Source characterization of nervous system active pharmaceutical ingredients in healthcare facility wastewaters

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ABSTRACT

Nervous system active pharmaceutical ingredients (APIs), including anti-depressants and opioids, are important clinically administered pharmaceuticals within healthcare facilities. This study provides source characterization data describing the composition and magnitude of nervous system APIs present in healthcare facility wastewaters. Concentrations and mass loadings of ten nervous system APIs and three nervous system API metabolites are reported for wastewaters from a hospital, nursing, assisted living, and independent living facility within a single municipality. Concentrations of nervous system APIs ranged from non-detectable levels for alprazolam in all four facility wastewaters to a high of 290 ng/L amitriptyline in nursing facility wastewater. The summed mean concentration of all thirteen analytes ranged from 402 ng/L in independent living facility wastewater to 624 ng/L in assisted living facility wastewater. Wastewater flow rates from each facility were combined with concentration data to estimate the daily mass loading of nervous system APIs leaving each facility through wastewater discharge to the municipal sewer system. The total mass loading of all thirteen analytes for the hospital, nursing, assisted living, and independent living facility wastewater was 228, 44, 29.5, and 28.1 mg/day, respectively. The total mass loading of nervous system APIs contributed to the municipality’s wastewater from all four facilities was 330 mg/day.

1. Introduction

National reconnaissance programs have established pharmaceuticals as environmental microcontaminants in groundwater, surface water, wastewater, and biota (Kolpin et al., 2002; Barnes et al., 2008; Focazio et al., 2008; Ramirez et al., 2009). The presence of pharmaceuticals in the environment occurs because humans and animals excrete administered pharmaceuticals unmetabolized, partly metabolized, or as metabolites directly into the environment or indirectly into the environment through wastewater conveyance and treatment systems that were not designed to remove trace organic contaminants. While pharmaceuticals are ubiquitous in wastewater influents and effluents, the contribution of pharmaceuticals to municipal wastewater from specific community sectors is not yet defined.

Healthcare facility wastewaters are a particularly under characterized source of pharmaceuticals to municipal wastewaters. Characterization of the pharmaceutical loading introduced to municipal wastewaters from healthcare effluents is of interest for risk management purposes due to the importance of healthcare facilities in providing medical care. As the debate concerning the importance of healthcare facilities as sources of pharmaceuticals to the environment is on-going, source characterization data is needed to inform risk assessments. This data is also required before decisions can be made concerning how, where, and whether or not to implement source reduction approaches or bring up advanced treatment systems for pretreatment.

The debate concerning whether or not healthcare facilities are a source of pharmaceuticals to the environment is caused by a combination of healthcare facility pharmaceutical consumption data, the abundance of healthcare facilities, and reports of pharmaceutical compounds measured in wastewater leaving hospitals. The approximately 9200 hospitals and clinics in the United States (NAICS, 2010) purchased slightly under 23% of the total pharmaceuticals sold in the US by one 2008 market estimate (IMS Health Incorporated, 2007). Studies exploring pharmaceutical residues in hospital wastewater also demonstrated the presence of individual compound classes of pharmaceuticals including analgesics/anti-rheumatics (Kümmerer, 2001), antibiotics (Brown et al., 2006), lipid regulators (Kümmerer, 2001), X-ray contrast media (Hai and
Kümmerer, 2006), anesthetics (Kümmerer, 2001), hormone antagonists (Liu et al., 2010), and chemotherapeutics (Lenz et al., 2007) in hospital wastewater. Recently, more complicated multi-residue analytical methods aimed at screening level characterization of pharmaceuticals in hospital wastewaters have begun to appear in the published literature, as well (Sim et al., 2009; Gómez et al., 2006; Gomez et al., 2007b; Langford and Thomas, 2009).

Despite the emerging concentration data for pharmaceuticals in hospital wastewaters, very few studies have provided actual mass loading estimates for the magnitude of pharmaceuticals leaving hospitals. Herberer and Feldmann were one of the first to report mass loadings for pharmaceuticals leaving hospitals. In their 2005 report, the mass loadings of carbamazepine and diclofenac released through wastewater from a military hospital were estimated to be 514 and 887 mg/day, respectively. Thomas et al. have also provided mass loadings data for two hospitals in Norway for three analgesics (paracetamol, ibuprofen, and diclofenac), one beta blocker (metoprolol), four antibiotics (tetracycline, trimethoprim, ciprofloxacin, sulfamethoxazole) and three naturally occurring estrogens (17β-estradiol, estriol, estrone). They report a combined mass loading of 90 and 64 g/day for the two hospitals (Thomas et al., 2007).

While the Herberer and Feldmann and Thomas et al. reports are a positive contribution to improving the understanding of hospitals as sources of pharmaceuticals to municipal wastewater, these reports present data for only a small portion of the pharmaceuticals and pharmaceutical metabolites present in hospital wastewater. Additionally, other types of healthcare facilities (including nursing, assisted living, stand alone emergency, rehabilitation, and independent living facilities as examples) need to be evaluated for the composition and magnitude of these compounds in wastewater from nursing care, assisted living, and independent living facilities, while three sampling locations were needed to capture the total discharge of the hospital.

Sampling occurred on April 14th and 15th, 2008. ISCO samplers collected 100 mL of wastewater every 15 min for 24 h into an ice-chilled 10 L polypropylene bottle. The collected wastewater samples at each location were subsampled into 1 L pre-labeled amber glass bottles in the field using a peristaltic pump. Clean tubing was used to subsample each facility's wastewater. Hospital wastewater from the three sample locations was combined in a 35 L glass vessel prior to subsampling. Two liters of subsampled wastewater from each facility were sent to the National Exposure Research Laboratory at the United States Environmental Protection Agency (US EPA) in Cincinnati, OH for analysis. Samples were shipped overnight on ice in coolers. Upon receipt in Cincinnati the samples were stored at 4 °C and extracted within three days of collection.

### 2.3. Sample extraction

All samples were split into 500 mL subsamples, filtered through a 0.7 μm filter, and spiked with 0.25 μg of each isotopically labeled procedural IS, 10 mg of Na₂EDTA, and 50 μg of ascorbic acid prior to extraction. Field blanks, laboratory blanks, and spiked distilled water controls were extracted along with the samples on 150 mg Oasis HLB MCX cartridges preconditioned with 6 mL amiben glass bottles in the field using a peristaltic pump. Clean tubing was used to subsample each facility's wastewater. Hospital wastewater from the three sample locations was combined in a 35 L glass vessel prior to subsampling. Two liters of subsampled wastewater from each facility were sent to the National Exposure Research Laboratory at the United States Environmental Protection Agency (US EPA) in Cincinnati, OH for analysis. Samples were shipped overnight on ice in coolers. Upon receipt in Cincinnati the samples were stored at 4 °C and extracted within three days of collection.

### 2.4. Extract analysis

A Waters Aquity UPLC coupled to a Micromass Quattro Micro triple quadrupole mass spectrometer was used to analyze the extracts for the ten nervous system APIs and three metabolites using positive electrospray ionization. 10 μL of extract was

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**Table 1**

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th># beds</th>
<th>Mean wastewater flow (L/day)</th>
<th>NAICS #</th>
<th>NAICS Facility Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>hospital</td>
<td>375</td>
<td>500,000</td>
<td>622110</td>
<td>general medical and surgical hospital</td>
</tr>
<tr>
<td>nursing</td>
<td>300</td>
<td>100,000</td>
<td>623110</td>
<td>nursing care facility</td>
</tr>
<tr>
<td>assisted living</td>
<td>225</td>
<td>50,000</td>
<td>623311</td>
<td>continuing care</td>
</tr>
<tr>
<td>independent living</td>
<td>225</td>
<td>70,000</td>
<td>623312</td>
<td>retirement communities</td>
</tr>
</tbody>
</table>

# – number of, NAICS – North American Industry Classification System.
injected through full loop injection onto a BEH C18 column (100 mm x 1.0 mm x 1.7 μm) equipped with a 0.2 μm in-line filter and maintained at 40 °C to chromatographically resolve the extracts at a flow rate of 100 μL/min. The complete analysis was divided into three injections, including injection 1 (I1) for paroxetine, alprazolam, amitriptyline, benztprine, norfluoxetine, fluoxetine, desmethylsertraline, sertraline; injection 2 (I2) for oxycodone and amphetamine; and injection 3 (I3) for carbamazepine.

A binary mobile phase of water with 0.3% formic acid (A) and 2:1 acetonitrile:methanol (B) and a linear gradient were used for all injections. For I1, B was held at 10% from 0 to 0.5 min, increased to 30% at 2.5 min, held at 30% until 9.5 min, further increased to 90% at 10.5 min, held at 90% until 11 min, returned to 10% at 11.5 min, and then held at 10% until the end of the run at 15 min. For I2, B was held at 10% from 0 to 0.5 min, increased to 50% at 7.5 min, further increased to 90% at 9.5 min, brought back to 10% at 10.5 min, and held at 10% until the end of the run at 14 min. For I3, B was held at 20% from 0 to 0.5 min, increased to 40% at 1 min, further increased to 85% at 6 min, held at 85% until 6.5 min, returned to 20% at 7 min, and held at 20% until the end of the run at 10 min.

Analyte detection was based upon retention time and multiple reaction monitoring (MRM) of two precursor-product transitions per analyte (with the exception of one transition for norfluoxetine). Analyte specific MRM transitions and instrument specific operation parameters are detailed in a separate publication (Batt et al., 2008). External calibration was used to quantify the detected analytes and procedural IS were used to ensure acceptable recovery of analytes in the extraction and analysis methods. The acceptable recovery range was 60–140% and only analytes with recoveries in the acceptable range are reported in this article. Additionally, all reported data was corrected to account for matrix effects based upon the procedural IS recovery in the sample matrix.

2.5. Mass loading estimations

Mass loadings of pharmaceuticals from individual facilities were determined by multiplying an analyte’s concentration in healthcare facility wastewater by the mean daily flow measured from the facility.

3. Results

Mean concentrations and recoveries for each nervous system API observed in the four healthcare facility wastewaters are presented in Table 2. The estimated daily mass loading of each nervous system API is given in Fig. 1. Matrix spiked recoveries ranged from a low of 35% for propoxyphene in independent living facility wastewater to 193% for 10-hydroxy-amitriptyline in the nursing facility wastewater. Overall, 88% of matrix spike samples analyzed in this research demonstrated acceptable recoveries between 60 and 140% for the analyte of interest. The concentration ranges and estimated mass loading of each nervous system API are presented in Sections 3.1–3.8.

3.1. Oxycodone

Oxycodone concentrations measured in healthcare facility wastewater ranged from non-detectable to 61 ng/L with the highest observed concentration occurring in hospital wastewater. The total estimated mass loading for oxycodone from the hospital, assisted living and independent living facility was 28, 0.4, and 1.0 mg/day, respectively.

3.2. Carbamazepine

Carbamazepine is one of the most commonly reported APIs in wastewater treatment plant influents (Heberer et al., 2002; Miao and Metcalfe, 2003; Lajeunesse and Gagnon, 2007). Carbamazepine concentrations measured in this study ranged from non-detectable in nursing facility wastewater to a high of 133 ng/L in independent living facility wastewater. The observed mass loading of carbamazepine was 18, 1.4, and 7.6 mg/day for the hospital, assisted living, and independent living facility, respectively.

3.3. Benzatropine, alprazolam, and propoxyphene

Benzatropine and alprazolam were not detectable in healthcare facility wastewater and did not have any measurable mass release from the facilities. Propoxyphene concentrations observed in this study ranged from non-detectable in nursing facility wastewater to a maximum concentration of 38 ng/L in assisted living facility wastewater. Significant matrix interferences prevent quantitative reporting for propoxyphene in hospital and independent living facility wastewaters, although a signal for the compound was present. Due to the observed matrix interferences, only the assisted living facility’s mass loading of propoxyphene to the municipal wastewater system could be estimated at 1.2 mg/day.
3.4. Amitriptyline and 10-hydroxy-amitriptyline

Amitriptyline was detected in each healthcare facility’s wastewater over a concentration range of 24–298 ng/L. 10-hydroxy-amitriptyline, a metabolite of amitriptyline, was also detected in each facility’s wastewater, but nursing facility wastewater data is not reportable due to matrix spike recoveries above 140%. The detected and reportable range of 10-hydroxy-amitriptyline in the hospital, assisted, and independent living facility wastewaters ranged from 9 ng/L to 91 ng/L.

The total mass loading of amitriptyline from the hospital, nursing, assisted, and independent living facility was 43, 27, 9.1, and 2.7 mg/day for a total loading of 81.8 mg/day. The mass loading of 10-hydroxy-amitriptyline was 37, 1.5, and 0.9 mg/day for the hospital, assisted living, and independent living facilities for a total loading of 39.4 mg/day. The resulting 121.2 mg/day combined loading for amitriptyline and its metabolite from the four facilities represents the highest observed loading of a nervous system API in this study.

3.5. Fluoxetine and norfluoxetine

Fluoxetine ranged from 38 ng/L in assisted living facility wastewater to 215 ng/L in the nursing facility wastewater. A signal for norfluoxetine was observed in each facility’s samples; however, the concentration for norfluoxetine is not reported due to contamination of the field blanks. The cause of the observed contamination remains uncertain. The mass loading for fluoxetine from the hospital, nursing, assisted, and independent living facility was 24, 17, 2.0, and 5.8 mg/L, respectively.

3.6. Paroxetine

The independent living facility wastewater was the only facility wastewater to have a measurable concentration of paroxetine at a maximum concentration of 29 ng/L and a mass loading of 2.0 mg/day. The three remaining facility wastewaters had non-detectable levels of paroxetine.

3.7. Sertraline and desmethylsertraline

Sertraline and the sertraline metabolite, desmethylsertraline, were detected in wastewater from the hospital, assisted living, and independent living facility. The concentration of sertraline ranged from non-detectable in nursing facility wastewater to a high of 129 ng/L in independent living facility wastewater. Desmethylsertraline concentrations in healthcare wastewaters were similar to sertraline, ranging from non-detectable in nursing facility wastewater to a high of 93 ng/L in assisted living facility wastewater. A signal for desmethylsertraline in independent living facility wastewater was observed, but a matrix spike recovery below 60% prevented quantitative reporting of this metabolite.

Sertraline’s mass loading from the hospital, assisted living, and independent living facility was 38, 5.1, and 8.3 mg/day, respectively. The hospital and assisted living facility wastewaters also contained 21 and 4 mg/day of the metabolite for a total mass loading of sertraline and desmethylsertraline from all four facilities of 76.4 mg/day.

3.8. Amphetamine

Amphetamine was detected in two of the four facility wastewaters at a maximum concentration of 102 ng/L in assisted living facility wastewater. Amphetamine is well reported in the literature as methods to evaluate the use of illicit drugs in wastewater matrices have recently emerged (van Nuijs et al., 2009; Boles and Wells, 2010; Kasprzyk-Hordern et al., 2010) However, to the best of our knowledge this is the first report of amphetamine in healthcare facility wastewaters.

The mass loading of amphetamine from the two facilities with detectable concentrations of amphetamine was 18 mg/day from the hospital and 4.8 mg/day from the assisted living facility. It is interesting to note that while the concentration of amphetamine in assisted living facility wastewater was three times higher than the concentration of amphetamine in hospital wastewater, the total loading from the hospital was more than four times higher than the assisted living facility due to the difference in wastewater flow from the two facilities.

4. Discussion

Only concentration ranges of two analytes from our dataset (carbamazepine and fluoxetine) have been previously reported in wastewater from a healthcare facility (hospital effluents) to the best of our knowledge. Gomez et al. reported a concentration range for carbamazepine in hospital wastewater effluents from 1 ng/L to...
210 ng/L (Gómez et al., 2006; Gomez et al., 2007a). Gomez et al. also report the concentration of fluoxetine in effluent from a hospital with a range of 4–100 ng/L (Gomez et al., 2007b). These reports agree with the concentrations range of carbamazepine (nd-133 ng/L) and fluoxetine (38–215 ng/L) reported in this study, suggesting that these two compounds in healthcare facility wastewater will be expected at concentrations below 250 ng/L.

Low observed concentrations of nervous system APIs in healthcare facility wastewaters also result in low observed mass loadings of these compounds from these facilities. Outside of this study, mass loading data for the evaluated nervous system APIs appear limited to a single report on the mass loading of carbamazepine in a military hospital's wastewater system. Herberger and Feldmann determined the mass loading of carbamazepine from the hospital in Germany to be approximately 500 mg/day (Herberger and Feldmann, 2005). While the German hospital had fewer beds than the hospital evaluated in this study, Herberger and Feldmann (2005) reported mass loading is higher than the 18, 14, and 7.6 mg/day mass loading reported for the hospital, assisted living, and independent living facility, respectively. The variable mass loading of carbamazepine between the two studies indicates that individual facilities contribute varying levels of pharmaceuticals due to differences in pharmaceutical usage and wastewater flow rates between facilities.

While concentration and mass loading data of the reported nervous system APIs in healthcare facility wastewater are limited, literature reports for the monitored analytes in wastewater influents have established the presence of nervous system APIs at concentrations ranging from non-detectable to low ng/L levels. Wastewater influent concentrations of the three analyzed metabolites (norfluoxetine, 10-hydroxy-amitriptyline, and desmethyleratine), sertraline, and paroxetine are consistently lower than 75 ng/L when detected on an infrequent basis (Lajeuennesse et al., 2008; Vasskog et al., 2008; Metcalfe et al., 2010). Oxycodone and fluoxetine concentrations reported for wastewater influents are slightly higher, but consistent, with maximum reported concentrations of 220 and 177 ng/L, respectively (Chiaia et al., 2008; Metcalfe et al., 2010). Concentrations of carbamazepine, amitriptyline, and amphetamine are highly variable in wastewater influents ranging from non-detectable levels to concentrations exceeding 5 µg/L for each analyte (Ternes, 2001; Kasprzyk-Hordern et al., 2009).

Because nervous system APIs reported in this study are not present in healthcare facilities at several orders of magnitude higher than literature reports for influent, healthcare facilities do not appear to be a more significant source of nervous system APIs to municipal wastewaters than other sectors including residential. At their low observed concentrations, the contribution of nervous system APIs from healthcare facilities will be proportional to the wastewater flow from the facilities compared to the total wastewater flow in the system. Since the four healthcare facilities evaluated in this study contribute less than 2% of the total wastewater flow entering the municipal wastewater system, based on the concentration and wastewater flow data we conclude that these four facilities are not a significant source of nervous system APIs to the municipality's wastewater system. However, in small municipalities or in municipalities where a healthcare facility is located directly up sewer from the municipal wastewater treatment plant, healthcare facility discharges may be important to environmental loadings of nervous system APIs. Additionally, because the summed concentrations of each facility's wastewater were consistently between 400 and 600 ng/L for all of the analytes (466, 470, 624, and 402 ng/L for the hospital, nursing, assisted living, and independent living facility, respectively), pharmaceutical concentrations in wastewater from healthcare facilities may provide a conservative estimate of concentrations in wastewater from residential developments.
investigated in this study are present at ng/L concentrations in wastewaters from a hospital, nursing, assisted living, and independent living facility. The same part-per-trillion concentration magnitude has been reported for these compounds in wastewater treatment system influents from other studies, indicating that healthcare facilities are not significant sources of nervous system APIs to municipal wastewaters compared to other still unidentified sources. When pharmaceutical compounds are present at part-per-trillion levels in healthcare and wastewater influents, the contribution of the analytes from healthcare facilities will be proportional to the flow rate from these facilities. However, it is likely that the observed concentrations in healthcare facilities will be a conservative estimate of residential contributions of nervous system APIs to wastewater.

Despite not being a significant source of nervous system APIs to the municipal wastewater, the facilities did contribute 228, 44, 29.5, and 28.1 mg/day of nervous system APIs from the hospital, nursing, assisted living, and independent living facility, respectively. The provided data informs risk assessment discussions by providing source characterization data for nervous system APIs not previously reported for hospital, nursing, assisted living, and independent living facility wastewaters.

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Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.jenvman.2010.10.058.

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NAICS. Health Care and Social Assistance. http://www.naics.com/naics62.htm, [last accessed 27.05.10].