Quantitative and Semi-Quantitative Determination of PPCPs and Their By-Products in Wastewater by Orbitrap MS

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Key Words
Environmental analysis, pharmaceuticals, personal care products, Exactive Plus, Orbitrap, high-resolution mass spectrometry, accurate mass

Goal
To develop an analytical method to determine the concentrations of pharmaceutical and personal care products (PPCPs) and their transformation products in wastewater during treatment processes.

Introduction
There is growing environmental concern regarding the health impact of trace levels of pharmaceuticals and personal care products (PPCPs) in the environment. PPCPs and endocrine disrupting chemicals (EDCs) detected in surface and drinking waters, as well as in treated wastewater, are an issue of increasing international attention due to potential environmental impacts.[1,2] These compounds are distributed widely in surface waters due to anthropogenic activities, improper disposal, and agricultural runoff. This presents a major challenge to drinking water treatment facilities, which are required to provide potable water that meets regulatory requirements for human consumption.

The goal of this work is to develop an analytical method capable of determining PPCPs and their by-products in wastewater treatment plant (WWTP) samples. This workflow was applied in a survey of 35 permeate samples obtained from a pilot anaerobic membrane bioreactor (AnMBR). Permeate is the filtrate from an AnMBR. Depending on the pore size of the membrane filter used and the waste stream being treated, the permeate may require further treatment prior to the final discharge to the environment.

Experimental
Chemicals, Sample Collection, and Preparation
For this study, wastewater permeate samples were chosen due to their complex matrix, which poses a challenge for conventional analytical methods. These samples were collected from an anaerobic membrane bioreactor pilot plant located at the Wastewater Technology Centre (Environment Canada, Burlington, Ontario). A total of 35 permeate samples were taken from January 2012 to March 2013 encompassing one summer and two winter sample sets. The reactor was operated at 20, 35, and 55 °C using these sample sets to investigate the effect on the removal of PPCPs in permeates. Grab samples were contained in 1 L amber bottles without headspace and stored in dark, cold storage (4 °C) until analysis, typically about four weeks.

Ethylenediaminetetraacetic acid disodium salt (EDTA Na_2, ACS grade), ammonium acetate, and neat standards of native target compounds were purchased from a reputable supplier. Fifteen deuterium (D) and ^{13}C-labeled standards were purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada) and Cambridge Isotope Laboratories (Andover, MA, US) and were used as surrogates to carry out isotope dilution mass spectrometric (IDMS) analysis. Five levels of analytical standard solutions were prepared by diluting intermediate solutions with Fisher Chemical™ HPLC-grade methanol (CH_3OH). High purity water used for aqueous mobile phases and sample preparation was produced by passing reverse osmosis water through a Thermo Scientific™ Barnstead™ Nanopure™ water purification system.

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Ontario Ministry of the Environment and Climate Change, Laboratory Services Branch (LaSB) method E3454 was used to prepare samples for targeted compound analysis and nontargeted compound screening. Method E3454, which has been accredited to the ISO 17025 standard since 2004, uses hydrophilic-lipophilic balanced solid-phase extraction (SPE) cartridges (6 mL, 200 mg) to extract analytes in one single neutral (pH 6.95) extraction.

Samples were prepared in batches of 12 that included three quality control (QC) samples (i.e., laboratory blank and two method spikes) and nine field samples to maximize operational efficiency. Duplicate method spike samples were used to monitor intra-run method precision and matrix effects. For the sample preparation, 200 mL of permeate samples were used. Prior to extraction, 1 g of EDTA Na_2, 500 µL of surrogate solution and 10 mL of 0.25 M ammonium acetate solution were added into each sample, homogenized on a roller (Wheaton Science, NJ, USA) for 10 min, and the pH value adjusted to 6.95 ± 0.05 using 10% (w/v) sodium hydroxide or 10% (v/v) sulfuric acid solution.

Each SPE cartridge was conditioned by adding 5 mL of water, 5 mL of methanol, and finally 5 mL 5% (v/v) ammonium hydroxide in methanol. A 200 mL aliquot of sample was loaded onto the SPE column. The SPE cartridges were rinsed with 5 mL of 10% (v/v) methanol/water, dried by air, and the analytes eluted from the SPE cartridges with 5 mL methanol. Then, 1 mL of the eluate was evaporated to dryness with nitrogen at ambient temperature, and reconstituted by using 0.1 mL of the instrument internal standard solution, which comprised isotopically labeled bisphenol-A and sulfamethazine prepared in methanol.

Five levels of calibration standards (2–100 ng/mL for level 1 and multiples of 5x, 10x, 50x, and 100x, respectively, for levels 2, 3, 4, and 5) were prepared by using a mixture of surrogate solution and each of different levels of target compound solutions, evaporating to dryness under nitrogen, and reconstituted to 0.3 mL using the instrument internal standard solution before instrumental analysis. The linearity of the calibration curves for each target compound was 0.99900 or better.

HPLC Separation

Sample analysis was achieved on a Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC consisting of an HRG-3400RS binary pump, WPS-3000 autosampler, and a TCC-3400 column compartment. Separation was performed by injecting 5 µL extracts onto a 2.1 × 100 mm (3 µm particle size) Thermo Scientific™ Betasil™ C18 column for positive mode MS analysis and a Thermo Scientific™ Hypersil GOLD™, 2.1 × 100 mm (3 µm particle size) column for negative mode MS analysis (part numbers 71503-102130 and 25003-102130, respectively). Mobile phase and gradient information is listed in Table 1.

One positive mode HPLC separation and two negative mode HPLC separations were used for the analysis of PPCPs and their by-products.

Table 1. HPLC mobile phase and gradient used in the analysis.

Parameter	Setting			
Column oven temperature	35 °C			
Flow rate	450 µL/min			
Mobile phase (Positive)	A: 5 mM ammonium formate/0.1% formic acid in methanol/water (10:90, v/v) B: Methanol/water (90:10, v/v)			
Mobile phase (Negative I)	A: Acetonitrile/water (10:90, v/v), pH 6.95±0.3 B: Acetonitrile			
Mobile phase (Negative II)	A: 5 mM ammonium acetate in acetonitrile/water (10:90, v/v), pH 6.95±0.3 B: Acetonitrile			
HPLC gradient	Time (min)	% A	% B	Curve
	0.0	95	5	5: linear
	2.0	25	75	5: linear
	10.0	5	95	7: concave upward (mid slope)
	15.0	5	95	5: linear
	15.2	95	5	5: linear

Mass Spectrometry

The HPLC was interfaced to a Thermo Scientific™ Exactive™ Plus hybrid quadrupole-Orbitrap mass spectrometer using a heated electrospray ionization (HESI-II) interface. High-purity nitrogen (>99%) was used in the HESI-II source (35 arbitrary units). Spray voltages used were 2500 and –3200 V for positive and negative modes, respectively. Mass spectrometric data was acquired from 95 to 950 Dalton at a resolving power of 140,000 (full width at half maximum, at *m/z* 200, R_{FWHM}), resulting in a scan rate of greater than 1.5 scans/s when using automatic gain control target of 1.0 x 10^6 and a C-trap inject time of 100 ms.

Data Analysis

Thermo Scientific™ TraceFinder™ software was used to perform quantitative analysis for 56 PPCPs. The same software was also used to perform non-targeted screening with a database of 312 pharmaceutically active compounds and their metabolites, steroids, hormones, surfactants, and perfluorohydrocarbons. TraceFinder software was used to search for adduct ions $(M+H)^+$, $(M+NH_4)^+$, and $(M+Na)^+$ in the positive mode and the $(M-H)^-$ molecular ion in the negative mode for compounds listed in the database. The software then created an extracted ion chromatogram (XIC) using a mass extraction window (MEW) of 5 ppm. Analytes were automatically identified using an XIC area threshold of 50,000 (approximately 25–50 pg/mL (ppt) depending on compound), a 5 ppm mass accuracy for the mono-isotopic mass (M) and at least two isotopic peaks ((M+1) and (M+2)), and a relative intensity of 90% ± 10% from the theoretical values. Results obtained from TraceFinder software were also exported to Thermo Scientific™ SIEVE™ software to carry out a ChemSpider™ search for nontargeted compounds and principal component analysis (PCA) to characterize the effect of both seasonal changes and treatment parameters.

Results and Discussion

Method Performance

Quantitative analysis determined that 43 target PPCPs comprising pharmaceuticals like antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs), as well as personal care products such as insect repellent and antimicrobial agents, were present (Table 2). Antibiotics (for example, ciprofloxacin and sulfa drugs) were found have the lowest median concentrations compared to other therapeutic classes. As depicted in Figure 1, the highest median concentration was reported for the antidepressant drug; however, since this group only has one representative (carbamazepine), it is difficult to draw any conclusions.

Table 2. Quantitative results for PPCPS with >75% occurrence in the 35 samples analyzed.

Compound Name	Usage	Occurrence	LOD (ng/L) N=7	Concentration (ng/L)		
				Min	Max	Median
Caffeine	Stimulant	100%	4.01	295	25,200	5450
Carbamazepine	Antiepileptic/antidepressant	100%	0.17	696	11,200	2520
DEET	Insect repellent	100%	0.90	219	1810	652
Lidocaine	Anesthetic/anti-arrhythmic	100%	0.28	175	3410	648
Lincomycin	Antibiotic	100%	0.24	51.8	9290	636
Ketoprofen	Analgesic/anti-inflammatory	100%	1.37	45.6	351	127
Bezafibrate	Lipid regulator	100%	0.33	34.1	324	71.6
Sulfamethazine	Antibiotic	97%	0.28	11.6	114	31.2
Bisphenol A	Commercial additive	95%	5.41	1600	2.80 x 10^6	9420
Acetaminophen	Analgesic/anti-inflammatory	95%	3.76	352	7.86 x 10^5	8030
Diclofenac	Analgesic/anti-inflammatory	95%	1.94	2.70	20,800	1270
Norfloxacin	Antibiotic	95%	0.77	191	1030	433
Triclocarban	Antimicrobial/antifungal	95%	0.56	10.4	1270	297
Triclosan	Antibacterial/antifungal	87%	1.67	207	126,000	3300
Estrone	Estrogen	85%	2.36	5.10	1640	265
Oxolinic acid	Antibiotic	85%	4.81	78.9	6420	162
Oxybenzone	Sunscreen	82%	3.60	1.80	14,300	295
Norethindrone	Ovulation inhibitor	82%	0.49	46.4	1460	275
Ciprofloxacin	Antibiotic	79%	4.08	934	57,600	4000
Estriol	Estrogen	79%	4.08	26.9	23,100	657
Ibuprofen	Analgesic/anti-inflammatory	77%	1.34	14.9	125,000	4370

LOD: Limit of detection

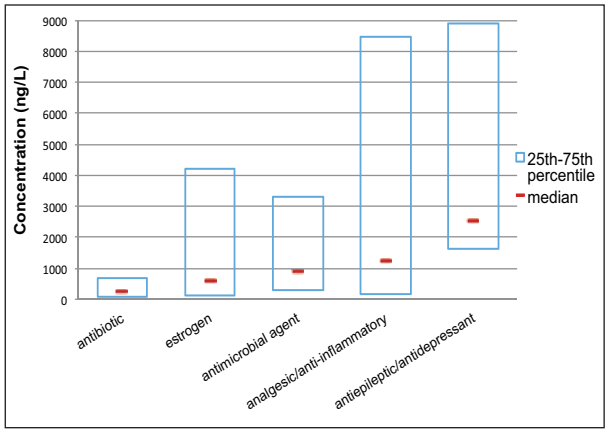

Figure 1. Median concentrations for selected groups of PPCPs.

Semi-Quantitative Determination of PPCPs

Triclosan (TCS, antimicrobial agent) and carbamazepine (CBZ, anticonvulsant drug) were used for the demonstration of by-product formation during wastewater treatment processes. These are representative members of the two most studied groups of PPCPs. The effects of treatment temperatures and seasonal changes on the permeate samples were first investigated using principal component analysis (PCA). This was done by creating a dataset that consisted of mass spectral data for the 35 permeate samples and analytical standards. From the mass spectral data, the amount of the compounds in each sample was determined and used in the PCA. The variance was calculated from these 35 samples as represented by the first three orthogonal (uncorrelated) factors. The contribution of each sample to the dataset was then calculated as a linear combination of weighted values (scores) of the three orthogonal factors and plotted along the eigenvectors. Samples with similar contributions to the dataset will have similar scores along the eigenvectors and form a cluster in the plot. As shown in Figure 2, scores for samples treated at 20 °C (red, summer) and 55 °C (brown, winter) were similar, while scores for samples obtained from 35 °C (green, winter) and standards (blue) were quite different. This is an indication that treatment temperatures probably had exerted more effect on samples than seasonal changes.

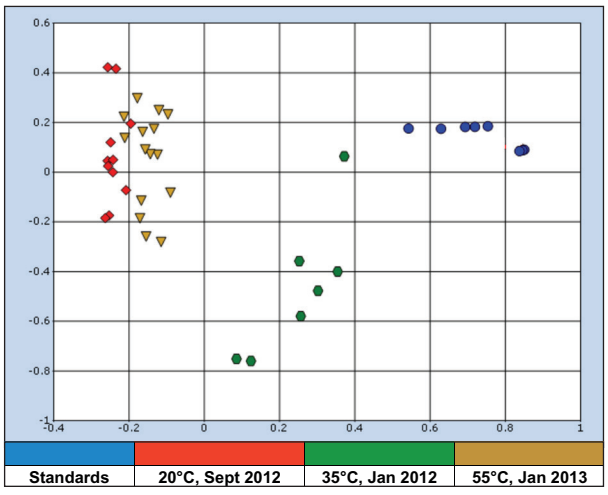

Figure 2. Overall effect of treatment temperatures.

Despite the vast number of TCS by-products proposed,[4] five compounds (dichlorohydroxy-diphenyl ether, 2- and 4-chlorophenol (Cl-Ph), methyl triclosan (Me-TCS), and 4- and 5-chloro triclosan, (Cl-TCS)) were identified in this experiment. Using area counts obtained from the analysis, relative populations of TCS, deuterium-labeled TCS (TCS-D3), Cl-Ph, Cl-TCS, and Me-TCS are shown in Figure 3, indicating that the population of Cl-Ph was minimal while other compounds reached their maximum at 35 °C.

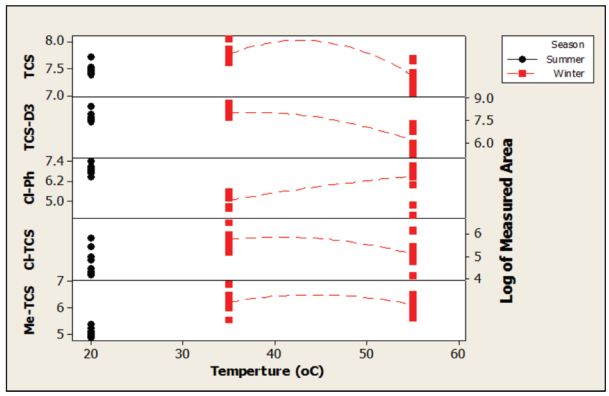

Figure 3. Relative concentration of TCS and the three TCS by-products found.

In comparison with the other PPCPs studied, a total of 16 CBZ by-products were identified in this work. Many of these CBZ by-products were isomers and had the same chemical formula and the same monoisotopic mass. Therefore, the identification using full-scan mass spectra became a challenge. As a result, the chromatographic peak was assigned to the most probable structure with the most dominated population in the literature. Semi-quantitative concentrations of CBZ, deuterium-labeled CBZ (CBZ-D10), and the three by-products found are shown in Figure 4.

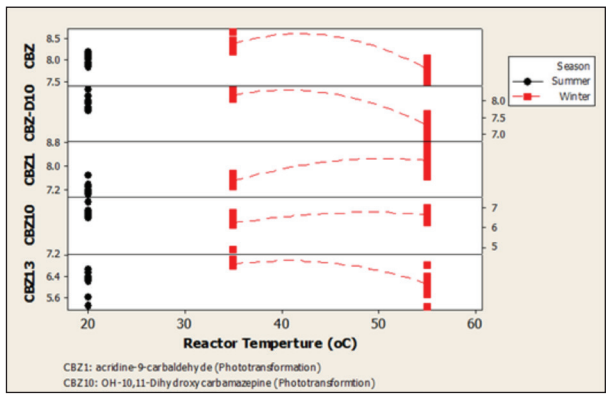

Figure 4. Relative concentration of CBZ and the three CBZ by-products found.

Conclusion

Quantitative results obtained using LC-Orbitrap MS show the prevalence of various PPCPs in wastewater, particularly for compounds with high usage and/or poor elimination (for example, caffeine, carbamazepine, DEET, lidocaine, lincomycin, ketoprofen, and bezafibrate).

For PPCP by-products, *in-situ* microbial degradation was the dominant pathway for triclocarban removal; while triclosan, diclofenac, and carbamazepine were eliminated via a combination of photodegradation and metabolism. Thirty by-products were detected in this pilot survey, including the toxic compounds chlorophenol isomers and acridone.

Semi-quantitative results, seasonal trends, and effect of treatment temperature on PPCP by-products were obtained using TraceFinder and SIEVE software.

Efforts to obtain analytical standards to complete the studies are on-going.

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