Chemicals and Reagents. Active pharmaceutical ingredients (API) reference standards were purchased in the highest purity available (97% or greater) from Sigma-Aldrich (Saint Louis, Missouri, USA) or Toronto Research Chemicals (North York, Ontario, Canada). A 10 µg/mL mixture of 6 compounds, 5 of which were stable isotopically labeled compounds (see Tables S.2.1 and S.2.2), was used as a procedural internal standard. Glassware used in the preparation and storage of standards was silanized using Sylon CT (Sigma-Aldrich) to avoid any loss of analytes due to sorption to glass. The acetonitrile (ACN) and methanol (MeOH) used for the chromatographic mobile phase were HPLC grade high-purity solvents purchased from Fisher Scientific (Fair Lawn, NJ). Reagent grade ACN and MeOH used in sample preparation, formic acid, hydrochloric acid (HCl), disodium ethylenediamine tetraacetate (Na_2EDTA), ascorbic acid, and ammonium hydroxide were also purchased from Fisher Scientific.

Sample Preparation. Samples were extracted using the same solid phase extraction method described in (Batt and Aga 2005). Sample pH was adjusted to between 2.8 and 3.0 using a dilute solution of HCl, and samples were then concentrated using 200 mg Oasis HLB cartridges (Waters, Milford, MA) . The cartridges were conditioned with 6 mL of ACN, followed by 6 mL of distilled water. Samples were passed through the cartridges at a rate of 3-5 mL/min using a Supelco vacuum manifold (Sigma-Aldrich), which allowed for the parallel extraction of up to 24 samples. Analytes were eluted with two 4 mL volumes of ACN into a silanized conical glass tube. The volume of each eluate was reduced to dryness under a gentle stream of nitrogen at 40°C. Samples were then reconstituted in 500 µL of 20% ACN in water and transferred to a polypropylene vial for immediate UPLC-MS/MS analysis.

Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry. Analysis was conducted using an Aquity Ultra Performance Liquid Chromatograph (UPLC) coupled to a

Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray ionization source (ESI) (Waters, Milford, Massachusetts, USA). Analytes were separated on a BEH Phenyl column (1.0 x 100 mm 1.7µm) equipped with 0.2 µm inline filter, also purchased from Waters. The flow rate was 100 µL/min, the column oven temperature was 40°C, and the full loop injection volume was 10 µL. Analysis was performed using a single injection with a binary gradient mobile phase, consisting of water with 0.3% formic acid (A) and a mixture of (2:1) methanol:acetonitrile (B). The initial mobile phase proportions were 12% (A): 88% (B), which were held for 9.0 minutes. B was then linearly increased to 95% in 8.0 minutes, which was then held for 1.0 minute. Initial mobile phase conditions were restored over 1.0 minute, and the column was allowed to equilibrate for 3 minutes, for a total run time of 22 minutes.

Individual tune files were created for each standard in continuous flow mode to determine the optimum capillary voltages, collision energies, and fragment ions, which are listed in Table S.2.1 for target analytes and Table S.2.2 for procedural internal standards. Multiple reaction monitoring (MRM) was used to collect data for the two most intense and/or specific product ions for each precursor ion. The desolvation temperature was 450°C, source temperature was 140°C, and the capillary voltage was 3.0 kV, with the instrument being operated in positive ion mode. Nitrogen was used as a desolvation gas at a flow rate of 450 L/h and cone gas at 50 L/h, and argon gas was used to induce dissociation for the acquisition of MS/MS data. The chromatographic run was divided into time segments (Table S.2.1), with a dwell time of 20 ms for each transition. Time Segment I was from 0.0 – 6.4, Segment II from 5.4 – 12.6, Segment III from 9.3 – 10.7, Segment IV from 11.4 – 15.0, and Segment VI from 16.0 – 18.0 minutes.

Detection and Quantification. Detection was based on retention time and product ion ratios collected from the MRM transitions. For a positive identification, both product ions had to

be present with a signal to noise ratio (S/N) of at least three and a product ion ratio within ±30% of the expected ratio. Quantification of target analytes was based on internal calibration curves constructed from a plot of the peak area ratio of the analyte signal to the signal of the assigned procedural internal standard versus concentration. Calibration standards were prepared at eight different concentrations to cover the dynamic range for all analytes (1, 5, 10, 25, 50, 100, 1000, and 3000 ng/mL) and non-weighted, linear calibration curves typically displayed correlation coefficients greater than 0.99.

Method Validation and Performance. The applicability of the previously reported extraction method was assessed by calculating the percent recovery and relative standard deviation (RSD) for 5 replicate distilled water samples fortified to 300 ng/L (Table S.2.3). Throughout the sample collection period, a spiked wastewater effluent sample was included in each extraction batch spiked at 1000 ng/L. This resulted in thirteen different matrix spike samples, with the average recovery being reported in Table S.2.3. The majority of the compounds behaved similarly in distilled water as they did in matrix spike samples, with the exceptions of lisinopril and enalaprilat, with lisinopril often displaying higher than acceptable recoveries in the matrix spike samples. This was likely due to not having an internal standard for these two compounds that could effectively compensate for matrix effects in these complex samples. All samples for which the associated batch matrix spike sample recovery was greater than 150% were reported as estimated. The method reporting limits (RL) for each analyte are reported in the main body of the manuscript. Since several isotopically labeled compounds were used as procedural internal standards, there is a possibility that the labeled standards may contain trace quantities of the unlabeled analytes that could interfere with quantification. Distilled water blanks at a volume of 500 mL were fortified with 500 ng/L of the procedural internal standards

and extracted along with each extraction batch. Blanks did not reveal any significant levels of the target compounds, indicating that contamination of the isotope standards was not a problem when spiked at this level.

Table S.2.1. A summary of the target analytes and their assigned procedural internal standards, respective retention times, MS/MS parameters, and precursor and product ions.

Compound Number	Compound Name	CAS Number	Procedural Internal Standard	RT (min)	Segment	Precursor Ion	CV	Product Ion 1	CE 1	Product Ion 2	CE 2
1	sulfamethazine	57-68-1	sulfamethoxazole-d4	4.4	I	279.1	27	155.8	18	185.9	15
2	sulfamethoxazole	723-46-6	sulfamethoxazole-d4	8.6	II	254.0	28	155.9	17	91.8	29
3	sulfadimethoxine	122-11-2	sulfamethoxazole-d4	13.3	IV	311.2	35	155.9	20	91.8	33
4	trimethoprim	738-70-5	trimethoprim-d9	4.4	I	291.1	46	229.8	25	122.9	30
5	ciprofloxacin	85721331	ciprofloxacin-d8	9.8	II	332.1	40	288.0	20	246.9	20
6	ofloxacin	82419-36-1	trimethoprim-d9	9.40	II	362.1	33	318.0	18	260.9	18
7	lincomycin	859-18-7	sulfamethoxazole-d4	3.1	I	407.2	35	125.9	28	359.1	19
8	enalapril	76095-16-4	enalapril-d5	14.1	IV	377.2	34	233.9	21	159.9	29
9	enaliprilat	76420-72-9	enalapril-d5	8.3	II	349.2	28	205.9	21	90.8	45
10	lisinopril	83915-83-7	enalapril-d5	4.0	I	406.2	40	83.9	25	246.0	25
11	florfenicol	73231-34-2	thiamphenicol	10.75	III	358.1	27	240.8	17	242.8*	17
12	testosterone	58-55-9	testosterone-d3	15.7	V	289.1	35	96.8	20	108.8	15
13	progesterone	80474-14-2	testosterone-d3	16.6	VI	315.1	35	96.8	20	108.8	25
14	melengestrol acetate	2919-66-6	testosterone-d3	16.7	VI	397.2	28	337.0	15	279.1	20

*Precursor ion for Product Ion 2 was 360.2. (Florfenicol is chlorinated, and the second most abundant product ion came from the second chlorine isotope peak.)

Table S.2.2. A summary of the employed procedural internal standards and their respective retention times and MS/MS parameters.

Procedural Internal Standard	Supplier	RT (min)	Segment	Precursor Ion	CV	Product Ion	CE
trimethoprim-d9	Toronto Research	4.4	I	300.2	46	233.9	25
sulfamethoxazole-d4	Toronto Research	8.4	II	258.0	28	95.8	29
ciprofloxacin-d8	Toronto Research	9.7	II	340.1	40	295.8	20
enalapril-d5	Toronto Research	14.1	IV	382.2	34	239.0	21
thiamphenicol	Sigma Aldrich	4.5	I	356.1	26	307.8	15
testosterone-d3	Toronto Research	15.7	V	292.2	35	96.8	20

RT = Retention Time (minutes)
CV = Cone Voltage (Volts)
CE = Collision Energy (eVolts)

Table S.2.3. A summary of the SPE percent recoveries and relative standard deviations (RSD) in distilled water and wastewater effluent matrix spike samples. For distilled water, n=5, for effluent, n=13 different wastewater samples.

Compound	Distilled Water		Effluent	
	Recovery 300 ng/L	RSD	Recovery 1000 ng/L	RSD
sulfamethazine	95	8	97	16
sulfamethoxazole	82	2	93	26
sulfadimethoxine	87	17	68	18
trimethoprim	81	6	103	16
ciprofloxacin	82	9	77	19
ofloxacin	114	8	117	24
lincomycin	105*	29*	121	92
enalapril	92	4	82	23
enaliprilat	59	15	130	38
lisinopril	54	19	635	72
florfenicol	107	9	128	49
testosterone	92	5	95	16
progesterone	84	16	106	13
melengestrol acetate	98	16	68	23

*Spike level for lincomycin was 1000 ng/L, as this compound was added to the method at a later date.