Supporting Information:

Pharmaceutical Formulation Facilities as Sources of Opioids and Other Pharmaceuticals to Wastewater Treatment Plant Effluents

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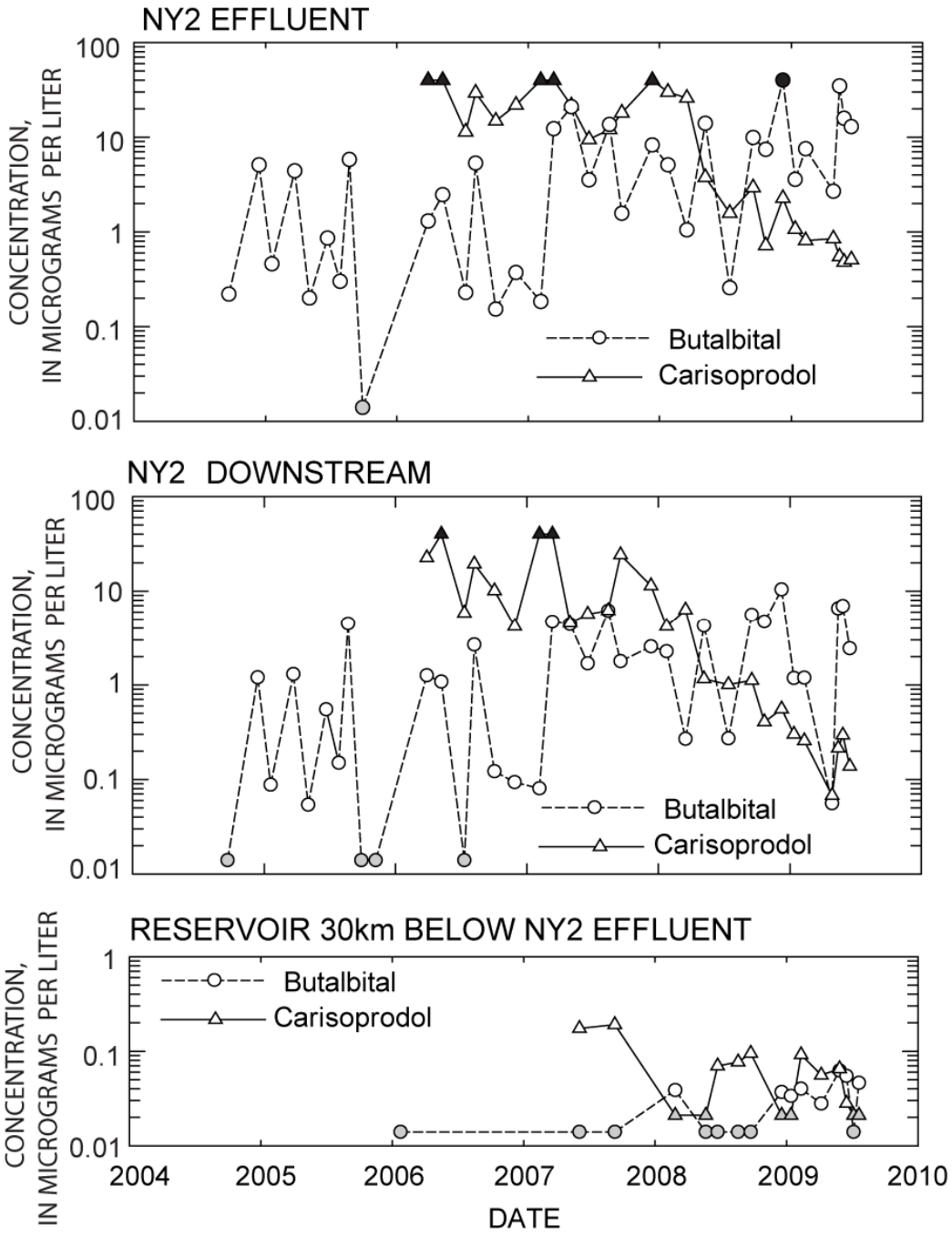
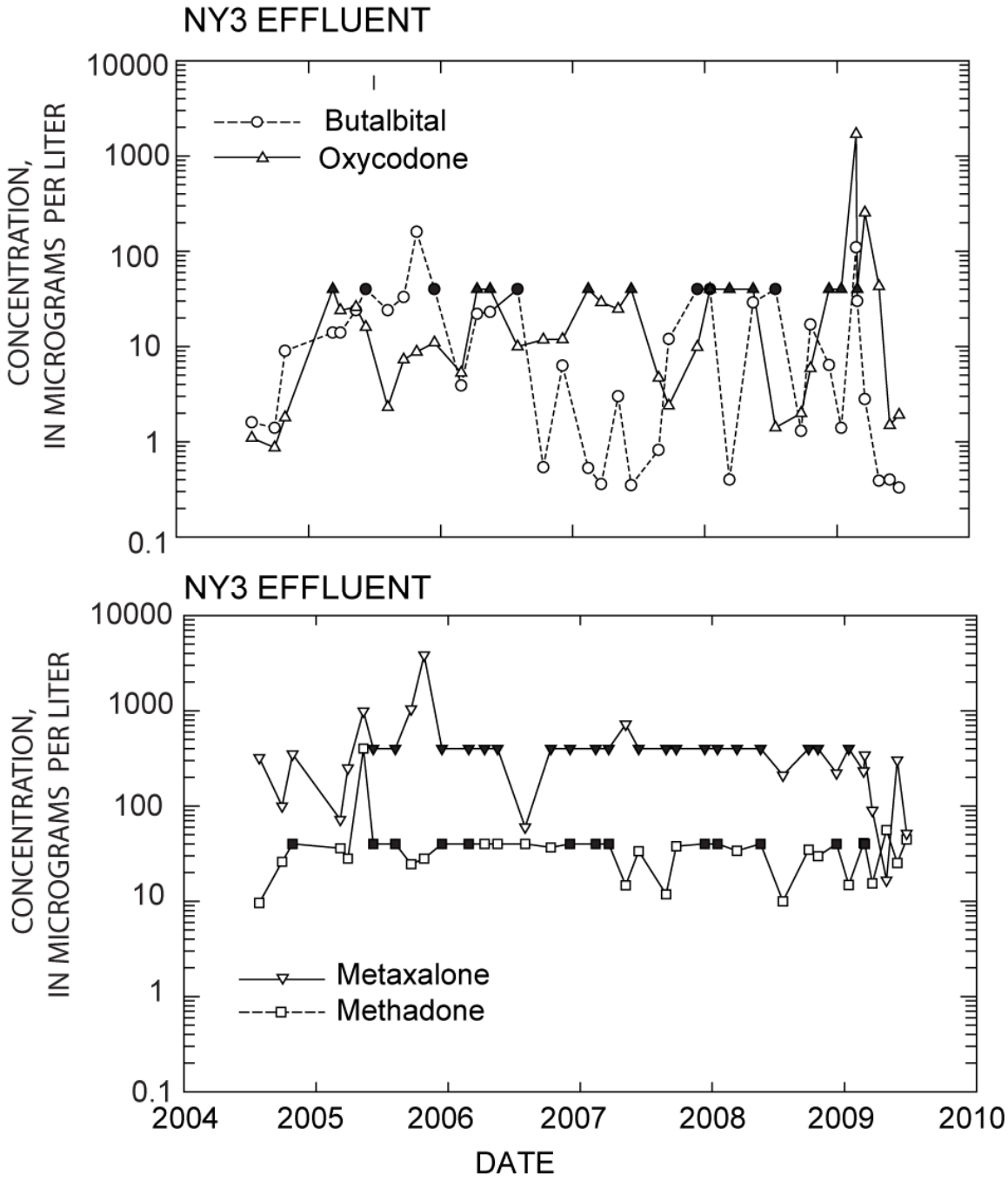
Twenty-nine pages, including 5 Figures and 16 Tables.

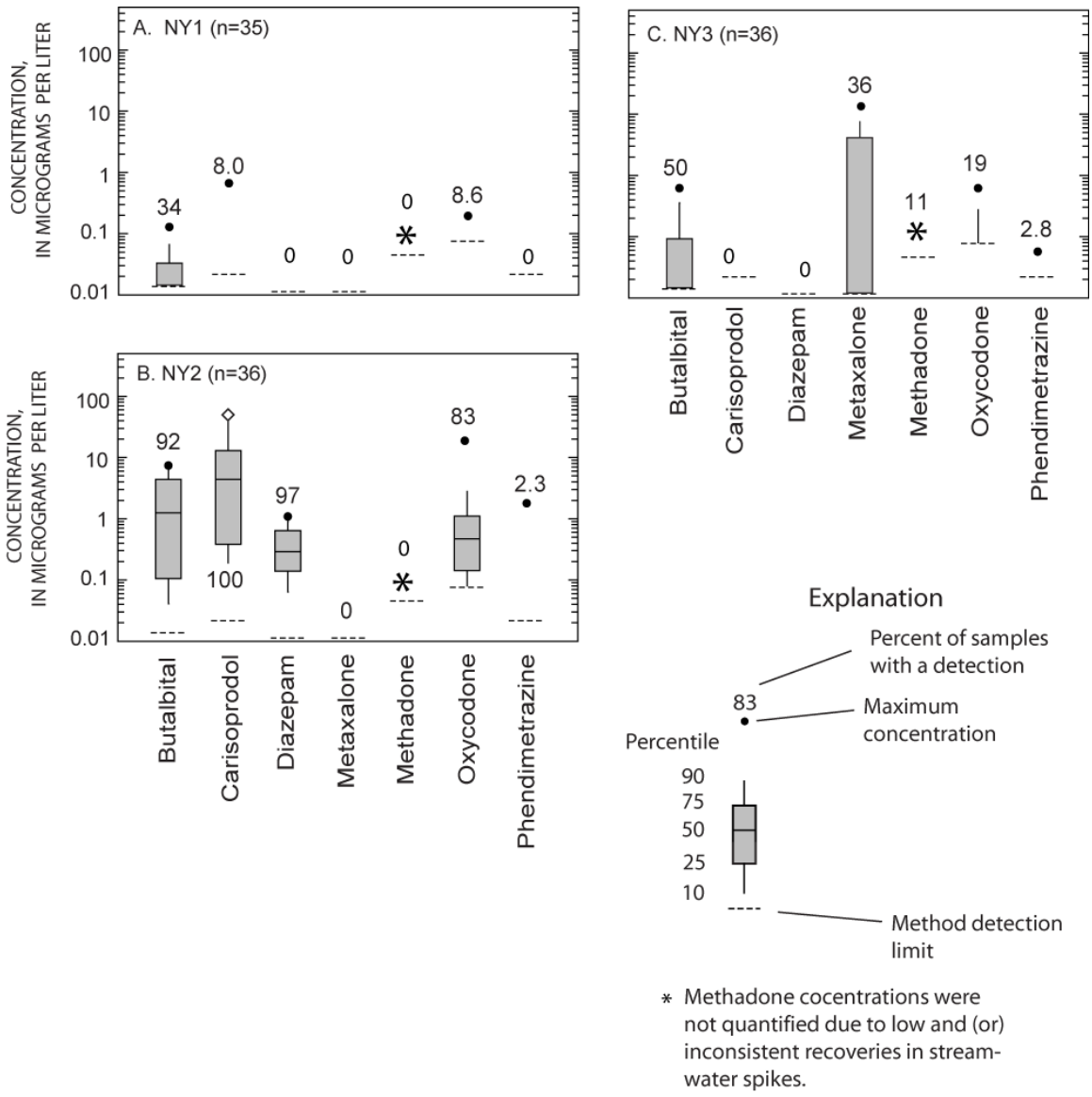
Pages S-2 through S-9 include figures S1-S4, and tables S1-S5, which are referred to directly in the Results and Discussion portion of the text.
Pages S-10 through S-26 provide details on the analytical method used for determination of 7 pharmaceuticals, and include tables S6-S16.



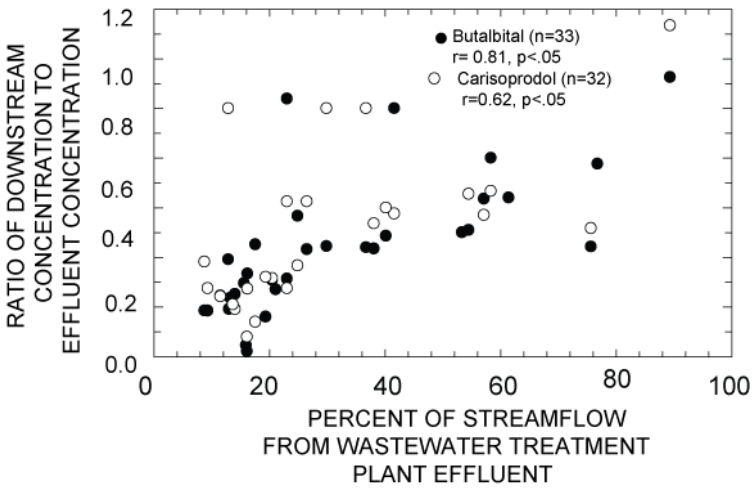
Supplemental Figure S-1. Concentrations of carisoprodol and butalbital for samples from NY2 effluent, NY2 downstream, reservoir site 30 km below NY2 effluent site. Symbols filled in black denote concentrations that exceeded uppermost calibration point of 40 micrograms per liter, and are plotted at 40 micrograms per liter. Symbols plotted in gray denote non-detects and are plotted at the method detection limit.



Supplemental Figure S-2. Concentrations of oxycodone and butalbital, and metaxalone and methadone in samples from NY3 effluent, 2004-2009. Symbols filled in black denote concentrations that exceeded uppermost calibration point of 40 mg/L (micrograms per Liter) for butalbital, methadone, and oxycodone, or 400 mg/L for metaxalone; these are plotted at the highest point on the calibration curve. Concentrations of butalbital, methadone and oxycodone plotted above 40 mg/L and metaxalone plotted above 400 mg/L prior to April 2009 represent samples that were quantified above the highest point on the calibration curve through 1) diluting samples, 2) extracting lesser amounts of sample, or 3) re-analyzing frozen archived samples.



Supplemental Figure S-3. Concentrations of seven pharmaceuticals analyzed in samples collected downstream of effluent from WWTPs (wastewater treatment plants) NY1, NY2, and NY3 during 2004-2009. Box plots depict range of concentrations, and are explained in Figure 1. Number above boxplot refers to percent detection. Numbers of samples for each site are indicated next to the site name. 25 samples were analyzed for carisoprodol at the site downstream of site NY1 effluent, and 26 samples were analyzed for carisoprodol downstream of NY2 and NY3 effluent sites. An asterisk (*) denotes that concentrations for methadone were not quantified due to poor and inconsistent recovery in spikes of streamwater; the percent of samples with a detection for methadone are given above the asterisk. Dashed line at bottom of boxplot or whisker denotes method detection limit.



Supplemental Figure S-4. Ratio of butalbital and carisoprodol concentrations in downstream water to concentration in NY2 effluent versus the percentage of WWTP effluent contribution to streamflow. r refers to Spearman rank correlation, and p is the corresponding probability value.

Supplemental Table S-1. Information on 23 wastewater treatment plants (WWTP) sampled once during 2006-2009 in a national survey of WWTP effluent and three WWTPs in New York State sampled multiple times during 2004-2009. All sites except IA2, NY1, and NY4 use activated sludge for biologic treatment; site NY4 uses rotating biologic contactor; sites IA1 and NY1 use trickling filter. Samples from sites CA1, MD1, NY1, NY2, NY3, and VT1 were collected as 24 hour flow-weighed composites.

Site Code	Location	Population Served	Pharmaceutical Source: hospital (H) or pharmaceutical manufacturer (P)	Effluent discharge rate (m^3/s)
National Survey				
AZ1	Arizona-1	500000	H	1.0
AZ2	Arizona-2	1000000	H	1.4
CA1	California	4000000	H	20
CO1	Colorado	110000	H	1.1
FL1	Florida	1250000	H	8.8
IA1	Iowa-1	25000	H	0.22
IA2	Iowa-2	42000		0.38
MD1	Maryland	1300000	H	7.9
MT1	Montana-1	30000	H	0.12
NV1	Nevada	300000	H	1.3
NY4	New York-4	800		0.0033
NY5	New York-5	200		0.0027
NY6	New York-6	1000		0.013
NY7	New York-7	1350		0.0088
NY8	New York-8	3500		0.051
NY9	New York-9	800	H	0.011
TX1	Texas-1	1200000	H	3.2
TX2	Texas-2	1400		0.013
TX3	Texas-3	37000	H	3.3
VT1	Vermont-1	31000		0.20
VT2	Vermont-2	7000	H	0.044
WI1	Wisconsin-1	1100000	H	4.40
WI2	Wisconsin-2	330000	H	1.80
New York Sites Sampled Multiple Times				
NY1	New York-1	10000		0.061
NY2	New York-2	3000	H,P	0.031
NY3	New York-3	400	P	0.0031

Supplemental Table S-2. Number of effluent and stream-water samples collected at sites NY1, NY2, and NY3 between 2004-2009, and types of treatment provided by each of these WWTPs.

Site	Number of Effluent Samples	Number of Downstream Samples	Secondary Biological Treatment	Tertiary Treatment	Disinfection
NY1	36	35	Trickling Filter	Sand Filtration	Chlorination/ Dechlorination
NY2	35	36	Two Stage Activated Sludge	Sand Filtration	Chlorination/ Dechlorination
NY3	38	36	Extended Aeration Activated Sludge	Sand/ Anthracite Micro-filtration	Ultraviolet

Supplemental Table S-3. Distance downstream and effluent dilution at stream sampling sites.

Site	Distance Downstream (km)	Median Effluent Discharge (L/s)	Median Streamflow (L/s)	Median Percent Streamflow from Effluent
NY1	0.01	53	330	15
NY2	1.2	30	120	24
NY3	6.1	2.8	1500	0.17

Supplemental Table S-4.
Pharmaceuticals formulated by the pharmaceutical formulation facility (PFF) or marketed by the PFF owner discharging to wastewater treatment plant (WWTP) NY2 and either 1) quantified in samples of NY2 effluent or 2) qualitatively identified in samples collected 2008-2009, or 3) not identified in samples of NY2 effluent. Compounds with 'na' for Source of Formulation Information were not identified as pharmaceutical products formulated at the PFF, but were included because they were qualitatively and may represent a degradate of pharmaceutical formulated at plant. N, identified as manufactured at the PFF or distributed by the corporation owning the PFF (*1*); M, identified as distributed by the corporation owning the PFF (*2*); F, identified as formulated at the plant by the FDA (*3*); na, not applicable.

Compound	CASRN[a]	Source Indicating Production or Marketing	Compound Type/Use
Pharmaceuticals Quantified in This Study			
Butalbital	77-26-9	N,M	Barbiturate
Carisoprodol	78-44-4	M	Muscle Relaxant
Diazepam	439-14-15	N,M	Benzodiazepine
Oxycodone	76-42-6	F,N,M	Opioid
Pharmaceuticals Qualitatively Identified in Samples Using a Standard, but not Quantified[b]			
2-Ethyl-2-phenylmalonamide	7206-76-0	na	Primidone degradate
Bupropion	34841-39-9	F,N,M	Antidepressant
Diltiazem	42399-41-7	N,M	Calcium channel blocker
Hydrocodone	125-29-1	N,M	Opiod
Meprobamate	57-53-4	N,M	Antianxiety, carisoprodol degradate
Methocarbamol	532-03-6	N,M	Muscle Relaxant
Methylphenidate	113-45-1	N,M	Psychostimulant (ritalin)
Phenobarbital	50-06-6	na	Metabolite of primindone
Primidone	125-33-7	N,M	Antiepileptic
Verapamil	52-53-9	N,M	Calcium channel blocker
Pharmaceuticals Identified as Formulated at Site, but not Identified in Samples			
Acetaminophen	103-90-2	F	Analgesic
Anagrelide	68475-42-3	F	Platelet reducing agent
Colchicine	64-86-8	F	Rheumatic treatment
Gabapentin	60142-96-3	F	GABA analog, pain relief
Hydrochlorothiazide	58-93-5	F	Thiazide diuretic
Ibuprofen	15687-27-1	F	NSAID
Meperidine	57-42-1	F	Opioid
Metformin	657-24-9	F	Anti-diabetic
Quinidine	56-54-2	F	Antiarrhytmic
Sulindac	38194-50-2	F	NSAID
Trazodone	19794-93-5	F	Piperazine antidepressant

[a] CASRS, CAS Registry Number® is a Registered Trademark of the American Chemical Society. CAS recommends the verification of the CASRNs through CAS Client Services℠ .
[b] Compound qualitatively identified using criteria given in table S-18 for samples collected 2008-2009.

Supplemental Table S-5.
Pharmaceuticals formulated by the pharmaceutical formulation facility (PFF) or marketed by the PFF owner discharging to wastewater treatment plant (WWTP) NY3 and either 1) quantified in samples of NY3 effluent or 2) qualitatively identified in samples collected 2008-2009, or 3) not identified in samples of NY2 effluent. Compounds with 'na' for Source of Formulation Information were not identified as pharmaceutical products formulated at the PFF, but were included because they were identified as TICs, and may represent a degradate of pharmaceutical formulated at plant. N, identified as manufactured at the PFF or distributed by the corporation owning the PFF (*1*); M, identified as distributed by the corporation owning the PFF (*4*); F, identified as formulated at the plant by the FDA (*3*); S, identified as formulated at the PFF by New York State FAIR report (*5*); na, not applicable.

Compound	CASRN[a]	Source Indicating Production or Marketing	Compound Type/Use
Pharmaceuticals Quantified in This Study			
Butalbital	77-26-9	N,M	Barbiturate
Metaxalone	1665-48-1	S,N	Muscle Relaxant
Methadone	76-99-3	F,N,M	Opioid
Oxycodone	76-42-6	N,M	Opioid
Phendimetrazine	634-03-7	N	Anorectic
Pharmaceuticals Qualitatively Identified in Samples Using a Standard, but not Quantified[b]			
Acetaminophen	103-90-2	F,S,N,M	Analgesic
Chlorpheniramine	132-22-9	N,M	Antihistamine
Codeine	76-57-3	F,N,M	Opioid
Dihydrocodeine	125-58-0	na	Opioid and opioid degradate
Fluoxetine	54910-89-3	F,N,M	SSRI antidepressant
Hydrocodone	125-29-1	F,N,M	Opioid
Meperidine	57-42-1	N,M	Opiod
Methylphenidate	113-45-1	N,M	Psychostimulant (ritalin)
o-Desmethyltramadol	7396-53-5	na	Tramadol degradate
Temazepam	846-50-4	F,N,M	Benzodiazepine
Tramadol	27203-92-5	F,N,M	Opioid
Pharmaceuticals Identified as Formulated at Site, but not Identified in Samples			
Cocaine	50-36-2	F	Dopamine reuptake inhibitor
Dextroamphetamine	51-64-9	F	Amphetamine
Morphine	57-27-2	F	Opioid

[a] CASRN, CAS Registry Number® is a Registered Trademark of the American Chemical Society. CAS recommends the verification of the CASRNs through CAS Client Services℠ .
[b] Compound qualitatively identified using criteria given in table S-18 for samples collected 2008-2009.

Method Description and Method Performance Information

INTRODUCTION

The results contained in this supplemental material section provides 1) a description of the analytical method used for the results discussed in this paper and 2) performance characteristics of the method, including a holding time study. The seven pharmaceuticals reported in this study were initially reported as tentatively identified compounds (TICS) in wastewater treatment plant (WWTP) effluent samples analyzed for 61 anthropogenic waste indicator compounds (*6*) in samples collected prior to July 2004. Six of these compounds were included in the initial implementation of the method (for samples collected between the July 2004-March 2006), and carisoprodol was added for samples collected after March 2006.

Authentic standards for the pharmaceuticals included in the study were subsequently obtained and a method implemented to provide quantitative data for the target pharmaceuticals in samples collected from these sites from July 2004 through October 2009. Unexpectedly, observed concentrations ranged over several orders of magnitude and were substantially higher than any reported for these or other pharmaceuticals in waters of the United States, therefore some method performance data was necessarily calculated after sample analysis began. The supplemental information provided herein is provided to enable the reader to evaluate the data generated by the method, and to put the data presented in this study in the context of method performance.

METHOD DESCRIPTION

Determination of the seven pharmaceuticals measured in this study (Table S-6) was based upon a previously described method that was used for the determination of 61 compounds typically found in domestic and industrial wastewater (*6*).

Sample Preparation, Apparatus and Instrumentation, and Standards

One-liter samples were filtered through 0.7-µm glass-fiber filters. After filtration, three surrogate compounds (Table S-6) were added to each environmental and/or quality-control sample to monitor sample-specific method performance. Samples were extracted by vacuum through 500 mg OASIS-HLB-SPE cartridges (Waters Inc., catalog number 186000115) using a custom extraction manifold (*6*). SPE Cartridges were eluted with dichloromethane:diethyl ether, 80:20 volume per volume. Internal standards were added to sample extracts before the sample extracts are evaporated to 0.4-mL, placed in a 1.5 mL autosampler vial and held at -4 degrees C until instrumental analysis. Laboratory reagent blank and spike samples were prepared with each set of up to 10 environmental samples.

Sample extracts were analyzed by capillary gas chromatography/mass spectrometry (GC/MS, Agilent Technologies model 6890 GC and model 5973 MS) and operated under full scan conditions using electron impact ionization at 70 electron volts.

The GC conditions were:

Column: HP Ultra II (5 % phenylmethyl silicone), 30 m x 0.25 mm, 0.50 μm film thickness.

Carrier gas: ultra high purity helium with a linear-flow velocity of 32 cm/sec.

Injection port temperature: 290 ^{o}C; 1 microliter volume.

Split vent open, 0.7 min.

GC oven temperature program: initial temperature, 40 ^{o}C; then ramp rate, 4 ^{o}C/min to 100 ^{o}C, then 8 ^{o}C/min to 350 ^{o}C; hold time, 2 min at 350 ^{o}C.

The mass spectrometer conditions were:

Ionization energy: 70 eV

Operation: Full-scan from 50 to 450 atomic mass units (amu) at 1 scan/sec.

Temperatures: source 230 ^{o}C; GC/MS interface, 290 ^{o}C.

Authentic standards were obtained for the seven pharmaceuticals that were included in the study. Metaxalone and phendimetrazine standards were obtained from Toronto Research Chemicals. All the remaining standards were obtained from Fisher Scientific. Purity for all compounds were 99% or better.

Analytes were required to meet qualitative identification criteria prior to quantification. The qualitative criteria include comparison with the authentic reference standard for confirmation of GC retention time and mass spectral performance. This requires that the suspected analyte GC retention time was within +/- 0.05 minute of that of the authentic standard and the mass spectral quantitation ion and two confirmation ion abundance ratios were within +/- 20% of the values determined by analysis of the authentic standard under identical experimental conditions. Analytes that meet these qualitative criteria were then quantified using the injection internal standard method using a 5–8 point calibration curve (*6*).

Table S-6. Pharmaceutical method compound measured retention time, quantitation ion, confirmation ions, surrogate compounds, and internal standard reference compounds. [min, minutes; m/z, mass-to-charge ratio]

Compound name	Retention Time (min)	Quantitation Ion (m/z)	Confirmation Ion (m/z)	Confirmation Ion (m/z)
Butalbital	30.96	168	167	181
Carisoprodol	33.53	245	158	184
Diazepam	38.70	256	283	221
Metaxalone	36.32	122	221	107
Methadone	36.13	294	72	165
Oxycodone	39.53	315	258	230
Phendimetrazine	27.74	191	51	85
Surrogates				
Caffeine-d_9	33.07	203	115	--
Decafluorobiphenyl	19.59	334	265	--
Fluoranthene-d_{10}	36.31	212	106	--
Internal Standards				
Acenaphthene-d_{10}	28.64	164	162	160
Chrysene-d_{12}	38.79	240	--	--
Perylene-d_{12}	41.97	264	132	--
Phenanthrene-d_{10}	32.63	188	--	--

Calibration Curves

The method used in this study was initially set up to detect expected low environmental concentrations of the target compounds. The initial maximum calibration point for most compounds was 40 μg/L, except diazepam (4 μg/L) and metaxalone (400 μg/L). Because concentrations of many compounds in WWTP effluent samples from NY2 and NY3 consistently exceeded the highest point on the calibration curve, the method was slightly modified in 2009 to provide the ability to quantify larger concentrations. Maximum calibration points were extended to allow for quantitation over a broader range of concentrations, and calibration curves were extended to 400 μg/L for all compounds except metaxalone (4000 μg/L).

For samples collected before August 1 2007, minimum calibration points were 0.4 μg/L for all analytes with the exception of diazepam (0.04 μg/L) and metaxalone (4 μg/L). After August 1 2007, calibration curves were extended to 0.04 μg/L for most analytes,

and to 0.004 μg/L for diazepam and 0.4 μg/L for metaxalone. Concentrations exceeding the calibration curve are reported as > the maximum calibration concentration. For select samples collected before April 2009, concentrations were quantified above the maximum calibration point by either 1) diluting samples, 2) extracting lesser amounts of sample, or 3) analysis of frozen archived samples using the higher calibration curves. Overall, much of the data from site NY3 effluent samples were affected by the calibration curve dynamic range limitation, with between 20-60% of samples right-censored for butalbital, metaxalone, methadone, and oxycodone. A few samples (less than 20%) for carisoprodol and butalbital were right-censored at 40 μg/L in NY2 effluent samples. Some low-level concentrations were estimated between the MDL and the lowest point on the calibration curve, particularly for samples collected before August 2007, but these estimated values were generally within 50% of the lowest point on the calibration curve.

METHOD PERFORMANCE

The measures of method performance discussed below reflect (1) method performance results collected over the entire period when environmental samples were analyzed and (2) specific method performance evaluations made after the range of concentrations likely to occur was better understood.

Reagent Set Spike Samples

Reagent set spike samples were reagent water samples fortified with known amounts of the seven pharmaceuticals and processed with a set of environmental samples. The final fortification concentration reflects the sensitivity of each compound in the analysis. Individual reagent spike samples were used to evaluate set-specific method performance in the absence of sample matrix components. The individual reagent set spike results can be used to assess overall method bias and precision by aggregating individual reagent set spike samples and calculating mean recoveries and relative standard deviations (RSDs).

Reagent set spike data were divided into two periods corresponding to different spiking concentrations. Reagent set spike data for analysis before 2009 are given in Table S-7, and those after 2009 are given in Table S-8. Spiking concentrations were higher for the reagent set spikes analyzed before 2009; spiking concentrations were decreased for analyses in 2009 to better coincide with concentrations typically present in samples. Analysis of reagent set spike recoveries over the duration of the study indicated no temporal trends, suggesting method performance stability over the course of the study. The difference in pre-2009 and 2009 reagent set spike recoveries and RSDs was likely due to the difference in spiking levels and to a lesser extent the difference in the number of spikes between the two periods.

Table S-7. Mean recovery and relative standard deviation of pharmaceuticals from reagent set spike samples analyzed before 2009. [RSD, relative standard deviation].

Pharmaceutical	Number of Spikes	Fortification Concentration, in micrograms per Liter	Mean Percent Recovery	Percent RSD
Butalbital	54	8	82	18
Carisoprodol	54	8	113	21
Diazepam	54	0.8	96	23
Metaxalone	54	80	102	14
Methadone	54	8	70	28
Oxycodone	54	8	72	34
Phendimetrazine	54	8	86	22

Table S-8. Mean recovery and relative standard deviation of pharmaceuticals from reagent set spike samples for 2009 [RSD, relative standard deviation]

Pharmaceutical	Number of Spikes	Fortification Concentration, in micrograms per Liter	Mean Percent Recovery	Percent RSD
Butalbital	24	0.2	124	22
Carisoprodol	24	0.2	140	27
Diazepam	24	0.2	111	17
Metaxalone	24	0.2	118	23
Methadone	24	0.2	66	25
Oxycodone	15	0.2	105	46
Phendimetrazine	19	0.2	83	21

Mean reagent set spike recoveries for both sets of reagent set spike data ranged from 66-140%, and RSDs were generally less than 30%. The only mean reagent set spike outside the 60-130% range was the low-concentration carisoprodol spike (140%). Only two RSDs were greater than 30%: RSDs for the oxycodone low-concentration and high-concentration spikes were 34 and 46%, respectively.

Matrix Spike Recoveries

Reagent set spike recoveries reflect method performance in the absence of coextracted sample matrix components. In order to better assess these contrasting matrix effects on the seven pharmaceuticals in this study, replicate samples from three different wastewater sources and a stream sample below one of these wastewater discharges were fortified with all or a select group of these pharmaceuticals and recoveries determined after correction for ambient compound concentrations from analysis of two or more unspiked replicates.

The four samples spiked were: 1) an NY3 effluent spiked at high concentrations (5-4000 μg/L), 2) an NY1 effluent spiked at moderate (0.8 to 80 μg/L) concentrations, 3) an NY4 effluent spiked at low (0.2 μg/L) concentrations, and 4) a stream sample collected upstream of the NY1 WWTP discharge (hereafter referred to as the NY1 upstream sample) spiked at low-moderate concentrations (0.08 to 8 μg/L). The number of replicate spikes ranged from five to 13 for each matrix spike. These matrices included various wastewaters with a wide range of expected pharmaceutical concentrations and a streamwater with no effluent discharge.

NY3 Effluent Matrix Spiking Results: A single composite sample of NY3 effluent collected in May 2009 was divided into six replicate one Liter samples, and fortified with five of the seven pharmaceuticals at high concentrations (5 to 4000 μg/L) to assess method performance for very high pharmaceutical concentrations in wastewater-enriched environmental samples. These samples were extracted and analyzed in a single analytical set. Any ambient environmental contributions of pharmaceuticals to the matrix spike replicates were corrected for by duplicate analysis of unspiked samples. Results for the seven spiked pharmaceuticals are listed in Table S-9.

Table S-9. Mean spike recoveries and percent relative standard deviations for analysis of six replicate matrix spikes of NY3 effluent fortified over a range of high concentrations. (RSD, relative standard deviation). Diazepam was not spiked in these samples so that data for this analyte is not available.

Pharmaceutical	Range of Fortification Concentration, in μg/L	Mean Percent Recovery	Percent RSD
Butalbital	12-402	87	2.8
Carisoprodol	20-800	94	13
Metaxalone	104-4000	77	11
Methadone	92-871	63	12
Oxycodone	24-804	70	11
Phendimetrazine	22-802	70	16

For the five pharmaceuticals with available data, mean recoveries and RSDs of pharmaceuticals fortified with high concentrations in samples of NY3 effluent (Table S-9) were similar to recoveries and RSDs observed in reagent spikes (Tables S-7, S-8). All analytes had recoveries between 60-130%, and RSDs were all less than 30%.

NY1 Effluent Matrix Spiking Results: A single composite sample of NY1 effluent collected in March 2007 was divided into five replicate one-Liter samples and fortified

with the analytes at moderate (0.8 to 80 μg/L) concentrations to assess method performance for moderate pharmaceutical concentrations in wastewater-enriched environmental samples. These samples were extracted and analyzed in a single analytical set. Any ambient environmental contributions of pharmaceuticals to the matrix spike replicates were corrected for by duplicate analysis of unspiked samples. Results for the seven spiked pharmaceuticals are listed in Table S-10.

Table S-10. Mean spike recoveries and percent relative standard deviations for analysis of five replicate matrix spikes of NY1 effluent fortified at moderate (0.8 to 80 μg/L) concentrations. [RSD, relative standard deviation]

Pharmaceutical	Fortification Concentration, in micrograms per Liter	Mean Percent Recovery	Percent RSD
Butalbital	8	95	7.2
Carisoprodol	8	104	6.1
Diazepam	0.8	94	4.6
Metaxalone	80	96	5.9
Methadone	8	59	27
Oxycodone	8	94	7.4
Phendimetrazine	8	66	26

Mean recoveries and RSDs of pharmaceuticals fortified with moderate concentrations in samples of NY1 effluent (Table S-10) were similar to recoveries and RSDs observed in reagent set spikes (Tables S-7, S-8) and those in the NY3 effluent samples (Table S-9). All but one of the mean recoveries were within the 60-130% range, and all RSDs were <30%. The only recovery outside the 60-130% range was methadone, at 59%. As was the case for NY3 effluent spikes, many of these pharmaceuticals had RSDs that were lower in these matrix spikes than in reagent set spikes. This is attributed to the analysis of these samples in a single set, whereas the reagent set spikes were analyzed in multiple sets over several years.

NY4 Effluent Matrix Spiking Results: A single composite sample of NY4 effluent collected in May 2009 was divided into 13 replicate one-Liter samples and fortified with the analytes at low (0.2 μg/L) concentrations to assess method performance for low pharmaceutical concentrations in wastewater-enriched environmental samples. Any ambient environmental contributions of pharmaceuticals to the matrix spike replicates were corrected for by triplicate analysis of unspiked samples. These 13 replicate samples were extracted in 5 different extraction sets and then analyzed in a single analytical set. Results for the spiked analytes are listed in Table S-11. These samples were also used for a holding time study (see below). Data for all the analytes but phendimetrazine are available; the data for phendimetrazine from the NY4 effluent spike holding time experiment are not included because there was a significant decrease in the concentration for this analyte over the 15 day holding time experiment. Thus, with the exception of

phendimetrazine, the aggregated results could also be used as a matrix spike recovery experiment.

Table S-11. Mean spike recoveries and percent relative standard deviations for analysis of 13 replicate matrix spikes of NY4 effluent fortified at low (0.2 μg/L) concentrations. [RSD, relative standard deviation] for six of the analytes.

Pharmaceutical	Fortification Concentration, in micrograms per Liter	Mean Percent Recovery	Percent RSD
Butalbital	0.2	120	10
Carisoprodol	0.2	99	5.1
Diazepam	0.2	110	6.5
Metaxalone	0.2	120	5.1
Methadone	0.2	91	31
Oxycodone	0.2	170	8.8

Five of the six analytes with available data had mean percent recoveries within the 60-130% range, and all but one of the RSDs were <30%. Recoveries for oxycodone (170%) were higher than most other analytes for these spikes, but the RSD was low (8.8%). Only the methadone RSD exceeded 30% (31%). For most analytes, the recoveries were higher and RSDs were similar to those for reagent set spikes (Table S-7 and S-8) and other matrix spikes (Tables S-9- S-11). The high mean recovery for oxycodone in this spike may reflect the low spiking level used in this experiment.

NY1 Upstream Matrix Spikes: A single composite sample of streamwater upstream of NY1 collected in March 2007 was divided into 5 replicate one-Liter samples and fortified with target anlaytes at low (0.08 to 8 μg/L) concentrations to assess method performance for low pharmaceutical concentrations in environmental waters with negligible wastewater content. These samples were extracted and analyzed in a single analytical set. Any ambient environmental contributions of pharmaceuticals to the matrix spike replicates were corrected for by duplicate analysis of unspiked samples. Results for the seven spiked pharmaceuticals are listed in Table S-12.

Table S-12. Mean spike recoveries and percent relative standard deviations for analysis of five replicate matrix spikes of a stream sample collected upstream of NY1 WWTP discharge fortified at low (0.08 to 8 μg/L) concentrations. [RSD, relative standard deviation; na, not applicable]

Pharmaceutical	Fortification Concentration, in micrograms per Liter	Mean Percent Recovery	Percent RSD
Butalbital	0.8	93	14
Carisoprodol	0.8	97	13
Diazepam	0.08	97	8.2
Metaxalone	8	87	8.5
Methadone	0.8	32	48
Oxycodone	0.8	57	36
Phendimetrazine	0.8	57	23

Four analytes (butalbital, carisoprodol, diazepam, and metaxalone) had mean recoveries between 60-130%, and five had RSDs<30%. Two pharmaceuticals (oxycodone and phendimetrazine) had recoveries of 57% and RSDs between 23 to 36%. Methadone had a low recovery (32%) and high RSD (48%) for this spike. Mean recoveries and RSDs of pharmaceuticals fortified with low concentrations in streamwater samples from upstream of the NY1 WWTP (Table S-12) were somewhat lower than the recoveries and RSDs observed in reagent set spikes (Tables S-7, S-8) and in the matrix spikes from effluent samples (Tables S9-S11) for some analytes.

Summary of Reagent Set and Matrix Spikes

Data from the different spiking experiments (two reagent set spike experiments at low and medium concentrations, three effluent spike experiments at low, medium and high concentrations, and a stream spike experiment at medium concentrations) show that median mean recoveries for all spikes were 94%, and median RSDs were 15%. Of the 14 reagent set spikes, only the mean recovery for the low concentration (0.2 μg/L) carisoprodol spike (140%) lies outside the 60-130% range, and only the two oxycodone spikes (low level at 46% and high level at 34%) have RSDs>30%. Of the 19 effluent spikes, two mean recoveries -- methadone for the moderate spike (59%) and oxycodone for the low-level spike (170%) have mean recoveries outside the 60-130% range. Only one of the 19 effluent spikes, the low level methadone spike, has an RSD >30% (31%).

The spiking results show that the effluent concentrations for the seven pharmaceuticals in this study have low bias and variability. Although the low-level reagent spikes indicate

that carisoprodol may have a positive bias for low concentrations, the other reagent spike and effluent spikes for carisoprodol ranged from 99-104%, suggesting no bias. The methadone spike recovery for the effluent spike at middle range (8 μg/L) concentrations indicates a slight low bias, yet the other two methadone effluent spikes for low (0.2 μg/L) and high (>=90 μg/L) range concentration (63 and 91%, respectively) show no bias. The results indicate a positive bias for oxycodone for low (0.2 μg/L) effluent concentrations, yet the two other oxycodone effluent spikes for moderate (8 μg/L) and high (>=24 μg/L) concentrations have recoveries of 94 and 70%, respectively. The high RSDs for reagent set spikes suggest that oxycodone concentrations may be more variable than other pharmaceuticals in this study, however the effluent spikes for oxycodone all have RSDs<30%.

The stream spike recoveries for butalbital, carisoprodol, diazepam and metaxalone were between 60-130%, and RSDs were less than 30%, indicating low bias and variability. Because of the low (32%) and variable (RSD of 48%) recovery for methadone stream spikes, stream water data for methadone are only reported qualitatively (as percent detection). The low recoveries for stream spikes for oxycodone and phendimetrazine (57%) suggest that the stream concentrations for these two pharmaceuticals may be biased low. In addition, the RSD for the oxycodone stream spike was 36%, indicating a higher variability for stream concentrations for this pharmaceutical than the others.

Method Detection Limit

The determination of method detection limits (MDLs) for each pharmaceutical was conducted according to the procedures of the U.S. Environmental Protection Agency (*7*), and are presented in Table S-13.

The MDLs were determined using a low-level reagent spike study that employed multiple experiments in order to ensure that the lowest appropriate spiking level was used to calculate each individual MDL. These experiments resulted in data that provides reagent set spike recoveries near the detection limit as well as MDLs, and are discussed together in this section**.** Two fortification concentrations were used. Ten reagent water samples were fortified at 0.05 μg/L for each compound, extracted, and analyzed. These samples were analyzed in the same analytical set. Seven reagent water samples were fortified at 0.2 μg/L for each compound, extracted, and analyzed. These samples also served as set spikes, and were prepared and analyzed in different analytical sets. Details on the mean recoveries, %RSDs, and calculated MDLs at both fortification concentrations are shown in Table S-13.

Table S-13. Mean recoveries, percent relative standard deviations, and method detection limits calculated from reagent water samples fortified at 0.05 µg/L (n=10) and 0.2 µg/L (n=7). MDL values in bold correspond to those presented in Table S-13. [RSD, relative standard deviation; MDL, method detection limit; ND, Not detected; --, data not calculated; µg/L micrograms per Liter]

Compound	0.05 µg/L fortification			0.2 µg/L fortification		
	Mean recovery, in percent	% RSD	MDL, in µg/L	Mean recovery, in percent	% RSD	MDL, in µg/L
Butalbital	112	9	**0.014**	110	5.6	0.036
Carisoprodol	97	15	**0.021**	100	5.9	0.035
Diazepam	125	7	**0.012**	110	5.4	0.034
Metaxalone	99	8	**0.011**	110	4.4	0.029
Methadone	57	13	--	74	10	**0.044**
Oxycodone	ND	--	--	94	14	**0.076**
Phendimetrizine	52	28	**0.021**	69	24	0.098

Five of the seven pharmaceuticals (butalbital, carisoprodol, diazepam, metaxalone, and phendimetrazine) were reliably detected in the 0.05 µg/L fortification samples, and the MDLs at this fortication were used as MDLs in the study (Table S-13). The remaining two pharmaceuticals (methadone, oxycodone, and methocarbamol) were detectable at 0.20 µg/L (Table S-13). Although a mean recovery could be calculated for methadone in the 0.05 µg/L fortification level, methadone was only detected in 7 of the 10 samples fortified at 0.05µg/L, so the results of the 0.20 µg/L fortification level were used for methadone. Mean recoveries and RSDs of the 5 pharmaceuticals reliably detected at the 0.05 µg/L fortification were comparable with the mean recoveries and RSDs at the 0.2 µg/L fortification and in the range typical for reagent set spikes (see Table S-7, S-8). Mean recoveries and RSDs at the 0.2 µg/L fortification were all comparable with mean recoveries and RSDs in the reagent set spike results (Table S-7, S-8).

The results for the low-level reagent spike/MDL study indicate that the concentrations chosen were appropriate for estimating the MDL. Based on the guidance provided in (*7*), the MDLs calculated for five analytes at the 0.05 µg/L fortification level were appropriate, given the sensitivity of the method for these five compounds. The spiking level for the MDLs used in this study are appropriate for these compounds, falling within the 1-5 times the calculated MDL.

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Estimation of Precision at Ambient Environmental Concentrations

In order to better understand precision in the analysis of environmental samples, 18 replicates of a homogeneous sample of NY3 effluent were analyzed and method precision calculated. These were prepared for analysis in five different preparation sets but instrumental analysis was conducted as a single set. These samples were also used for a holding-time study (see below), and thus the calculated precision better incorporates sources of variation associated with different preparation sets. The results for this experiment are shown in Table S-14. Because these data represent the environmental concentrations in NY3 effluent at the time of sample collection, all compounds were not present.

Table S-14. Mean concentrations and percent relative standard deviations calculated for ambient pharmaceuticals determined in18 replicate one-Liter sample aliquots from site NY3. [RSD, relative standard deviation]

Pharmaceutical	Average Concentration, in micrograms per Liter	% RSD
Butalbital	1.6	5.2
Metaxalone	4.23	5.1
Methadone	71.7	11
Oxycodone	3.7	21
Phendimetrazine	1.79	6.4

Most compounds (5 of 7) were detected in the sample of NY3 effluent, allowing for the assessment of precision for most of the pharmaceuticals included in the study; the RSDs for these 8 compounds ranged from 6.4% to 27%. This precision was comparable with other estimates of environmental precision made for pharmaceuticals and wastewater indicator compounds included in previous studies (*8*).

Summary of Method Performance

The results of the spiking experiments suggest the method performed acceptably for the seven target pharmaceuticals in effluent samples, and for six pharmaceuticals in surface water samples. Because of the low methadone stream spike recoveries, discussion of surface water concentrations is limited to six pharmaceuticals (butalbital, carisoprodol, diazepam, metaxalone, oxycodone, and phendimetrazine), with methadone only reported as qualitatively detected in stream samples. These results also indicate that concentrations

of oxycodone at low concentrations in streams and effluent may be somewhat more variable than other concentrations presented in this study.

Holding Time Studies

The method of analysis used in this study was based upon a recommended holding time (*6*) of up to 14 days for filtered water samples. However, since these samples were comprised of treated wastewater, separate holding time studies were conducted in order to assess whether the maximum recommended 14 day holding time would introduce systematic low bias into the results as a result of potential degradation of the seven target pharmaceuticals. Two studies were conducted using multiple splits of samples collected at the NY3 and NY4 WWTP sites, as previously discussed above. The NY4 sample splits were fortified with the seven pharmaceuticals and replicates analyzed over a 14 day period. The NY3 replicate samples were not fortified and were also analyzed over a 14 day period. Two experiments were conducted to include an assessment of long holding times for a range of wastewater treatment matrices, as the NY3 effluent was from an activated sludge wastewater plant, and the NY4 effluent was from a rotating biologic contactor wastewater plant. These data are useful for ensuring that holding time information was available for all seven pharmaceuticals included in the study, and to indicate whether results would be different for unfortified samples at high concentrations (NY3) and fortified samples at low concentrations (NY4).

NY3 Hold Time Study

Eighteen NY3 replicate effluent samples were analyzed to determine compound degradation in sample matrix with respect to time. None of these samples were fortified, because they contained high ambient environmental concentrations of five of the seven target pharmaceuticals. Three samples were extracted within 24 hours of sampling. Three samples were extracted three days from collection then three more on days five, seven, eleven, and fifteen. Linear regression analysis of concentration versus hold time was performed to calculate a degradation rate. This rate was used to determine the percent of analyte lost to degradation in fifteen days. The results of the holding time study for these unfortified samples are shown in Table S-16.

NY4 Holding time Study

Sixteen NY4 replicate effluent samples were studied to determine compound degradation in sample matrix with respect to time; all of the 16 replicate samples were collected within a short (less than 2 hour period) on the same day. Because the NY4 WWTP uses rotating biologic contactors for biologic treatment, it is expected that this matrix represents WWTPs that are less efficient at removing a variety of wastewater compounds than sites using activated sludge for biologic wastewater removal. Thirteen of the 16 replicate samples were fortified in the field at 0.2 μg/L for all seven target pharmaceuticals at the time of collection; three of the 16 replicate samples were not

fortified. The three unspiked samples and three spiked samples were extracted within 24 hours of sampling. Three samples were extracted three days from collection and three more seven days from collection. Two samples were extracted 11 days from collection and two more were extracted 15 days from collection. Linear regression analysis of concentration versus hold time was performed to determine a degradation rate for each pharmaceutical. This rate was used to determine the percent of analyte lost to degradation in 15 days. The rates for all seven target pharmaceuticals are shown in Table S-15.

Table S-15. Percent change in concentration for pharmaceuticals in unfortified NY3 effluent samples and fortified NY4 effluent samples after 15 days. Negative results indicate a decrease and positive results indicate an increase in concentration.

Pharmaceutical	Percent Change in Concentration, NY3 Effluent	Percent Change in Concentration, NY4 Effluent
Butalbital	-1.7	1.3
Diazepam	ND	-3.6
Metaxalone	-1.0	-3.9
Methadone	0.3	-6.9
Oxycodone	-7.3	-5.8
Phendimetrazine	1.2	-45
Carisoprodol	ND	-7.3

Summary of Holding Time Results

In both sample matrixes, four pharmaceuticals (carisoprodol, methadone, oxycodone, and metaxalone) had small (<8%) changes in concentrations over the 15 days for both NY3 and NY4 samples (Table S-15). Two other pharmaceuticals only detected in the NY4 effluent (carisoprodol and diazepam) also had small (<8%) changes in concentrations after 15 days (Table S-15). The remaining pharmaceutical, phendimetrazine had slight changes (1.2%) in concentrations in NY3 effluent samples, but large (45%) decreases in concentrations in NY4 effluent spikes at 15 days. The lower concentrations in NY4 effluent spikes for phendimetrazine indicate that this sample matrix may result in degradation of this analytes in samples over 15 days. Overall, for most of the target pharmaceuticals, there was little indication of positive or negative bias over 15 days, but some effluent matrices may result in the decrease in concentrations for phendimetrazine and over a 15 day holding time. The median holding times for the environmental samples included in the study was 8 days, and the holding time for 90% of the samples did not exceed 15 days. Thus, the combination of small changes in concentrations for most analytes and the low holding times for most samples indicate that holding time between sample collection and extraction did not greatly affect pharmaceutical concentrations reported in this study.

Additional Compounds Qualitatively Identified in Samples

All of the pharmaceuticals reported as 'Pharmaceuticals Qualitatively Identified in Samples Using a Standard, but not Quantified' in Tables S-4 and S-5 were identified qualitatively using standards in at least two samples collected between 2008-2009. Authentic standards were obtained for the additional 18 pharmaceuticals listed in Table S-4 and S-5, and these analytes met qualitative identification criteria. Four of these compounds (chlorpheniramine, codeine, hydrocodone and methocarbamol) were originally included in method development spike experiments for this study, but due to low and or inconsistent recoveries concentrations for these compounds are not reported, but their presence was noted in multiple samples from these sites.

The qualitative criteria include comparison with the authentic reference standard for confirmation of GC retention time and mass spectral performance. This requires that the suspected analyte GC retention time was within +/- 0.05 minute of that of the authentic standard and the mass spectral quantitation ion and two confirmation ion abundance ratios were within +/- 20% of the values determined by analysis of the authentic standard under identical experimental conditions. Ions used for qualification are listed in Table S-16.

Table S-16. Measured retention time, quantitation ion, and confirmation ions for qualitatively identified compounds.
[min, minutes; m/z, mass-to-charge ratio]

Compound name	Retention Time (min)	Quantitation Ion (m/z)	Confirmation Ion (m/z)	Confirmation Ion (m/z)
2-Ethyl-2-phenylmalonamide	33.97	163	148	120
Acetaminophen	31.03	109	151	80
Bupropion	29.71	100	139	224
Chlorpheniramine	35.05	203	205	167
Codeine	38.40	299	229	162
Dihydrocodeine	38.42	301	284	286
Diltiazem	42.40	58	71	150
Fluoxetine	33.55	309	148	104
Hydrocodone	38.90	299	242	214
Meperidine	32.18	71	247	246
Meprobamate	32.54	83	144	114
Methocarbamol	35.18	118	109	124
Methylphenidate	32.00	84	91	150
o-desmethyltramadol	35.20	58	121	249
Phenobarbital	34.67	204	232	117
Primidone	37.10	190	146	117
Temazepam	39.89	271	300	256
Tramadol	34.51	58	263	135
Verapamil	43.84	303	151	58

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