Routine, Targeted and Non-Targeted Analysis of Environmental Contaminants of Emerging Concern – Development and Validation of a UHPLC Orbitrap MS Method

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Overview

The purpose of this work was to develop a method for the targeted, quantitative analysis of 61 contaminants of emerging concern (CECs) and non-targeted screening of 312 CECs in wastewater treatment plant (WWTP) samples. The method used a solid phase extraction procedure (SPE), ultrahigh performance liquid chromatography (UHPLC) separation, Thermo Scientific™ Orbitrap™ mass spectrometry analysis (UHPLC-Orbitrap MS) and the Thermo Scientific™ TraceFinder™ software tool. An in-house compound database consisting of 312 CECs including parents products and their metabolites, conjugates and treatment by-products was used in the verification of the workflow as well as the non-targeted identification of CECs. Samples collected from WWTPs were used to demonstrate the effectiveness of this method for both targeted and non-targeted CEC analysis without using analytical standards.

Introduction

Contaminants of emerging concern in the environment are generally described as compounds that are unknown or unrecognized, undetected or not routinely monitored, and represent a diverse group of chemicals that may pose a risk to human health and the environment. New CECs were discovered by using recently available analytical technologies and implicated by a prior knowledge of the process details. Due to limited analytical capability and available resources (e.g., hardware, software, analytical standards and capacity), monitoring of CECs has been focused on selected analytes rather than a holistic approach which includes as many known chemical classes in the analysis as possible. Presented in this poster is a new analytical method that can be used in the quantitative analysis of 61 targeted and 312 non-targeted CECs. Analytical results obtained for a series of WWTP samples were used to evaluate and demonstrate the effectiveness of this method.

Methods

Sampling

Grab samples were collected from a pilot WWTP (Figure 1) and two WWTPs (Figure 2) using a nitrifying process and ultraviolet disinfection technologies. Following screening, primary sewage grab samples were taken from the aerated grit tank (Figures 1 and 2, S1) and thickened waste activated sludge tank (Figure 1, TWAS, S2). Primary effluent samples (Figure 2, S3) were taken after primary sedimentation (with settled solids removed at this stage) and after the first point of addition of ferric chloride to reduce total phosphorous through precipitation. Secondary and final effluent grab samples (Figure 2, S4 and S6) and permeate (Figure 1, S5) were also collected from the WWTPs. A total of ten samples were collected and stored at 4±2 ºC until ready for analysis.

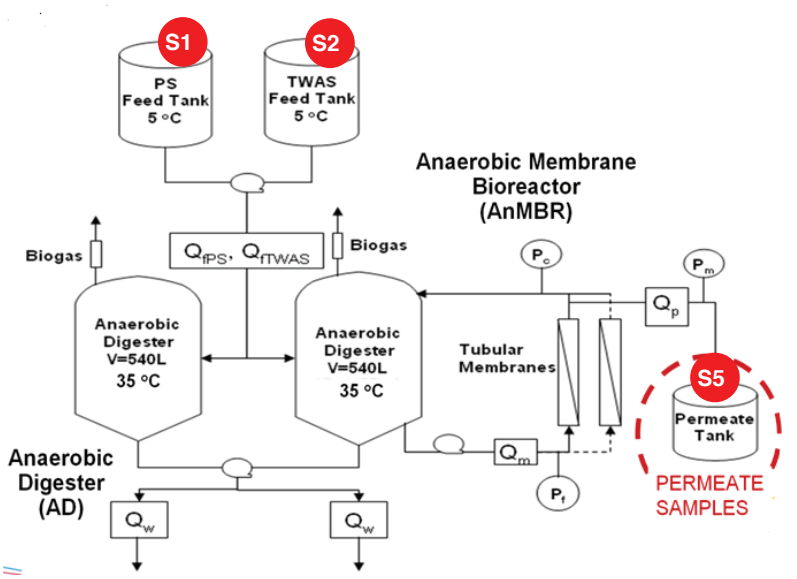
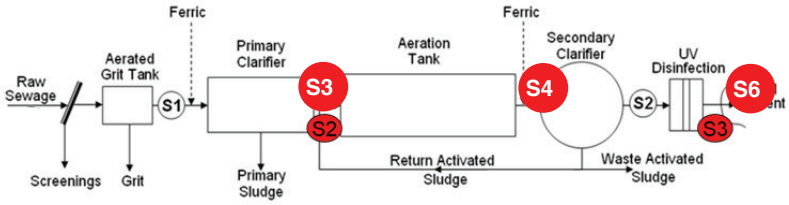


Figure 1. Schematic of the pilot WWTP identifying the three locations of sampling points.

Figure 2. Schematic of the WWTP identifying the three sampling locations.



Chemicals, Sample Preparation and UHPLC Orbitrap MS Analysis

HPLC grade acetonitrile (CH_3CN) and methanol (CH_3OH) were purchased from 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.

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). Native and isotopically-labelled intermediate standard solutions were prepared by mixing the corresponding stock solutions in CH_3OH. Five levels of analytical standard solutions were prepared by diluting intermediate solutions with CH_3OH.

Sample analysis was achieved on a Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC 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™ column (positive mode) and an Agilent XDB C-18, 2.1x100 mm coreshell technology column, respectively, for positive and negative mode Orbitrap MS analysis. Details of the UHPLC analysis is available on request (Ref. 1). The UHPLC was interfaced to a Thermo Scientific™ Exactive™ Plus Orbitrap MS using a heated electrospray ionization (H-ESI II) 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 2,500 and 3,200 V for positive and negative modes. Mass spectrometric data was acquired at a resolving power of 140,000 (defined as full-width-at-half-maximum peak width at *m/z* 200, R_{FWHM}), resulting a scanning rate of > 1.5 scans/sec when using automatic gain control target of $1.6x10^6$ and a C-trap inject time of 50 msec.

Data Analysis

TraceFinder software was used to perform targeted, quantitative analysis of 61 CECs. The same software was also used to perform non-targeted screening along with a database of 312 CECs consisting of pharmaceutically active compounds, steroids, hormones, surfactants and perfluorohydrocarbon. 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 an isotopic (M+1) peak threshold of 90% with relative intensity variation of < 10%. Typical screening time was about 65 sec/sample using the 312 CEC database. Analytical results were interpreted manually for the top 10^{th} percentile compounds and exported to Microsoft Excel® with which analytical data were compiled for the presentation.

Results

Targeted Compound Analysis

Table 1 lists results obtained from targeted compound analysis in the collected samples along with their respective method detection limits (MDL). A total of 21 of the 61 target compounds were found in the ten samples analyzed.

FIGURE 3. Sample preparation and analysis using UHPLC-Orbitrap MS.

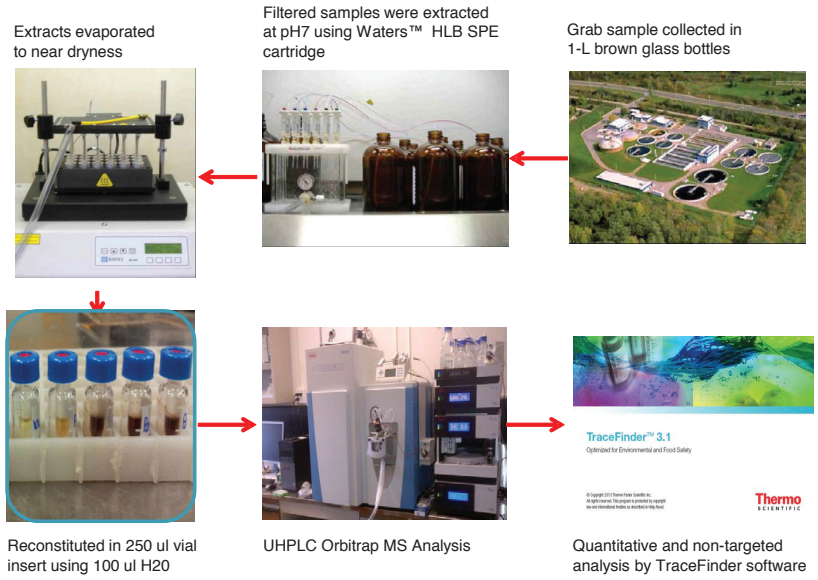


TABLE 1. Results of targeted compound analysis.

Compound	MDL	#1, PS	#1, PS	#2, PS	#1, TWAS	#1, PE	#2, PE	#2, SE	#1, FE	Permate	Permate
	ng/L										
Acetamidophenol	100	<MDL	<MDL	578.5	111.5	1952.5	4026.5	<MDL	<MDL	105.0	<MDL
Atenolol	50	<MDL	<MDL	<MDL	<MDL	143.5	579.5	288.5	<MDL	<MDL	<MDL
Atorvastatin	10	147.5	3419.5	3417.5	107.5	<MDL	165.0	<MDL	3419.5	110.0	72.5
Bezafibrate	20	<MDL	<MDL	<MDL	49.0	53.0	109.0	23.0	<MDL	<MDL	<MDL
Caffeine	20	147.5	75.5	525.0	34.5	4426.5	<MDL	35.0	<MDL	288.0	105.5
Carbadox	200	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Carbamazepine	2	332.5	<MDL	<MDL	107.0	24.0	235.5	115.0	<MDL	262.0	183.5
Ciprofloxacin	100	918.0	289.0	289.5	316.0	304.5	319.5	315.0	298.5	606.5	536.0
DEET	150	<MDL	<MDL	<MDL	<MDL	<MDL	338.5	<MDL	<MDL	<MDL	<MDL
Diclofenac sodium	100	<MDL	<MDL	<MDL	166.5	<MDL	148.5	<MDL	<MDL	<MDL	<MDL
Hydrocortisone	5	1781.5	<MDL	<MDL	7.5	8.0	11.0	9.5	<MDL	976.5	636.5
Lidocaine	10	38.0	<MDL	<MDL	114.0	<MDL	118.5	54.5	<MDL	32.5	35.5
Oxolinic Acid	20	<MDL	<MDL	<MDL	81.0	<MDL	125.5	49.0	<MDL	<MDL	<MDL
Progesterone	20	29.0	<MDL	<MDL	<MDL	<MDL	957.5	<MDL	<MDL	108.5	<MDL
Bisphenol A	200	4458.0	617.5	1252.5	522.0	1211.0	1675.5	213.0	249.0	3621.5	3383.5
Equilin	50	1619.0	449.5	<MDL	<MDL	678.0	1531.0	1337.0	<MDL	1441.0	345.5
Estriol	200	<MDL	472.5	<MDL	1006.0	216.5	<MDL	<MDL	<MDL	<MDL	<MDL
Gemfibrozil	10	<MDL	121.5	193.0	281.0	174.0	125.0	77.5	127.0	260.0	227.0
Oxybenzone	50	158.5	346.0	<MDL	170.0	237.0	166.5	170.0	196.0	159.5	159.5
Triclocarban	50	<MDL	734.0	429.0	366.5	1176.5	532.0	247.5	411.0	<MDL	<MDL
Triclosan	120	3068.5	<MDL	<MDL	2422.0	<MDL	<MDL	<MDL	343.5	777.5	912.0

Screening of Non-targeted Compounds

The identification of non-targeted compounds uses accurate mass of the monoisotopic peak M and isotopic (M+1) peaks, relative intensities of the M/(M+1) peaks and isotopic pattern of the halogenated compounds. Manual inspection of line shape of the XIC chromatogram, major fragment ions from the mass spectrum will also improve the confidence and credibility of analytical results. Figure 4 shows examples of true positive identification of 3-phenoxybenzoic acid, a common primary metabolite of the synthetic pyrethroid insecticides (4A); a halogen containing xenobiotic 3,5-dibromo-4-hydroxybenzoic acid (4B); and an artificial sweetener sucralose (Splenda®) (4C).

Figure 5 shows an example of false-positive identification of desethylatrazine, an environmental metobolite of the pesticide atrazine. The mono-isotopic peak M has a mass error of 0.2454 ppm and the XIC has a perfect Gaussian shape and can be considered as a positive identification. However, mismatch of chlorine isotopic pattern concludes that the compound in question is not desethylatrazine.

FIGURE 4. Examples of true-positive identification.

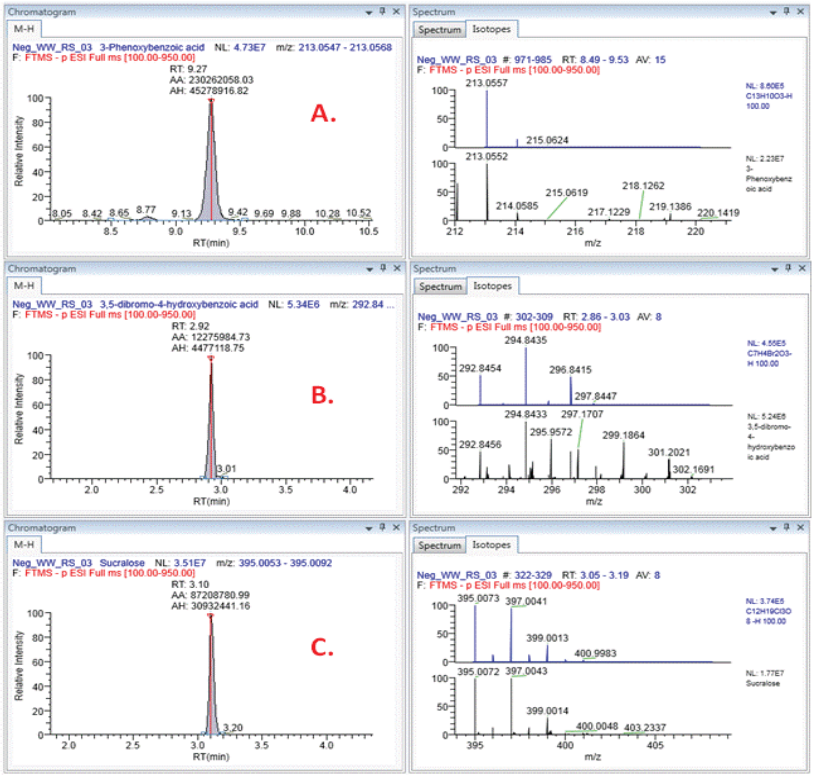


FIGURE 5. Example of a false-positive identification.

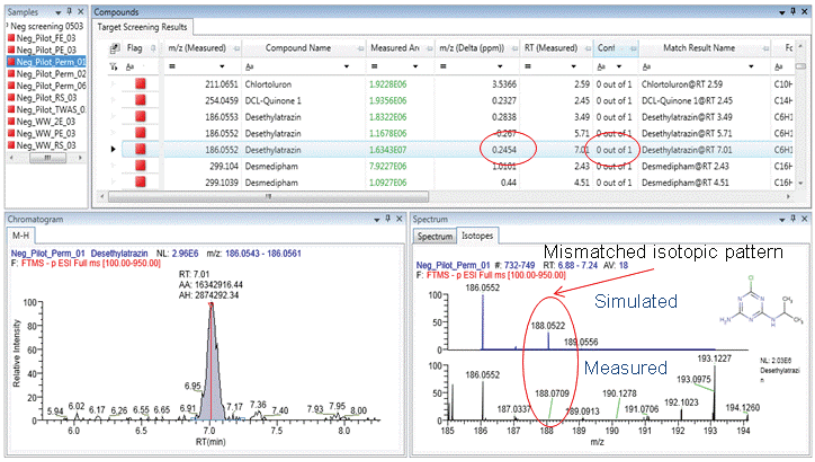


Table 2 lists screening results of non-targeted compounds found in these 10 WWTP samples along with their occurrence in both the positive and negative mode using the 312 CEC database. Of the 48 compounds in the top 10th percentile of area counts, bisphenol A was the only one positively identified.

TABLE 2. Results of non-targeted compound analysis (Occu.: Occurrence; CBZ: Carbamazepine).

Compound Name	Occu.	Compound Name	Occu.
Benzotriazol	100%	Isoproturon-didemethyl	80%
Methyl-Benzotriazol	100%	Primidon	80%
N,N-Didesvenlafaxin	100%	3-(4-Methylbenzylidene)-camphor	70%
N-Desvenlafaxine	100%	Valsartan	70%
O-Desvenlafaxine	100%	Acridone	60%
Tramadol	100%	Dimethachlor	60%
Venlafaxine	100%	2-(2-Hydroxy-5-methylphenyl)benzotriazole	50%
Lamotrigin	100%	2-Ethylhexyl-4-methoxycinnamate	50%
Metoprolol	100%	Clarithromycin	50%
Acridine	100%	Valsartan	80%
Dinoseb	100%	OH-Diclofenac	80%
n-Perfluorooctanoic acid	100%	2-(2,4-Dichlorophenoxyl)-phenol	80%
Galaxolidone	100%	5-Chloro-2-(2-chlorophenoxyl)-phenol	80%
Nonylphenol monoethoxylate	100%	5-Chloro-2-(4-chloro-2-hydroxyl-phenyl)phenol	80%
Perfluorooctane sulfonate	100%	5-Chloro-2-(4-chlorophenoxyl)-phenol	80%
Carbamazepine-10,11-epoxide	100%	Irbesartan	70%
10,11-Epoxide-CBZ	100%	Bentazon	70%
OH-CBZ	100%	Ethofumesate	70%
Fenofibric-Acid	100%	Oxazepam	70%
3-Phenoxybenzoic acid	90%	Prometon	60%
Sucralose	90%	Terbumeton	60%
Nonylphenol diethoxylate	90%	Phenazon (Antipyrine)	60%
Di-OH-CBZ	90%	Primidon	60%
Perfluorohexane sulfonate	90%	Fluconazole	60%

Conclusion

It is demonstrated that the combination Orbitrap MS hardware and TraceFinder software is a powerful tool in the analysis of contaminants of emerging concern, with improved data quality, confidence and credibility in analytical results. These include the lack of a analytical standards and hence, chromatographic retention time for the confirmation of non-targeted compounds dictated that caution must be taken to avoid the false-identification of CECs.

- True positive identification of targeted and tentative identification of non-targeted compounds including pharmaceutically active compounds, endocrine disrupting compounds and environmental metabolites;
- To achieve unambiguous identification of targeted and non-targeted analytes, a mass resolving power of 140,000 should always be used along with a 2.1x100 mm UHPLC column that produces chromatographic peak of full-width-at-half-maximum of 3–5 sec.
- Identification by accurate mass of M and isotopic (M+1) peaks and their relative intensity of M/(M+1) can achieve reliable results. Confirmation by fragment ion(s), as suggested by SANCO (Ref. 2), library search and complementary UHPLC information can be useful to improve the confidence and credibility of results.

References

1. “The Determination of Emerging Organic Pollutants in Environmental Matrices by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)”, Ontario Ministry of the Environment method E3454. Available via laboratoryservicesbranch@ontario.ca.
2. “Method validation and quality control procedures for pesticide residues analysis in food and feed” , Document N° SANCO/12495/2011.

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