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Trace Analysis of Antidepressant Pharmaceuticals and Their Select Degradates in Aquatic Matrixes by LC/ESI/MS/MS

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Treated wastewater effluent is a potential environmental point source for antidepressant pharmaceuticals. A quantitative method was developed for the determination of trace levels of antidepressants in environmental aquatic matrixes using solid-phase extraction coupled with liquid chromatography-electrospray ionization tandem mass spectrometry. Recoveries of parent antidepressants from matrix spiking experiments for the individual antidepressants ranged from 72 to 118% at low concentrations (0.5 ng/L) and 70 to 118% at high concentrations (100 ng/L) for the solid-phase extraction method. Method detection limits for the individual antidepressant compounds ranged from 0.19 to 0.45 ng/L. The method was applied to wastewater effluent and samples collected from a wastewater-dominated stream. Venlafaxine was the predominant antidepressant observed in wastewater and river water samples. Individual antidepressant concentrations found in the wastewater effluent ranged from 3 (duloxetine) to 2190 ng/L (venlafaxine), whereas individual concentrations in the waste-dominated stream ranged from 0.72 (norfluoxetine) to 1310 ng/L (venlafaxine).

Pharmaceuticals and personal-care products are of scientific and public concern as newly recognized classes of environmental pollutants.[1−3] Currently (2007) there are over 3000 registered pharmaceutical ingredients, including antidepressants, painkillers, antibiotics, antidiabetics, β-blockers, contraceptives, lipid regulators, and impotence drugs.[3] A primary route for human pharmaceuticals into the environment is through the discharge of treated wastewater effluent, which contains pharmaceuticals because humans excrete the unmetabolized fraction following prescribed usage.[2,4−10] Many of these pharmaceuticals, which often are pharmacologically active or endocrine modulating across multiple levels of biological organization, are not removed by wastewater-treatment processes.[2,6,8,9] Consequently, the untreated pharmaceuticals are discharged into lakes and rivers where aquatic flora and fauna are continuously exposed to varying concentrations of these and other contaminants.

Antidepressants are a commonly prescribed class of pharmaceuticals.[11] One class of antidepressants, known as selective serotonin reuptake inhibitors (SSRIs), has been widely marketed since the mid-1980s and is primarily prescribed to patients diagnosed with clinical depression; the SSRIs also are used to treat obsessive−compulsive disorder, panic disorder, social phobia, and attention-deficit disorder.[12,13] As of 2001, one SSRI, fluoxetine (more commonly known as Prozac), has been prescribed to over 34 million people worldwide.[12] Other SSRIs include sertraline (Zoloft), paroxetine (Paxil), citalopram (Celexa), escitalopram (Lexapro), the eutomer of citalopram, and fluvoxamine (Luvox) (Figure 1).

The possibility that the effects of pharmaceuticals with similar mechanisms could be additive has not been examined; therefore, if multiple pharmaceuticals with the same modes of action are present in an ecosystem, the effective environmental effect could be substantial.[1] In the case of SSRIs, this could be particularly important if the effects of other antidepressants that are prescribed when SSRIs are not effective are considered. These other antidepressants include venlafaxine (Effexor), duloxetine (Cymbalta), and bupropion (Wellbutrin) (Figure 1). Venlafaxine and duloxetine are selective serotonin and norepinephrine reuptake inhibitors (SSNRIs). Unlike SSRIs, which only modulate levels of serotonin, SSNRIs have the ability to affect the uptake of two

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(1) Daughton, C. G.; Ternes, T. A. *Environ. Health Perspect.* **1999**, *107*, 907−938.
(2) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.
(3) Richardson, S. D.; Ternes, T. A. *Anal. Chem.* **2005**, *77*, 3807−3838.
(4) Vieno, N.; Tuhkanen, T.; Kronberg, L. *Water Res.* **2007**, *41*, 1001−1012.
(5) Ternes, T. A. *Water Res.* **1998**, *32*, 3245−3260.
(6) Glassmeyer, S. T.; Furlong, E. T.; Kolpin, D. W.; Cahill, J. D.; Zaugg, S. D.; Werner, S. L.; Meyer, M. T.; Kryak, D. D. *Environ. Sci. Technol.* **2005**, *39*, 5157−5169.
(7) Peng, X.; Wang, Z.; Kuang, W.; Tan, J.; Li, K. *Sci. Total Environ.* **2006**, *371*, 314−322.
(8) Miao, X.-S.; Yang, J.-J.; Metcalfe, C. D. *Environ. Sci. Technol.* **2005**, *39*, 7469−7475.
(9) Lindqvist, N.; Tuhkanen, T.; Kronberg, L. *Water Res.* **2005**, *39*, 2219−2228.
(10) Clara, M.; Strenn, B.; Gans, O.; Martinez, E.; Kreuzinger, N.; Kroiss, H. *Water Res.* **2005**, *39*, 4797−4807.
(11) The top 200 prescriptions for 2006 by number dispensed. http://www.rxlist.com/top200.htm Accessed June 2007.
(12) Fong, P. P. In *Pharmaceutical and Personal Care Products in the Environment: Scientific and Regulatory Issues*; Daughton, C. G., Jones-Lepp, T. L., Eds.; ACS Symposium Series 791; American Chemical Society: Washington DC, 2001; pp 264−281.
(13) Brooks, B. W.; Foran, C. M.; Richards, S. M.; Weston, J.; Turner, P. K.; Stanley, J. K.; Solomon, K. R.; Slattery, M.; La Point, T. W. *Toxicol. Lett.* **2003**, *142*, 169−183.

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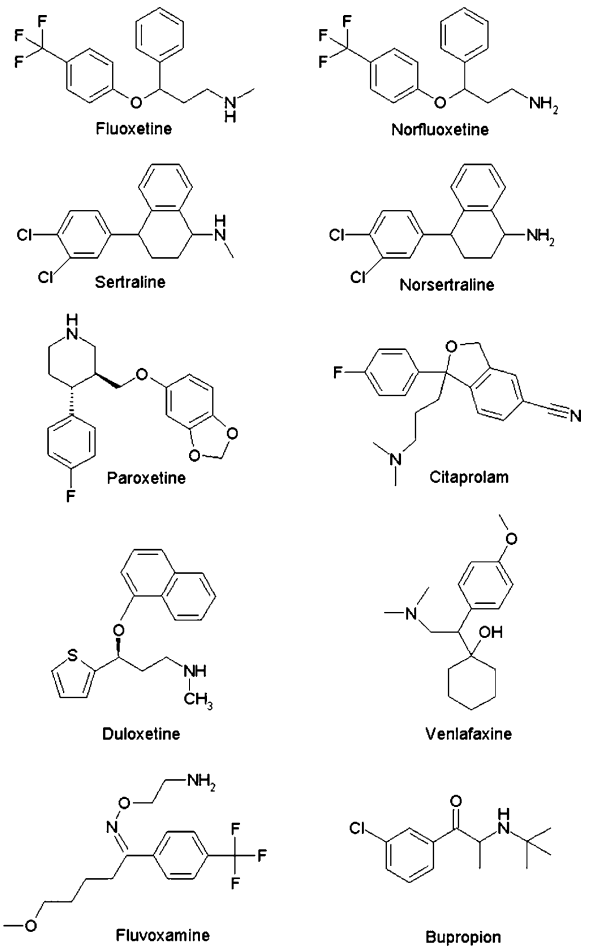

Figure 1. Chemical structures of the antidepressant pharmaceuticals.

neurotransmitters—serotonin and norepinephrine. Bupropion does not inhibit serotonin uptake. Instead, it acts by inhibiting norepinephrine and dopamine uptake.[14] Bupropion is also the active ingredient in Zyban, which is prescribed as a non-nicotine aid to smoking cessation.[11]

Six of the 200 most widely dispensed pharmaceuticals in 2006 were antidepressants, including citalopram (prescribed as Lexapro and Celexa), sertraline, duloxetine, venlafaxine, paroxetine, citalopram (both Lexapro and Celexa in top 200), and bupropion (both Wellbutrin XL and Zyban in the top 200).[11] Despite this widespread use, few analytical methods exist to detect antidepressants in environmental matrixes; thus, little is known about their distribution and fate in the environment. To date, most analytical methods for the determination of antidepressants and their degradates have been developed for biological matrixes.[15–17] If analytical methods for aquatic samples exist, they usually determine only fluoxetine.[2,18–20] Antidepressants and their degradates that enter the environment through various mechanisms (e.g., by way of wastewater effluent or land application of biosolids) may affect the metabolic pathways of selected aquatic, terrestrial organisms, or both. In a recent study, fish populations of *Lepomis macrochirus* (bluegill), *Ictalurus punctatus* (channel catfish), and *Pomoxis nigromaculatus* (black crappie) residing in a municipal effluent-dominated stream contained concentrations of fluoxetine, sertraline, norfluoxetine, and norsertraline greater than 0.1 ng/g in all muscle, liver, and brain tissues examined.[21] These levels are high enough to possibly affect physiological systems, because toxicological studies performed with fluoxetine indicate that adverse effects are observed in standardized aquatic toxicity tests at concentrations of 1 μg/L.[13] Another study has reported that SSRIs induced spawning in some crustaceans and bivalves at concentrations as low as 10^{-10} M, equivalent to ~0.03 μg/L.[22] More recently, fluoxetine, fluvoxamine, and sertraline were found to be toxic to algae in laboratory experiments.[23] A study performed on mianserin, a tetracyclic antidepressant, demonstrated estrogenic activity and endocrine disruption in zebrafish, further suggesting adverse effects in aquatic organisms by antidepressant pharmaceuticals.[24]

The aim of this study was to develop a reliable, quantitative, analytical method for a suite of eight commonly prescribed antidepressant pharmaceuticals and two antidepressant degradates in aqueous samples by liquid chromatography/tandem mass spectrometry. The validated methodology then was applied to two unique hydrologic sample sets: a suite of municipal wastewater–effluent samples collected from a metropolitan urban center and surface water samples collected from a waste-dominated stream. To the best of the authors' knowledge, this work is the first reported identification of venlafaxine, bupropion, and duloxetine in natural, aquatic environments.

EXPERIMENTAL SECTION

Standards and Reagents. Standards of fluoxetine hydrochloride (pure material) and paroxetine hydrochloride (pure material) were purchased from United States Pharmacopeia (Rockville, MD). Sertraline hydrochloride (>98%), *N*-desmethyl sertraline (>98%), duloxetine hydrochloride (>98%), D,L-venlafaxine hydrochloride (>98%), bupropion hydrochloride (>98%), and the internal standard sertraline-d_3 hydrochloride (>98%) were acquired from Toronto Research Chemicals (Toronto, ON, Canada). Norfluoxetine hydrochloride (97%), citalopram hydrobromide (>99%), fluvoxamine maleate (98%), and the labeled surrogates fluoxetine-d_5 hydrochloride (>98%) and norfluoxetine-d_5 hydrochloride (>98%) were obtained from Sigma Aldrich (St. Louis, MO).

Solid-Phase Extraction (SPE). All laboratory analyses were conducted at the National Water Quality Laboratory in Denver, CO. Unfiltered, 1-L aqueous samples were acidified with 0.1%

(14) Stahl, S. M.; Pradko, J. F.; Haight, B. R.; Modell, J. G.; Rockett, C. B.; Learned-Coughlin, S. *Primary Care Companion J. Clin. Psychiatry* **2004**, *6*, 159–166.
(15) Tournel, G.; Houdret, N.; Hedouin, V.; Deveau, M.; Gosset, D.; Lhermitte, M. *J. Chromatogr., B* **2001**, *761*, 147–158.
(16) Sutherland, F. C.; Badenhorst, D.; Jager, A. D. d.; Scanes, T.; Hunt, H. K.; Swart, K. J.; Hundt, A. F. *J. Chromatogr., A* **2001**, *914*, 45–51.
(17) Ramirez, A. J.; Mottaleb, M. A.; Brooks, B. W.; Chambliss, C. K. *Anal. Chem.* **2007**, *79*, 3155–3163.
(18) Vanderford, B. J.; Pearson, R. A.; Rexing, D. J.; Snyder, S. A. *Anal. Chem.* **2003**, *75*, 6265–6275.
(19) Cahill, J. D.; Furlong, E. T.; Burkhardt, M. R.; Kolpin, D. W.; Anderson, L. G. *J. Chromatogr., A* **2004**, *1041*, 171–180.
(20) Vanderford, B. J.; Snyder, S. A. *Environ. Sci. Technol.* **2006**, *40*, 7312–7320.
(21) Brooks, B. W.; Chambliss, C. K.; Stanley, J. K.; Ramirez, A.; Banks, K. E.; Johnson, R. D.; Lewis, R. J. *Environ. Toxicol. Chem.* **2005**, *24*, 464–469.
(22) Fong, P. P. *Biol. Bull.* **1998**, *April*, 143–149.
(23) Johnson, D. J.; Sanderson, H.; Brain, R. A.; Wilson, C. J.; Solomon, K. R. *Ecotoxicol. Environ. Saf.* **2007**, *67*, 128–139.
(24) van der Ven, K.; Keil, D.; Van Hummelen, P.; van Remortel, P.; Maras, M.; De Coen, W. *Chemosphere* **2006**, *65*, 1836–1845.

Table 1. Mass Spectrometer Parameters and Ion Transitions Used for Identification and Quantitation for Isotopically Labeled and Native Antidepressants[a]

compound	retention time (min)	precursor ion	declustering potential (V)	primary product ion (PI 1)	collision energy primary ion (eV)	secondary product ion (PI 2)	collision energy secondary ion (eV)	MS^3 ion(s) for PI 1	MS^3 ion(s) for PI 2
fluoxetine	11.8	310	20	44	35	148	11	nd	nd
norfluoxetine	11.6	296	25	134	8	30	15	nd	nd
sertraline	12.1	306	20	275	15	159	35	159, 129, 197	nd
norsertraline	11.8	292	10	275	12	159	40	159, 129, 197	nd
paroxetine	11.5	330	50	192	27	70	50	70	68
citalopram	11.1	325	35	262	25	109	50	234	83
fluvoxamine	11.8	319	30	258	12	71	30	nd	nd
duloxetine	11.6	298	25	44	30	154	7	nd	137
bupropion	10.3	240	20	184	15	166	25	166	131
venlafaxine	10.3	278	20	260	15	121	15	215	nd
fluoxetine-d_5	11.8	315	20	44	10	148	35	nd	nd
sertraline-d_3	12.1	309	20	275	15	159	35	159, 129, 197	nd

[a] nd , fragment ions not detected; MS^3 = MS−MS−MS trapped ion scan mode.

formic acid and spiked with known amounts of the labeled surrogates fluoxetine-d_5 and norfluoxetine-d_5 (typically, the expected final concentration was 100 ng/L). The Waters Oasis HLB 0.5-g, 6-mL solid-phase extraction cartridges (Milford, MA) were conditioned by first wetting the sorbent with 5 mL of water, followed by 5 mL of methanol. The 1-L acidified sample then was added to the cartridge at a flow rate of 15 mL/min. The cartridge was then washed with 5 mL of 70% methanol in 2% ammonium acetate. The analytes of interest were eluted from the cartridge with 10 mL of 70% methanol in 2% acetic acid. The 10-mL extract then was dried under a stream of nitrogen to a volume of 0.1 mL with a Turbo-Vap (Zymark, Hopkinton, MA), using nitrogen at a pressure of 35 kPa, in a 40 °C water bath. The 0.1-mL extract was spiked with a known amount of the internal standard sertraline-d_3 hydrochloride (500 ng/L) and reconstituted with aqueous buffer (0.1% formic acid) to 1 mL.

SPE Spike and Recovery. Spike and recovery experiments were performed to determine the accuracy and precision of the SPE method. For these experiments, two sets of seven replicate 1-L samples for each of five aqueous matrixes, consisting of unfiltered Solution 2000 (Aqua Solutions, Inc., model 2002AL) reagent water, groundwater, river water, wastewater effluent, and wastewater influent, were spiked with known amounts of antidepressant pharmaceuticals. The Solution 2000 water was from a unit within the laboratory, and groundwater was collected from a domestic well in Evergreen, CO. The river water was collected at a U.S. Geological Survey (USGS) station on the South Platte River, Denver, CO. Influent and effluent grab samples were collected from the City of Boulder, CO, wastewater-treatment plant; sample collection times were not adjusted for hydraulic residence time within the plant. One set of seven replicate 1-L samples of each Solution 2000, groundwater, river water, effluent, and influent were spiked to give a final concentration of 0.5 ng/L, and a second set was prepared to contain a final concentration of 100 ng/L of each of the antidepressant pharmaceuticals. The endogenous concentrations in the river water, raw influent, and final effluent, if any, were subtracted from the measured concentration of each spiked sample before calculating recoveries.

Liquid Chromatography−Mass Spectrometry. A 100 μL aliquot of each antidepressant pharmaceutical sample extract was separated by an Agilent 1100 LC (Palo Alto, CA). A 3.5-μm, 3.0 × 150 mm Eclipse XDB-Phenyl column (Agilent) heated to 60 °C was used for all separations. The LC solvents included Solution 2000 water with 0.1% (v/v) formic acid (>98%, EMD Chemicals, Darmstadt, Germany) and high-performance liquid chromatography grade acetonitrile (Burdick and Jackson, Muskegon, MI). The flow rate was 200 μL/min. The gradient is as follows:

time (min)	% acetonitrile	time (min)	% acetonitrile
0	10	9	85
3	10	9.1	95
3.1	45	14	95
5	45	14.1	10
8	85	20	10

The LC was directly interfaced to the electrospray ionization (ESI) source coupled to an Applied Biosystems/MDS Sciex 2000 QTrap (Framingham, MA). The QTrap is a hybrid triple-quadrupole/linear ion trap mass spectrometer that has MS/MS and MS/MS/MS capabilities. The QTrap ion source was operated in positive ESI mode, and multiple reaction monitoring (MRM) transition mode was used for sample analysis. Two MRM transitions (Table 1), a quantitation ion and a confirmation ion, were acquired for each analyte. Optimal instrumental source parameters are as follows: ion spray voltage, 4000 V; nebulizer gas pressure, 20 psig; heater gas pressure, 70 psig; collision gas pressure, 6 psig; and source temperature, 450 °C. The declustering potentials and collision energies were analyte dependent but ranged from 10 to 50 V and 7 to 50 eV, respectively (Table 1).

Quantitation and Confirmation. The two MRM transitions were quantified by internal standard calibration using standards prepared in methanol/0.1% formic acid. Weighted ($1/x$), linear regression was used to generate calibration curves from eight calibration standards, and the intercept was not forced through zero. Calibration standards ranged from 0.1 to 500 ng/L (equivalent to aqueous concentrations, assuming a 1-L sample) for each analyte, and contained 500 ng/L internal standard, sertraline-d_3. Points included in the calibration curves were required to be within 20% of the theoretical concentration. Calibration curves were analyzed at the beginning and end of each sample batch

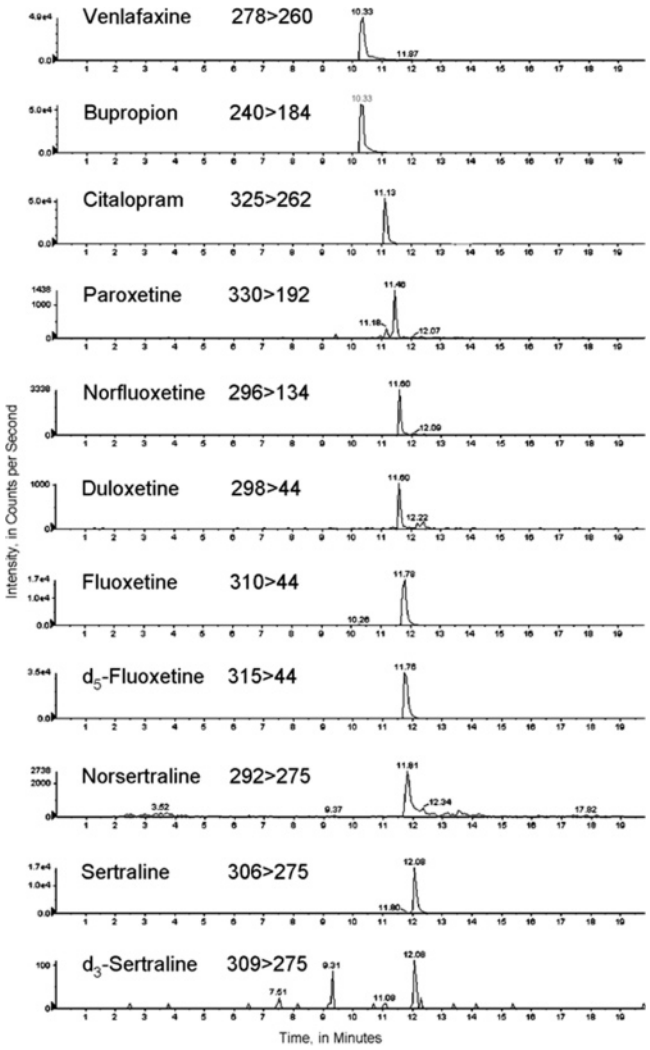

Figure 2. Typical chromatogram for an aqueous sample containing antidepressant pharmaceuticals (wastewater effluent). Only one of the two monitored ion transitions is shown.

with methanol solvent blanks and calibration verification standards analyzed within the set after approximately every fifth sample. Confirmation of antidepressent identification was performed by quantitating both the primary and secondary product ion transitions monitored for each analyte (Table 1). The ratio of the values for both transitions were in good agreement with the ratio of monitored transitions of standards, typically <10% variation.

Method Detection and Quantitation Limits. The method detection limit (MDL) for the entire method (extraction, isolation, detection, and quantitation) was determined as outlined by Grant et al.[25] where seven replicate 1-L aqueous samples were spiked to give a final concentration of one to five times the estimated MDL (~0.50 ng/L). The replicate aqueous samples then were analyzed, and the MDL was calculated by multiplying the standard deviation of the replicate analyses by the one-sided *t*-value corresponding to 6 degrees of freedom and a 99% confidence level. The limits of quantitation were defined as the concentrations that yielded signal-to-noise values of ≥10.

Environmental Sample Collection. Aqueous (stream and wastewater effluent) samples were collected unfiltered in 1-L amber glass jars. After collection, the samples were shipped on ice overnight and stored at 4 °C until extraction.

Grab samples of the wastewater effluent were collected from the Metropolitan Council Environmental Services Wastewater Treatment Plant (St. Paul, MN) in January 2006. The municipal wastewater treatment plant employs advanced secondary treatment with activated sludge and serves a population of 1.8 million and ~800 industries. It treats an average of 190 million gallons/day, with a capacity of 251 million gallons/day. Duplicate grab samples of the effluent were collected on Friday, January 20 at 7:30 a.m., 2:30 p.m., and 11:30 p.m. Subsequent duplicate samples were collected at 9:30 a.m. on the following 3 days: Tuesday, January 24, Thursday, January 26, and Sunday, January 29.

A Lagrangian sampling design was used to collect samples from Pecan Creek (Denton, TX) in August and September 2005 on five different occasions (August 10, August 18, August 25, September 8, and September 22). Samples were collected from three different locations downstream from the Pecan Creek Water Reclamation Plant. The Pecan Creek plant employs secondary treatment with activated sludge, sand filtration, and ultraviolet disinfection. The collection sites were 5, 643, and 1762 m downstream, respectively, from the location of the effluent discharge. All samples were shipped to the laboratory and stored frozen in polyethylene bottles until analysis. The time of travel (hours) of a discrete water parcel to be sampled was calculated from an empirical relation previously determined by a rhodamine dye study.[26] The total flow in Pecan Creek is predominantly wastewater effluent during the summer months.[27]

RESULTS AND DISCUSSION

Liquid Chromatography–Mass Spectrometry. Initial infusion experiments identified the precursor and product ions for each of the two transitions used to identify and quantify each compound (Table 1). In all cases, the precursor ion was $[M + H]^+$, most likely a protonation of the common amino moiety. The primary product ion (quantitation ion) was the most abundant fragment ion produced from the precursor ion, and likewise the secondary product ion (confirmation ion) was the second most abundant fragment ion produced (Table 1). Antidepressant identity was confirmed by quantitating both product ion transitions for each analyte. The resulting concentrations for each transition were compared for good agreement (within 10%), and the concentration associated with the primary product ion was reported.

The chromatographic separation of the antidepressant pharmaceuticals determined in this study is shown in Figure 2 for the final effluent collected from the Metropolitan Wastewater Treatment Plant (fluvoxamine not detected). Better chromatographic separation was achieved using a phenyl column as compared to the more conventional reversed-phase C_{18} column, as well as zirconia-based reversed-phase and hydrophilic interaction chromatography columns. This observation is attributed to the $\pi-\pi$ interactions of the phenyl moiety present on the column's stationary phase to the benzene ring(s) (at least one benzene ring

(25) Grant, C. L.; Hewitt, A. D.; Jenkins, T. F. *Am. Lab.* **1991**, 15–33.

(26) Taylor, R. D. Water Quality Aspects of an Intermittent Stream and Backwaters in an Urban North Texas Watershed. Ph.D. Dissertation, University of North Texas, Denton, TX, 2002.

(27) Brooks, B. W.; Stanley, J. K.; White, J. C.; Turner, P. K.; Wu, K. B.; Point, T. W. L. *Environ. Toxicol. Chem.* **2004**, *23*, 1057–1064.

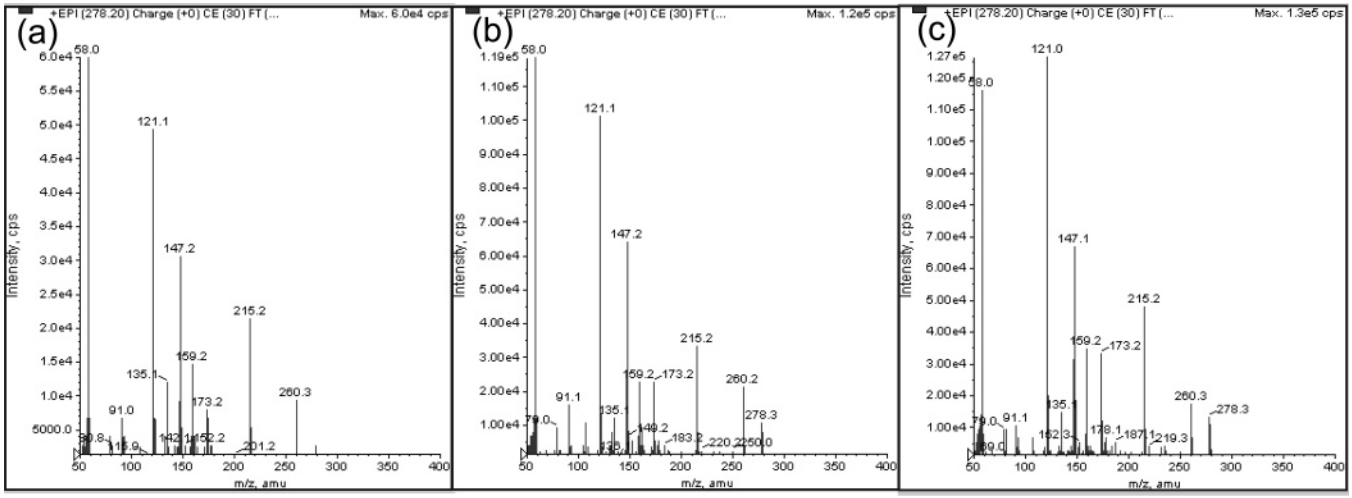

Figure 3. (a) Reference library EPI spectrum of venlafaxine with a collision energy of 30 eV. (b) EPI spectrum of venlafaxine in a river water extract (collision energy 30 eV, fit 0.985, reverse fit 0.978, and purity 0.973). (c) EPI spectrum of venlafaxine in a wastewater effluent extract (collision energy 30 eV, fit 0.972, reverse fit 0.964, and purity 0.962).

is present in each antidepressant) and possibly other π-bond containing function groups, such as carbonyls, that may be present. Chromatograms with authentic standards of antidepressants (not shown) indicated the following overall elution order: venlafaxine ≤ bupropion < citalopram < fluvoxamine (not shown) < paroxetine < norfluoxetine ≤ duloxetine < fluoxetine = fluoxetine- d_5 < norsertraline < sertraline = sertraline-d_3.

Because the third quadrupole of a QTrap mass spectrometer is a linear ion trap, additional scanning modes are available as compared to a conventional triple quadrupole. In particular, two of these scanning modes, MS/MS/MS trapped ion (MS^3) scans and enhanced product ion (EPI) scans, were explored for further analyte confirmation, which is especially important when working with complex environmental matrixes, such as wastewater. As shown in Table 1, not all antidepressants produced stable MS^3 ions, and even if a MS^3 transition were possible, the trace signals present in environmental samples were often not abundant enough to be detected by this scanning mechanism. In contrast, EPI scanning proved to be an effective, additional confirmatory tool. In EPI scanning, precursor ions are selected in Q1, fragmented in Q2, and the product ions are trapped in Q3, while conducting a conventional MRM experiment. The EPI mass spectrum consists of the trapped ions present in Q3. A library of EPI scans can be generated using authentic standards and later can be used as a confirmatory tool to EPI scans produced from samples. For example, Figure 3a shows the stored library spectrum of venlafaxine created with the analytical standard using collision energy of 30 eV. When using information-dependent acquisition (IDA), if the precursor ion for venlafaxine (m/z 278) is detected greater than a specified threshold, an EPI scan is triggered. Panels b and c in Figure 3 show the EPI spectra for venlafaxine in river water and wastewater effluent extracts obtained in IDA mode. The fit, reverse fit, and purity for venlafaxine as compared to the reference library were 0.985, 0.978, and 0.973, respectively, in the river water extract and were 0.972, 0.964, and 0.962, respectively, in wastewater effluent extracts, thus demonstrating that IDA-triggered EPI spectra provide unambiguous confirmation of antidepressant identity in complex samples.

Sample Preparation Optimization. Initially, aqueous samples were analyzed by direct injection, where a known amount of antidepressant standard was spiked into an aqueous sample, centrifuged, and a portion of the supernatant was collected and analyzed. Injection of a 100-μL aliquot into the LC/ESI/MS/MS proved insufficient because endogenous environmental concentrations of most of the measured antidepressants were at or less than the estimated direct-injection detection limits (~10 ng/L); however, direct injection could be viable for samples with analyte concentrations greater than 10 ng/L.

Oasis HLB solid-phase extraction cartridges were selected for the sample isolation and concentration procedure, demonstrating higher recoveries than C_{18} or Oasis MCX cartridges in preliminary experiments. Unfiltered, 1-L aqueous samples were extracted onto the 0.5-g, 6-mL cartridge except when extracting wastewater influent samples, where only 100 mL of sample was extracted. The wastewater influent samples often contained a higher concentration of suspended solids as compared to other aqueous samples and would often plug the extraction cartridges if a 1-L sample was used. Also, recoveries were improved by ~5% by acidifying the aqueous samples with 0.1% formic acid prior to extraction.

The accuracy and precision of the method was determined from spike and recovery experiments performed with antidepressant pharmaceuticals and degradates at high (100 ng/L) and low (0.5 ng/L) concentrations in Solution 2000 water, groundwater, Platte River water, Boulder wastewater effluent, and Boulder wastewater influent. Spike and recovery experiments at the low concentration were not performed in the wastewater influent because the endogenous concentrations of the antidepressants were typically at least 1 order of magnitude greater than the added amount. The absolute recoveries of the parent antidepressants from all unfiltered, aqueous matrixes spiked at low and high concentrations ranged from 72 to 118% and from 70 to 118%, respectively (Table 2). However, recoveries of the antidepressant degradates norfluoxetine and norsertraline were substantially more variable from the high concentration spike. The absolute recoveries for these two compounds from all aqueous matrixes

Table 2. Mean Percent Recoveries (% ± RSE) of the Individual Antidepressants and Degradates Obtained after Extraction from Aqueous Samples (Native Analyte Concentrations, if Present, in ng/L)[a]

compound	solution 2000 low spike	solution 2000 high spike	ground water low spike	ground water high spike	native conc in river water	river water low spike	river water high spike	native conc in waste water effluent	waste water effluant low spike	waste water effluent high spike	native conc in waste water influent	waste water influent[b] high spike	MDL[c] (ng/L)
fluoxetine	114 ± 8	101 ± 3	117 ± 4	96 ± 6	10.9	na	117 ± 3	45.3	na	110 ± 2	27.1	102 ± 5	0.25
norfluoxetine	118 ± 3	25 ± 4	93 ± 6	25 ± 6	1.9	na	98 ± 7	13.6	na	61 ± 8	14.2	12 ± 5	0.28
sertraline	108 ± 7	89 ± 4	104 ± 4	90 ± 5	4.5	na	84 ± 4	55.2	na	113 ± 4	60	70 ± 4	0.21
norsertraline	114 ± 4	53 ± 4	112 ± 6	40 ± 5	5	na	98 ± 4	37.5	na	33 ± 3	14.3	38 ± 9	0.32
paroxetine	99 ± 8	95 ± 3	107 ± 6	118 ± 2	0.9	85 ± 5	111 ± 3	15.8	na	95 ± 5	10.5	87 ± 6	0.32
citalopram	107 ± 6	85 ± 4	118 ± 7	108 ± 2	36.1	na	105 ± 3	125	na	86 ± 7[c]	78.9	82 ± 7	0.45
fluvoxamine	80 ± 3	72 ± 3	84 ± 6	84 ± 4	nd	87 ± 5	89 ± 3	2.5	na	100 ± 5	25.3	89 ± 5	0.24
duloxetine	88 ± 8	84 ± 4	86 ± 5	90 ± 4	<LOQ	115 ± 10	83 ± 2	2.5	na	97 ± 5	4.1	98 ± 6	0.24
bupropion	98 ± 6	110 ± 3	96 ± 7	107 ± 1	120	na	117 ± 3	221	na	115 ± 9[d]	72.5	85 ± 6	0.33
venlafaxine	123 ± 5	100 ± 2	113 ± 5	99 ± 2	310	na	108 ± 8[d]	873	na	113 ± 4[e]	930	118 ± 6[e]	0.29
fluoxetine-d_5 (surrogate)	106 ± 3	105 ± 2	96 ± 4	109 ± 4	nd	93 ± 8	96 ± 3	nd	83 ± 8	88 ± 3	nd	93 ± 8	0.19

[a] conc, concentration; na, native concentration at least an order of magnitude greater than the spiked amount, so sample not analyzed; nd, analyte not detected; LOQ, limit of quantitation; RSE, relative standard error. [b] Influent treated differently; only concentrated by 2 orders of magnitude (100 to 1 mL), all others 1000 to 1 mL. [c] MDL determined with the Evergreen, CO, groundwater because it did not contain native analytes of interest. [d] Aqueous sample only concentrated 2 orders of magnitude (100 to 1 mL), endogenous concentration too high. [e] Aqueous sample only concentrated 1 order of magnitude (10 to 1 mL), endogenous concentration too high.

ranged from 93 to 118% in the low-concentration spike samples and from 12 to 98%, in the high-concentration spike samples. Experiments were attempted to improve the extraction efficiency of norfluoxetine and norsertraline by verifying that losses did not result from breakthrough on the SPE phase, increasing the elution volume, and trying different elution solvents (98% methanol/2% acetic acid; 70% methanol/0.1% formic acid). Despite these efforts, the extraction efficiency at the high concentration did not improve. Norfluoxetine and norsertraline were retained in the method because they are important primary degradates that retain pharmacological activity; however, reported concentrations of these compounds are likely conservative estimates of actual environmental concentrations, especially at higher (100 ng/L) concentrations. The precision of the method, as indicated by the relative standard error (RSE), ranged from 1 to 5% for all antidepressants in the five replicate samples analyzed.

The MDL was determined from seven replicate blank groundwater samples that were spiked with each compound to a final concentration of 0.5 ng/L. The MDL determined for the antidepressant pharmaceuticals and degradates ranged from 0.19 to 0.45 ng/L (Table 2). The limit of quantitation (LOQ) for the SPE method was defined as the analyte concentration required to produce a signal-to-noise ratio of 10:1 within the environmental matrix and was typically on the order of 0.9 ng/L.

Application to Environmental Samples. The performance of the SPE LC/MS/MS method was evaluated by analyzing wastewater effluent samples and stream samples suspected to contain environmentally relevant concentrations of antidepressant pharmaceuticals.

Wastewater Effluent. Antidepressant concentrations found in the Metropolitan Wastewater Treatment Plant effluent ranged from 1.9 (duloxetine) to 2190 ng/L (venlafaxine) (Figure 4a and b). Effluent grab samples were collected three times within the same day to determine whether concentrations of antidepressants exhibited diurnal variations. Figure 4a shows that there is not a substantial change in concentration for the antidepressants, except for venlafaxine, which increased in concentration from 1430 ng/L observed at 7:30 a.m. to 1800 ng/L at 2:30 p.m. to 2190 ng/L at 11:30 p.m. The mean concentration of venlafaxine for the three time periods is 1800 ng/L, with a standard deviation of 380 ng/L. Based on the results observed for other pharmaceuticals at other wastewater treatment plants,[28] a variation of ±21% is likely typical of large wastewater treatment plant processes in samples collected over a short time. Additionally, effluent grab samples were collected three times over a 1-week period to examine whether considerable daily variations were present. Similarly, there were no substantial increases or decreases observed in antidepressant concentrations over this 7-day period (Figure 4b).

As already mentioned, limited studies have examined a suite of antidepressants in wastewater matrixes. Vanderford and Snyder[20] did include fluoxetine and norfluoxetine in their survey of pharmaceuticals and found an average of 25 ng/L fluoxetine and 3.9 ng/L norfluoxetine in six wastewater effluent samples collected in Las Vegas, NV. In the present study, fluoxetine and norfluoxetine were found to have averages of 58 and 5 ng/L, respectively (Figure 4a,b).

Stream Samples. As expected, individual antidepressant concentrations were less in the streamwater than those observed in the wastewater effluent with individual antidepressant concentrations ranging from 0.72 (norfluoxetine) to 1310 ng/L (venlafaxine). Venlafaxine, citaprolam, and bupropion were found at concentrations substantially higher than the other antidepressants measured, a pattern similar to that observed in wastewater effluent. In Table 3, average concentrations (±95 confidence interval) of individual antidepressants are reported for all samples collected on five different occasions from the three sites in Pecan Creek (Table 3). The concentrations of antidepressants were observed to be relatively stable within the stream reach and between days; the average concentrations were not found to be statistically different at the 95% confidence interval. This repeated sampling confirms the presence of antidepressants in a waste-dominated stream.

(28) Benotti, M. J.; Brownawell, B. J. *Environ. Sci. Technol.* **2007**, *41*, 5795–5802.

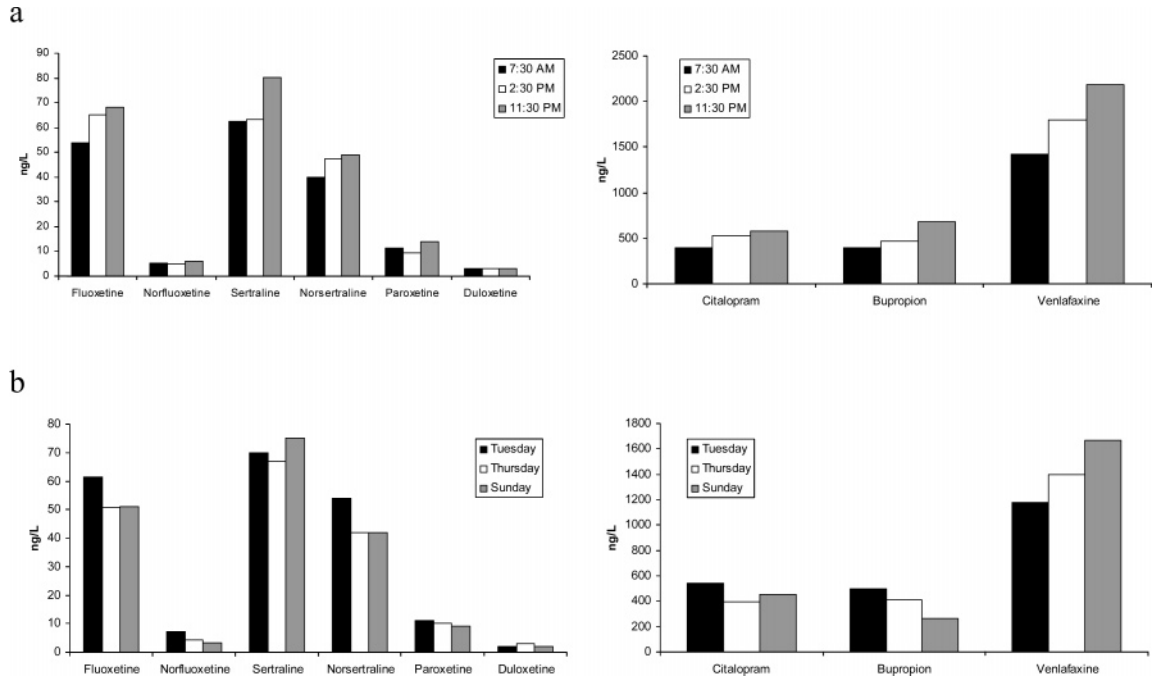

Figure 4. Effluent grab samples (a) collected the same day and (b) collected at the same time (9:30 a.m.) over 3 days.

Table 3. Average Concentrations (in ng/L) ± 95% Confidence Interval for Samples Collected from Pecan Creek[a]

	fluoxetine	norfluoxetine	sertraline	norsertraline	paroxetine	citalopram	duloxetine	bupropion	venlafaxine
site 1 (5 m)[a]	12 ± 3	0.83 ± 0.01	36 ± 5	5 ± 3	2.1 ± 0.4	90 ± 20	1.5 ± 0.2	50 ± 20	600 ± 200
site 2 (643 m)	20 ± 10	1.0 ± 0.5	49 ± 9	7 ± 3	3 ± 1	40 ± 30	2 ± 2	60 ± 40	1000 ± 400
site 3 (1762 m)	12 ± 5	0.9 ± 0.2	33 ± 8	3 ± 1	2.2 ± 0.2	80 ± 30	1.2 ± 0.9	50 ± 10	900 ± 300

[a] Values reported in parentheses refer to the distance downstream from the Pecan Creek Water Reclamation Plant.

Previous reports have found a median concentration of 12 ng/L fluoxetine in contaminated U.S. streams[2] and an average of 2.6 and 1.3 ng/L for fluoxetine and norfluoxetine, respectively, in a wastewater-dominated stream in Las Vegas, NV.[20] The range of concentrations of fluoxetine and norfluoxetine in this study were 12−20 and 0.83−1.0 ng/L, respectively. Also note that fish populations collected from Pecan Creek in proximity to the monitored sites contained concentrations of fluoxetine, sertraline, norfluoxetine, and norsertraline greater than 0.1 ng/g in all muscle, liver, and brain tissues examined.[21]

The results of this study confirm the authors' hypothesis that conventional wastewater treatment does not completely remove antidepressant pharmaceuticals and that wastewater treatment plants are a point source of antidepressants to the environment. In these environmental samples, typical aqueous concentrations of individual antidepressants were in the nanogram per liter range except for venlafaxine, which was found in microgram per liter concentrations in the wastewater samples and in selected stream samples. Further research is required to determine whether observations from this small sample set are representative of environmental concentrations and trends of antidepressants nationwide. Interestingly, the concentrations of venlafaxine and bupropion often were at least 1 order of magnitude greater than the more commonly investigated antidepressants fluoxetine and sertraline. To the best of our knowledge, this study represents the first documentation of venlafaxine, bupropion, and duloxetine as environmental contaminants.

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