Qualitative and Quantitative Analysis of Contaminants of Emerging Concern in Biosolids Using Dilute-and-Shoot UHPLC-Orbitrap MS Method

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Overview

Purpose: Develop a workflow to (1) do quantitatively analyze contaminants of emerging concerns (CECs) in biosolids samples, and (2) screen for 381 targeted CECs in samples.

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

Results: Quantitative results of CECs in typical biosolids samples are presented. Targeted screening of CECs in biosolids showed the presence of different categories of CECs including parent pharmaceuticals and personal care products (PPCPs), e.g., DEET, Triclosan (TCS), Triclocarban (TCC), musks, Carbamazepine (CBZ), their degradation products, and surfactants.

Introduction

A rapid dilute-and-shoot method for the quantitative determination of targeted CECs, e.g., endocrine disrupting chemicals, pharmaceuticals, personal care products, as well as their degradation by-products has been developed. Using ultrasonic based sample preparation and HPLC-Orbitrap MS analysis without any sample cleanup, this method has been optimized for the determination of 49 CECs present in biosolids and terrestrial biomes exposed to biosolids amended soils (BAS). The quantitative information on the CECs in biosolids and biological tissues would allow for the assessment, when and where appropriate, of potential uptake and bioaccumulation. In addition, full scan HRMS data provides information on the possible environmental transformation by-products for possible environmental accumulation and ecological effects that would not be available with other technology.

Methods

Sample Preparation

For this study, model biosolid samples and biosolids amended samples were used in the evaluation of the method. Grab biosolid samples were contained in 1L-amber bottles without headspace and stored in dark, cold storage (4°C) until analysis. The same biosolids were also used to prepare BAS at Ryerson University and used to observe the fate of CECs from October 2013 to March 2014.

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).

Biosolids and BAS samples were dried in fumehood for 96 hours, sieved through a 200 micron mesh, homogenized and stored in freezer until ready for extraction. Sample extraction was done using 5.0 g of sample in glass centrifuge tubes, 20 mL of the extraction solvent A (acetonitrile: 0.1% acetic acid in H2O, 70:30 (v/v), 1 mM ethylenediaminetetraacetic acid (EDTA) and isotopically labelled surrogates. The tubes were shaken for 5 min and sonicated for 20 min, shaken for another 5 min and centrifuged for 8 min at 3500 rpm. The supernatant was transferred into another glass centrifuge tube (50 mL). The cycle was repeated using solvent B (acetonitrile:Acetone, 50:50 (v/v)). The combined extracts volumes were brought up to 50 mL, centrifuged for 3 min at 5000 rpm and 10 mL of the extract was evaporated to dryness. The residues were dissolved in 100 μL of the internal standard then injected into the HPLC-Orbitrap MS for analysis.

High Pressure Liquid Chromatography Separation

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 mL extracts into a Thermo Scientific™ Betasil™ and a Thermo Scientific™ Hypersil™ Gold, 2.1x100 mm column, respectively, for positive and negative mode Orbitrap MS analysis. Three 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

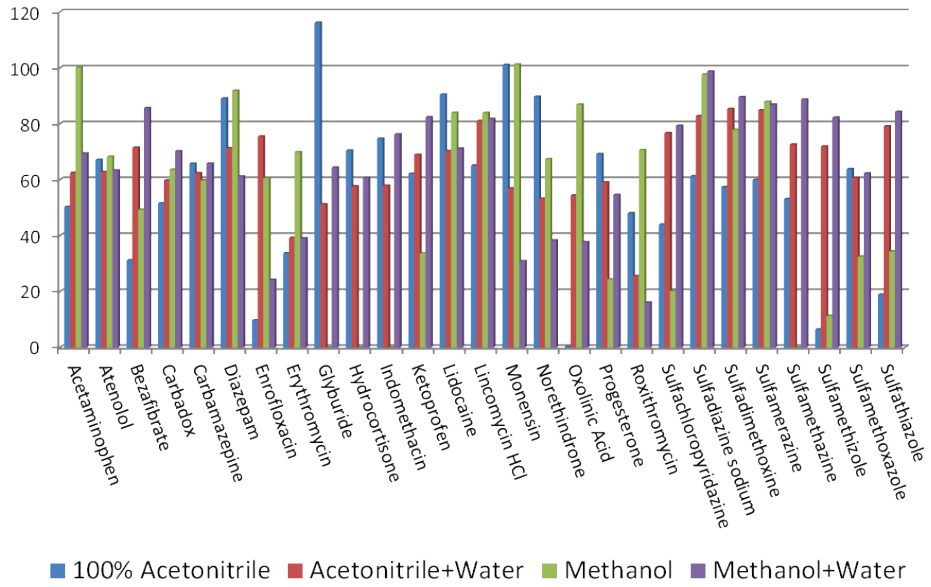
Mass Spectrometry

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, respectively. Mass spectrometric data was acquired at a resolving power of 140,000 (full-width-at-half-maximum , at *m/z* 200, R_{FWHM}), resulting a scanning rate of > 1.5 scans/sec when using automatic gain control target of $1.0x10^6$ 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 PPCPs and their metabolites, steroids, hormones, perfluorohydrocarbons, surfactants, and organophosphorus flame retardants. Quantitative analysis identified targeted compounds by retention time (RT) obtained from extracted ion chromatogram (XIC) using a mass extraction window (MEW) of 5 ppm. Non-targeted screening searched compounds listed in a database using $(M+H)^+$, $(M+NH_4)^+$ and $(M+Na)^+$ adduct ions in the positive mode and $(M-H)^-$ quasi-molecular ion in the negative mode, and created XICs for each compound. Those non-targeted analytes with area counts larger than 200,000 (approximately 25–50 pg/mL depending on compound), had a 5 ppm mass accuracy for the mono-isotopic mass (M) and two isotopic peaks ((M+1) and (M+2)), and a relative intensity of 90% ± 10% from the theoretical values were considered to be identified. Results obtained from TraceFinder software were also exported to Thermo Scientific™ SIEVE™ software to carry out a ChemSpider™ search.

FIGURE 1. Optimization of extraction solvent



Current extraction procedure has been validated for the analysis of 49 targeted compounds. Table 2 showed the performance data for these 49 PPCPs.

TABLE 2. Method performance for targeted compound analysis. MDL (method detection limit) is derived from eight replicate spikes. (RSD: relative standard deviation; REC: recovery)

Compound	RSD	MDL	Rec	Compound	RSD	MDL	Rec
19-Norethisterone	10	27	75	Hydrocortisone	41	42	56
Acetamidophenol	2.4	21	57	Ibuprofen	3.7	51	114
α-Estradiol	13	572	112	Indomethacin	4.6	15	92
α-Ethynyl Estradiol	3.9	68	97	Ketoprofen	16	18	64
Atenolol	4.7	39	91	Lidocaine	8.4	6	73
β-Estradiol	3	121	98	Lincomycin HCl	7.4	11	80
Bisphenol A	20	135	76	Naproxen	13	44	95
Caffeine	9.9	26	72	Norfloxacin	9.9	27	76
Carbadox	16	99	88	Ofloxacin	6.1	39	89
Carbamazepine	8.2	6	80	Oxolinic Acid	8.7	63	100
Chloramphenicol	5.6	7	73	Oxybenzone	14	14	54
Chlorotetracycline	9.3	110	132	Oxytetracycline HCl	8.3	57	128
Ciprofloxacin	5.6	35	88	Progesterone	5.9	20	96
Clofibric acid	1.9	7	94	Roxithromycin	13	65	141
DEET	16	10	67	Sulfachloropyridazine	10	14	76
Diazepam	8	33	57	Sulfadiazine sodium	15	269	50
Diclofenac sodium	6.6	16	88	Sulfadimethoxine	9.4	11	66
Doxycycline HCl	15	94	87	Sulfamerazine	17	22	73
Enrofloxacin	10	56	78	Sulfamethazine	7.1	9	74
Equilin	3.9	20	98	Sulfamethizole	6.7	9	74
Esterone	2.8	23	93	sulfamethoxazole	7.1	12	91
Estriol	9.6	81	94	Sulfathiazole	9.4	13	80
Gemfibrozil	12	15	116	Trimethoprim	20	70	98
Glipizide	7.7	9	78	Tylosin	9.9	287	97

Quantitative Determination of PPCPs in Biosolids Samples

Quantitative determination of targeted PPCPs in biosolids are shown in Table 3. Five compounds, i.e., bisphenol A, caffeine, CBZ, TCC and TCS, were found in all six samples at the high ppb range.

Table 4 showed targeted screening results from the same samples with 100% occurrence. These include known treatment by-products of CBZ, TCC and TCS, artificial sweeteners, surfactants, musks were abundant along with organphosphorus flame retardant and quaternary ammonium surfactants.

TABLE 3. Results of quantitative determination of different biosolids

Compound	#1	#2	#3	#4	#5	#6
Bisphenol A	30,200	9,220	3,680	84,280	85,700	47,750
Caffeine	356	2,500	807	1,230	1,260	1,170
Carbamazepine	3,490	3,520	3,600	3,300	3,600	3,500
Clofibric acid	91	73	36	84	34	106
DEET	174	218	190	273	214	210
Esterone	1,984	2,400	938	<MDL	631	<MDL
Estriol	<MDL	955	<MDL	<MDL	<MDL	<MDL
Lidocaine	190	105	80	123	94	<MDL
Oxybenzone	326	81	31	<MDL	418	484
Triclocarban*	2,947	2,770	2,040	1,510	2,080	1,130
Triclosan*	3,290	3,070	2,290	1,680	2,580	1,390

***Semi-quantitative results**

TABLE 4. Results of targeted screening of different biosolids

Compound Name	RT (Min.)	Compound Name	RT (Min.)
Ethofumesate	1.6	Dihexadecyldimethylammonium	11.8
Fenofibric-Acid	3.8	Dodecyltrimethylammonium	10.1
Metoprolol	3.9	Galaxolide	11.7
Neotame	2.5	Galaxolidone	11.2
Spiroxamine	10.9	Hexadecyltrimethylammonium	10.8
Sucralose	2	Isoproturon	2.5
4-Chloro-2-(2,4-dichloro-phenoxyl)-phenol	10.6	Mefenamic acid	9.2
4- & 6-Chloro-triclosan	10.9	Methyl-Benzotriazol	5.1
Acridine	3.1	Metoprolol	3.8
acridone-N-carbaldehyde	5.8	Myristyltrimethylammonium	10.6
Benzotriazol	3.4	N-Desvenlafaxine	3.5
Benzyldimethyldodecylammonium	10.4	Nonylphenol diethoxylate	11.6
Benzyldimethylhexadecylammonium	10.9	Nonylphenol monoethoxylate	9.2
Benzyl-dimethyl-tetradecylammonium	10.7	O-Desvenlafaxine	3.5
Carbamazepin-10,11-dihydroxy	5.3	Phenazon (Antipyrine)	7.5
Carbamazepine-10,11-epoxid	5.4	Primidon	3.5
Dibutyl Phthalate	11.1	Tonalide	11.7
Didecyldimethylammonium	10.8	Tramadol	3.5
Diethyl Phthalate	9.3	Tributyl Phosphate	11.1
Diethylhexyl Phthalate	12.8		

Conclusion

- Quantitative results of PPCPs were obtained using HPLC-Orbitrap MS.
- Semi-quantitative results showed the presence of surfactants, musks and treatment by-products in biosolids.
- Efforts to obtain analytical standards to complete the studies are on-going.

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