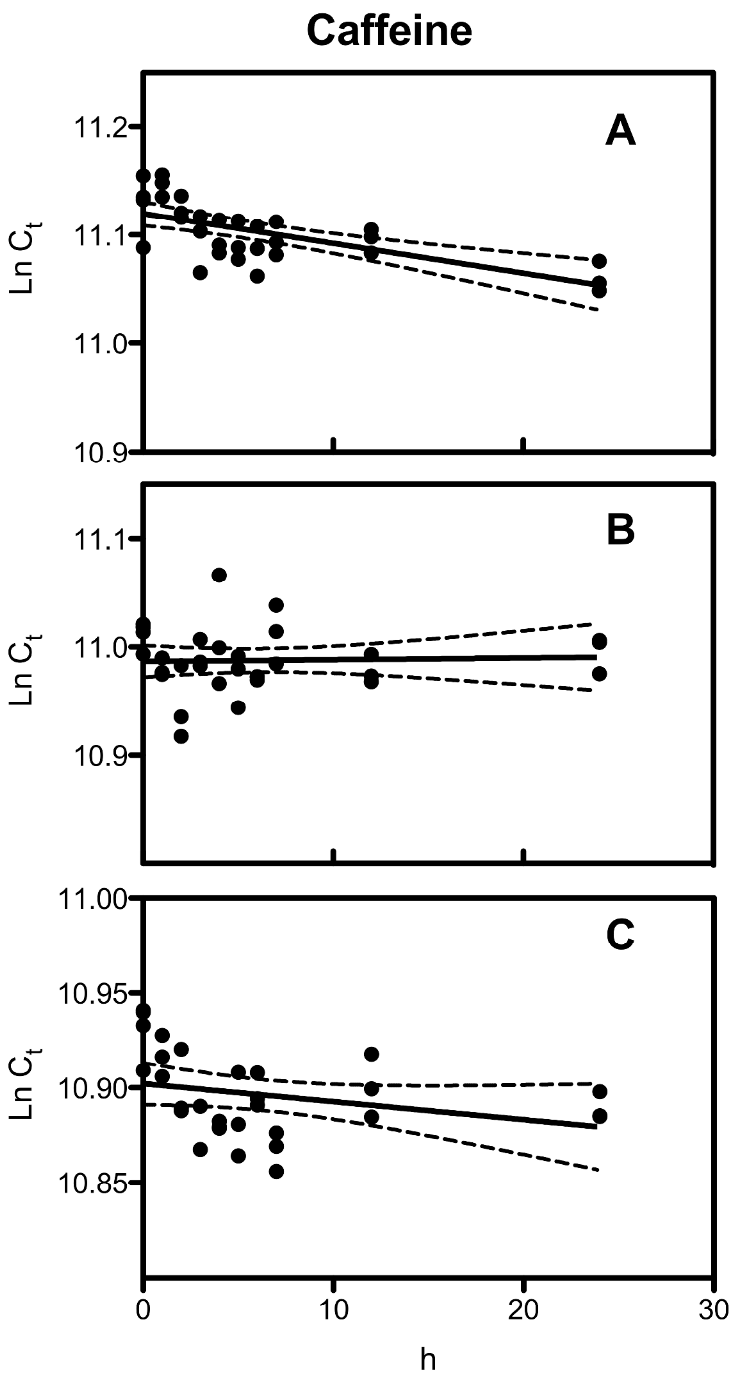
FIGURES

Figure S1. Creatinine concentrations over 24 h at 4ºC for three different days (A, B, and C). Dashed lines represent the 95%CI about the slope.[1]



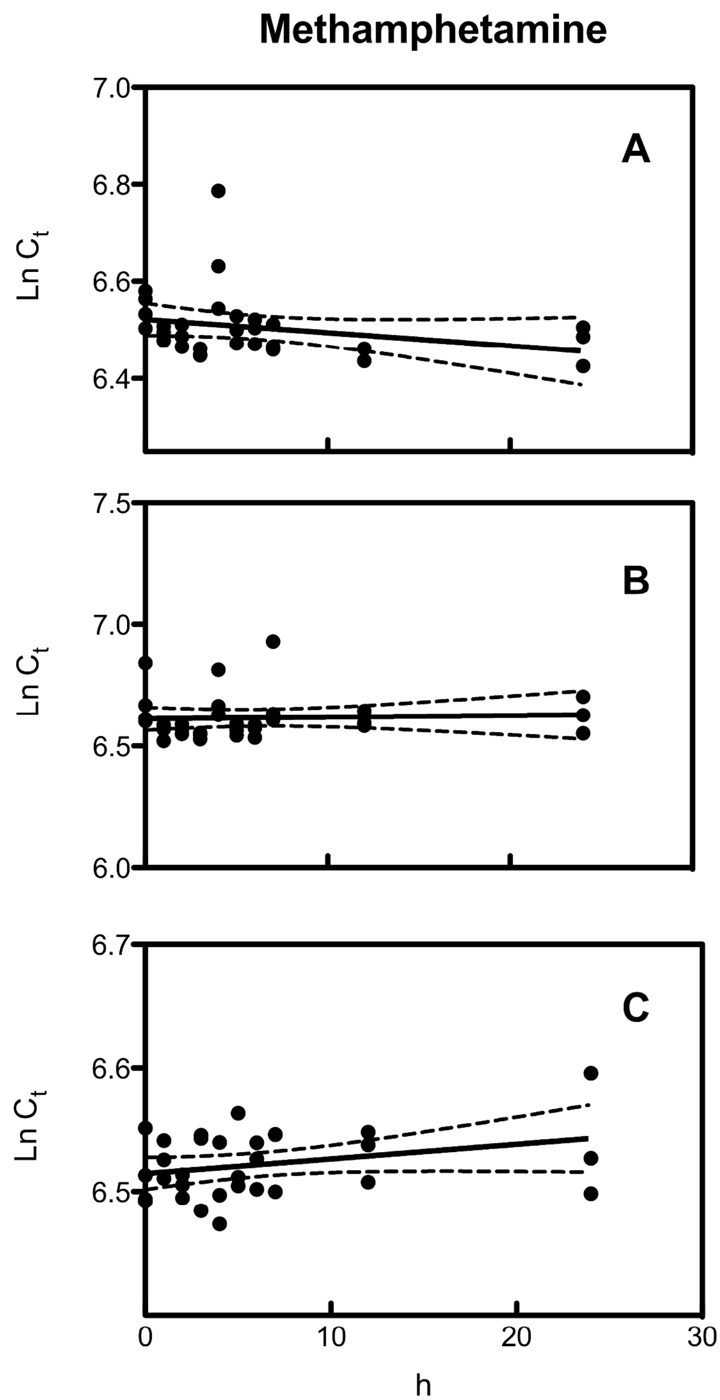
[1]n =3 at each time point except time = 0 where n=4

Figure S2. Caffeine concentrations over 24 h at 4ºC for three different days (A, B, and C). Dashed lines represent the 95%CI about the slope.[1]



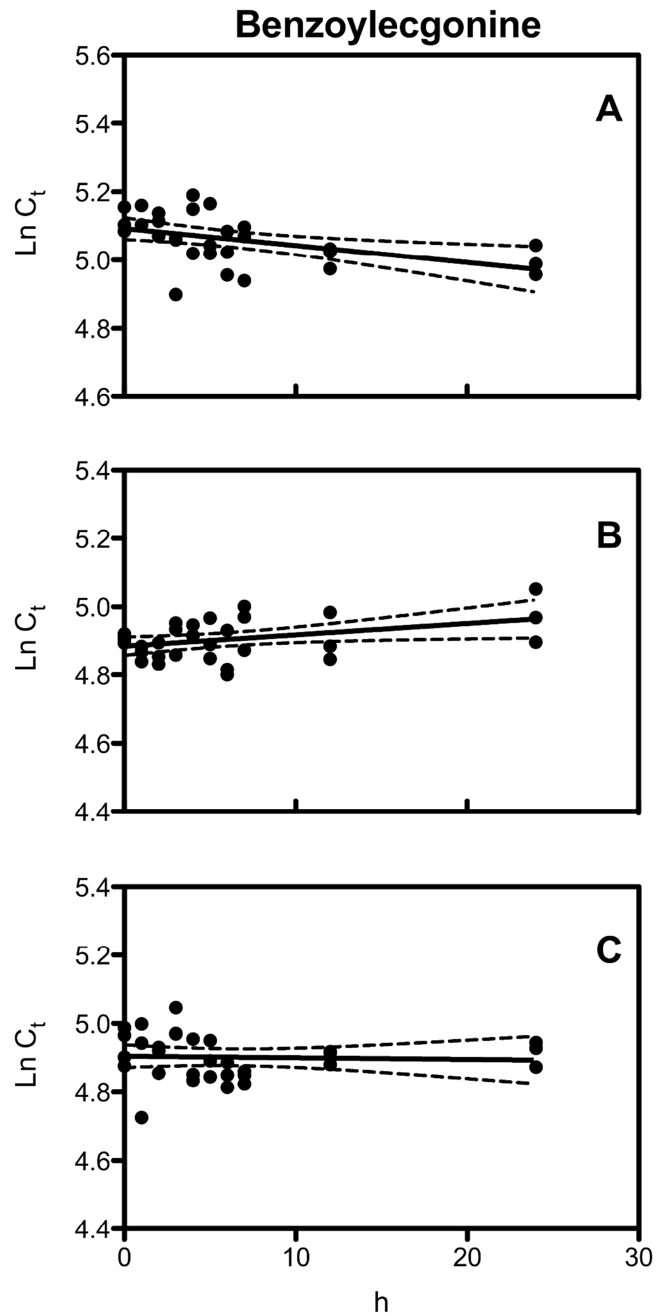
[1]n =3 at each time point except time = 0 where n=4

Figure S3. Methamphetamine concentrations over 24 h at 4ºC for three different days (A, B, and C). Dashed lines represent the 95%CI about the slope.[1]



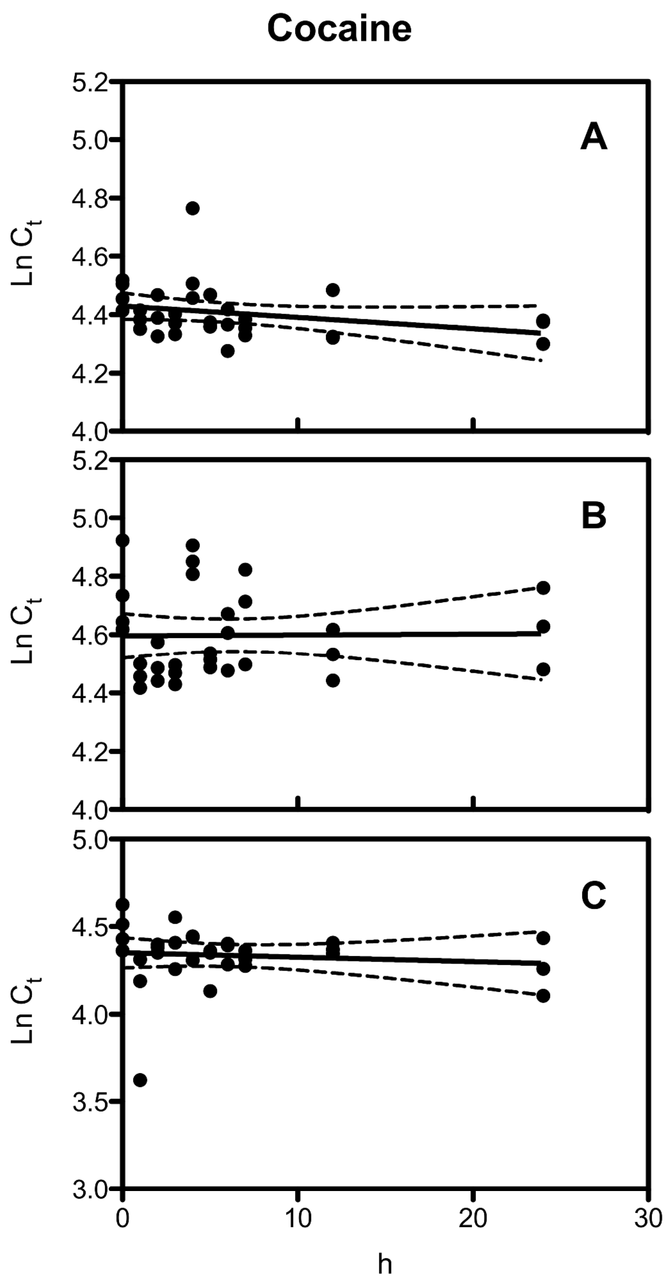
[1]n =3 at each time point except time = 0 where n=4

Figure S4. Benzoylecgonine concentrations over 24 h at 4ºC for three different days (A, B, and C). Dashed lines represent the 95%CI about the slope.[1]



[1]n =3 at each time point except time = 0 where n=4

Figure S5. Cocaine concentrations over 24 h at 4ºC for three different days (A, B, and C). Dashed lines represent the 95%CI about the slope.[1]



[1]n =3 at each time point except time = 0 where n=4

Figure S6. Hourly concentrations of creatinine, caffeine, benzoylecgonine, cocaine, and methamphetamine.

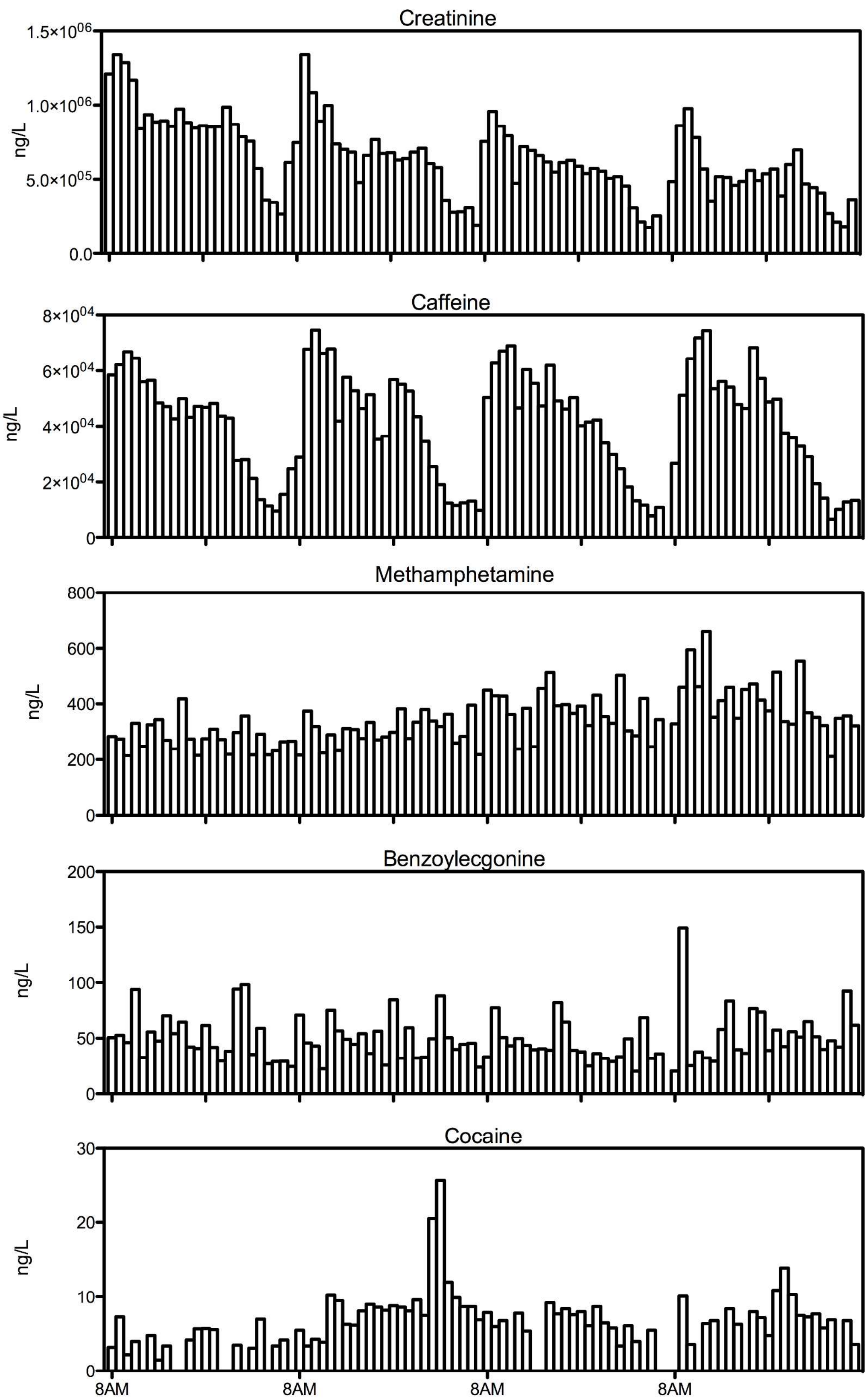


Figure S7. Ratios (mg/mg) of analytes to creatinine after applying degradation rate constant from stability study.