Quantitative and Semi-Quantitative Determination of PPCPs and Their By-products in Wastewater Treatment Plants Samples Using UHPLC-Orbitrap MS and Data Mining Technologies

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Overview

Purpose: Develop an analytical method to (1) determine PPCP concentrations in wastewater samples, and (2) examine the transformation of selected PPCPs during treatment processes.

Methods: Samples prepared by solid phase extraction (SPE) and analyzed by high performance liquid chromatography-Orbitrap mass spectrometry (HPLC-Orbitrap MS).

Results: Quantitative results of selected pharmaceuticals and personal care products (PPCPs) like DEET, Triclosan (TCS), Triclocarban (TCC), Diclofenac (DCL), Carbamazepine (CBZ) and semi-quantitative of their degradation products were obtained.

Introduction

Results obtained from a simple and powerful workflow that can readily determine PPCPs and their by-products in wastewater treatment plant (WWTPs) samples will be presented. This workflow was applied in a survey of 43 permeate samples obtained from a pilot anaerobic membrane bioreactor (AnMBR). Quantitative results show the prevalence of various PPCPs in wastewater, particularly for compounds with high usage and/or poor elimination (e.g., caffeine, carbamazepine (CBZ), DEET, lidocaine, lincomycin, ketoprofen, and bezafibrate). For PPCP by-products, we identified that in-situ microbial degradation was the dominant pathway for triclocarban (TCC) removal; whilst triclosan (TCS), diclofenac (DCF) and CBZ were eliminated via a combination of photodegradation and metabolism. Thirty by-products were detected in this pilot survey, including the toxic compounds chlorophenol and acridone.

Methods

Sample Preparation

For this study, permeate samples were chosen due to their complex matrix which poses as a challenge for conventional analytical method. These samples were collected from a pilot anaerobic membrane bioreactor (AnMBR) pilot plant located at the Wastewater Technology Centre (Environment Canada, Burlington, Ontario). A total of 35 permeate samples permeate tank from January 2012 to March 2013. During this time, the reactor were operated at different temperatures at 20, 35 and 55 °C using samples collected, respectively, in summer, winter and winter, to investigate the effect on the removal of PPCPs in permeates. Grab samples were contained in 1L-amber bottles without headspace and stored in dark, cold storage (4°C) until analysis.

Neat standards of native target compounds were purchased from Sigma-Aldrich (Oakville, ON, Canada). Deuterium (D) and ^{13}C-labelled standards were purchased from CDN Isotopes (Pointe-Claire, QC, Canada) and Cambridge isotope Laboratories (Andover, MA, US). Five levels of analytical standard solutions were prepared by diluting intermediate solutions with CH_3OH HPLC grade acetonitrile (CH_3CN) and methanol (CH_3OH) were purchased from Thermo Fisher Scientific (Ottawa, ON, Canada). 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 (Mississauga, ON, Canada). Laboratory Services NBranch (LaSB) method E3454[1] was used to prepare samples for targeted compound analysis and non-targeted compound screening. Waters OASIS® (Mississauga, ON, Canada) HLB solid phase extraction (SPE) cartridge (6 cc, 500 mg) was used in the extraction. Method E3454 has been accredited by the Canadian Association for Laboratory Accreditation (CALA) since 2004.

Liquid Chromatography (or more generically Separations)

Sample analysis was achieved on a Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC consisting of a HRG-3400RS binary pump, WPS-3000 autosampler, and a TCC-3400 column compartment. Separation was made by injecting 5 μL extracts into a Thermo Scientific™ Betasil™ and a Thermo Scientific™ Hypersil™ Gold, 2.1x100 mm columns, respectively, for positive and negative mode Orbitrap MS analysis.

One positive mode HPLC 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

Column oven temperature: 35°C; Flow rate: 450 mL/min				
Mobile phase (Positive)	A: 5 mM $HCOONH_4$/0.1% HCOOH in 10:90/CH_3OH:H_2O B: 90:10/CH_3OH:H_2O			
Mobile phase (Negative I)	A: 10:90/CH_3CN:H2O, pH 6.95±0.3 B: CH_3CN			
Mobile phase (Negative II)	A: 5 mM CH_3COONH_4 in 10:90/CH_3CN:H2O, pH 6.95±0.3 B: CH_3CN			
HPLC Gradient	Time (min)	% A	% B	Curve
	0.0	95	5	5
	2.0	25	75	5
	10.0	5	95	7
	15.0	5	95	5
	15.2	95	5	5

Mass Spectrometry

The HPLC was interfaced to a Thermo Scientific™ Exactive Plus™ Orbitrap MS using a heated electrospray ionization (HESI) interface. The Orbitrap MS system was tuned and calibrated in positive and negative modes by infusion of standard mixtures of MSCAL5 and MSCAL6. High purity nitrogen (>99%) was used in the ESI source (35 L/min). Spray voltages used were 2500 and 3200 V for positive and negative modes. Mass spectrometric data was acquired at a resolving power of 140000 (defined as full-width-at-half-maximum peakwidth at *m/z* 200, R_{FWHM}), resulting a scanning rate of > 1.5 scans/sec when using automatic gain control target of 1.0×10^6 and a C-trap inject time of 100 msec.

Data Analysis

Thermo Scientific™ TraceFinder™ software were used to perform quantitative analysis for 56 PPCPs. The same software was also used to perform non-targeted screening along with a database of 312 compounds consisting of pharmaceutically active compounds and their metabolites, steroids, hormones, surfactants and perfluorohydrocarbons. TraceFinder software is used to search for adduct ions $(M+H)^+$, $(M+NH_4)^+$ and $(M+Na)^+$ in the positive mode and $(M-H)^-$ molecular ion in the negative mode for compounds listed in the database. The software then creates 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. Typical screening time was about 65 sec/sample using the 312 CEC database. Results obtained from TraceFinder software were also exported to Thermo Scientific™ SIEVE™ software to carry out ChemSpider™ search. Principal component analysis was carried out using the SIEVE software too.

Results

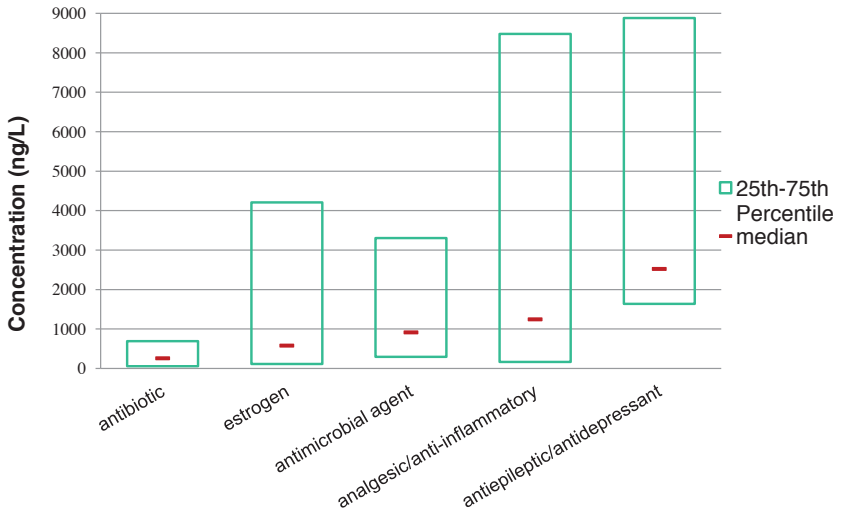
Quantitative Analytical Results

Quantitative analysis determined 43 target PPCPs comprised of pharmaceuticals like antibiotics, non-steroidal anti-inflammatory drug (NSAID); as well as personal care products such as insect repellent and antimicrobial agents (Table 2). Antibiotics (e.g. ciprofloxacin and sulfa drugs) found have the lowest median concentration compared to other therapeutic classes. As depicted in Figure 1, the highest median concentration is reported for the antidepressant drug; however since this group only has one representative (i.e., CBZ), it is difficult to draw any conclusion.

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

Compound Name	Usage	CAS #	Occur.	Concentration (ng/L)		
				Min	Max	Median
Caffeine	Stimulant	58-08-2	100%	2.95E+02	2.52E+04	5.45E+03
Carbamazepine	Antiepileptic/antidepressant	298-46-4	100%	6.96E+02	1.12E+04	2.52E+03
DEET	insect repellent	134-62-3	100%	2.19E+02	1.81E+03	6.52E+02
Lidocaine	anesthetic/anti-arrhythmic	137-58-6	100%	1.75E+02	3.41E+03	6.48E+02
Lincomycin	Antibiotic	154-21-2	100%	5.18E+01	9.29E+03	6.36E+02
Ketoprofen	analgesic/anti-inflammatory	22071-15-4	100%	4.56E+01	3.51E+02	1.27E+02
Bezafibrate	lipid regulator	41859-67-0	100%	3.41E+01	3.24E+02	7.16E+01
Sulfamethazine	Antibiotic	57-68-1	97%	1.16E+01	1.14E+02	3.12E+01
Bisphenol A	commercial additive	80-05-7	95%	1.60E+03	2.80E+06	9.42E+03
Acetaminophen	analgesic/anti-inflammatory	103-90-2	95%	3.52E+02	7.86E+05	8.03E+03
Diclofenac	analgesic/anti-inflammatory	15307-86-5	95%	2.70E+00	2.08E+04	1.27E+03
Norfloxacin	antibiotic	70458-96-7	95%	1.91E+02	1.03E+03	4.33E+02
Triclocarban	antimicrobial/antifungal	101-20-2	95%	1.04E+01	1.27E+03	2.97E+02
Triclosan	antibacterial/antifungal	3380-34-5	87%	2.07E+02	1.26E+05	3.30E+03
Estrone	estrogen	53-16-7	85%	5.10E+00	1.64E+03	2.65E+02
Oxolinic acid	antibiotic	14698-29-4	85%	7.89E+01	6.42E+03	1.62E+02
Oxybenzone	sunscreen	131-57-7	82%	1.80E+00	1.43E+04	2.95E+02
Norethindrone	ovulation inhibitor	68-22-4	82%	4.64E+01	1.46E+03	2.75E+02
Ciprofloxacin	antibiotic	85721-33-1	79%	9.34E+02	5.76E+04	4.00E+03
Estriol	estrogen	50-27-1	79%	2.69E+01	2.31E+04	6.57E+02
Ibuprofen	analgesic/anti-inflammatory	15687-27-1	77%	1.49E+01	1.25E+05	4.37E+03

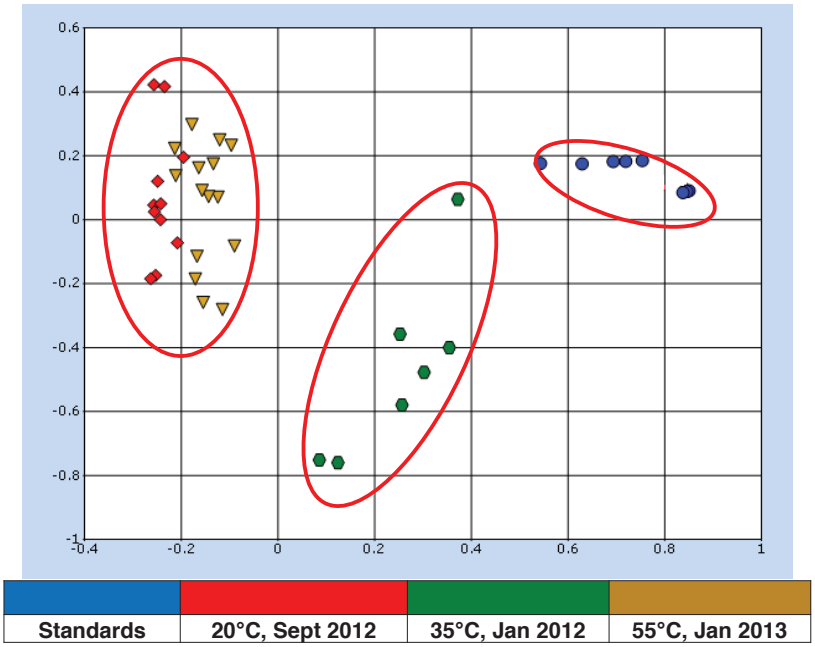
FIGURE 1. Median concentrations for selected groups of PPCPs



Semi-Quantitative Determination of PPCPs

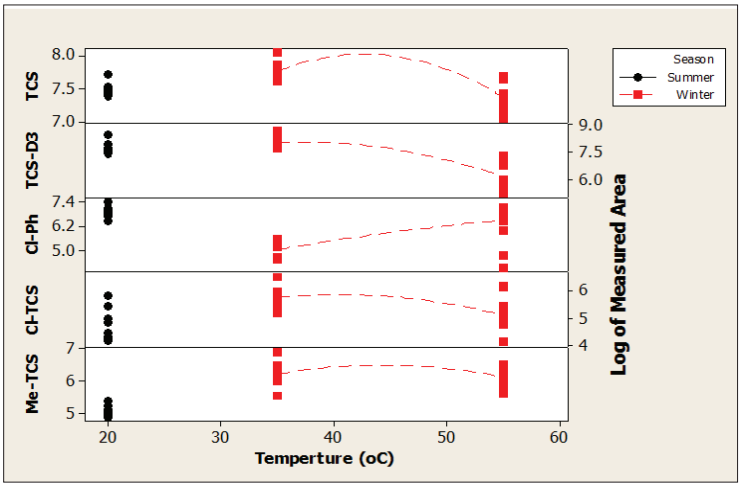
In this presentation, TCS (antimicrobial agent) and CBZ (anticonvulsant drug) will be used for the demonstration of by-product formation during wastewater treatment processes. They are representative of pharmaceuticals and are the two most studied groups of medicines. The effect of treatment temperatures and seasonal changes were first investigated using principal component analysis. 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. An indication that treatment temperatures exerted more effect on samples than seasonal changes.

FIGURE 2. Overall effect of treatment temperatures



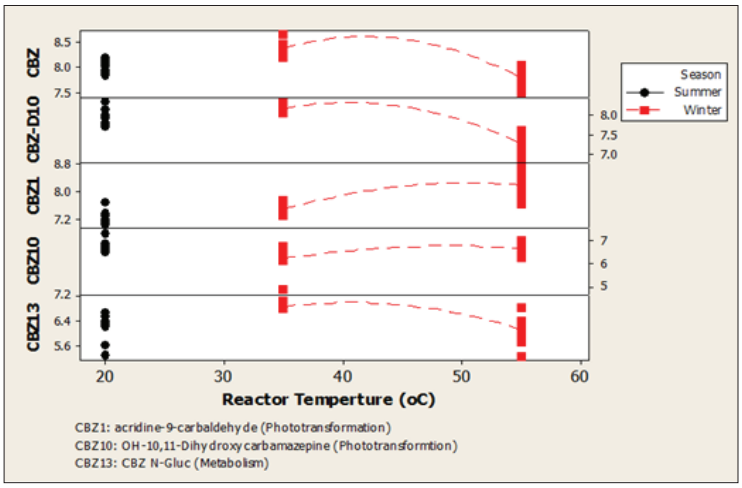
Despite the vast number of TCS by-products proposed in the literature [2], five compounds (i.e., dichlorohydroxy-diphenyl ether, 2- and 4-chlorophenol (Cl-Ph), methyl Triclosan (Me-TCS), and 4- and 5-chloro Triclosan, (Cl-TCS)). Semi-quantitative concentrations of TCS, deuterium labelled TCS (TCS-D3), Cl-Ph, Cl-TCS and Me-TCS are shown in Figure 3, indicating population of Cl-Ph were minimum 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 other PPCPs studied, CBZ had the most by-products identified (16) in this work. The unequivocal identification for CBZ by-products thus became a challenge as many of these compounds had the same chemical formula and therefore, the same monoisotopic mass measured by the Orbitrap MS. Without available reference standards, the chromatographic peak was assigned to the most probable structure with the most dominated population in the literature. Semi-quantitative concentrations of CBZ, deuterium labelled 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 of PPCPs were obtained using HPLC-Orbitrap MS.
- 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.

References

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