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

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Goal
Develop an HRAM-based LC-MS/MS workflow to quantitatively analyze contaminants of emerging concern (CECs) in biosolids samples and to screen for 381 non-targeted CECs.

Introduction
There is growing environmental concern regarding the health impact of trace levels of CECs, such as pharmaceuticals and personal care products (PPCPs) and endocrine disruptors (EDCs) in water resources. Detected in surface and drinking waters, as well as in treated wastewater, these compounds are an issue of increasing international attention due to their potential environmental impacts.[1,2] They are distributed widely in surface waters from wastes excreted by human and animal, as well as improper disposal of expired medications, and are a potential concern to environment and human health. This presents a major challenge to water treatment facilities.

Quantitative information on the CECs in biosolids and biological tissues is readily available from triple quadrupole mass spectrometry (LC-QQQ-MS/MS) and allows for the assessment, when and where appropriate, of potential uptake and bioaccumulation. Unlike LC-QQQ-MS/MS, full-scan high-resolution, accurate-mass (HRAM) mass spectrometry provides not only quantitative data for targeted compounds but also information on the non-targeted compounds, such as environmental transformation by-products, for possible environmental loadings and ecological effects that would not be available in a targeted assay.

Experimental
Sample Preparation
For this study, model biosolid samples and biosolids-amended soil (BAS) samples were used in the evaluation of the method. Grab biosolid samples were collected 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 samples 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 C/D/N 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 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.

Biosolids and BAS samples were dried in a fume hood for 96 hours, sieved through a 200 micron mesh, homogenized, and stored in a 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 water, 70:30 (v/v)), 1 mM ethylenediaminetetraacetic acid (EDTA), and isotopically labeled surrogates. The tubes were shaken for 5 minutes and sonicated for 20 minutes, shaken for another 5 minutes, and centrifuged for 8 minutes 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)).

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The volumes of the combined extracts were brought up to 50 mL, centrifuged for 3 minutes at 5000 rpm, and 10 mL of the extract was evaporated to dryness. The residues were reconstituted in 100 µL of the internal standard solution prepared in pure water, then injected into the high-pressure liquid chromatography (HPLC)-Orbitrap Exactive MS for analysis.

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 Thermo Scientific™ Betasil™ column for positive mode MS analysis and a Thermo Scientific™ Hypersil GOLD™, 2.1 × 100 mm column for negative mode MS analysis. Three HPLC separations were used for the analysis of pharmaceuticals and personal care products (PPCPs) and their by-products. The mobile phase and gradient conditions are provided in Table 1.

Mass Spectrometry

The HPLC was interfaced to a Thermo Scientific™ Exactive Plus™ Orbitrap™ MS using a heated electrospray ionization (HESI II) interface. The Orbitrap MS system was tuned and calibrated in positive and negative modes, respectively, by infusion of solutions of MSCAL5 and MSCAL6 ProteoMass ESI Calibration Kit (Sigma-Aldrich, St. Louis, MO). High purity nitrogen (>99%) obtained from a nitrogen generator (Parker Hannifin Corporation, Haverhill, MA) was used in the HESI II 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 in a scan 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 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 381 compounds consisting of PPCPs and their known metabolites, steroids, hormones, perfluorohydrocarbons, surfactants, and organophosphorus flame retardants. The database consisted of molecular formula and LC retention time of compounds with analytical standards. 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 the database using molecular formula to predict $(M+H)^+$, $(M+NH_4)^+$ and $(M+Na)^+$ adduct ions in the positive mode and $(M-H)^-$ quasi-molecular ions in the negative mode to generate isotopic pattern for identification 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), 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.

Results and Discussion

Method Performance

Figure 1 shows extraction method parameters with 100% acetonitrile, acetonitrile/water (0.1% acetic acid in water, 70:30 (v/v), 1 mM EDTA), 100% methanol and methanol/water (0.1% acetic acid in water, 70:30 (v/v)). Both acetonitrile and methanol extraction showed similar recovery. To facilitate the evaporation step used during the sample preparation, samples were extracted using 2 × 20 mL of acetonitrile/H_2O, 70:30 (v/v), in an ultrasonic bath for 45 min each.

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

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
	2.0	25	75	5
	10.0	5	95	7
	15.0	5	95	5
	15.2	95	5	5

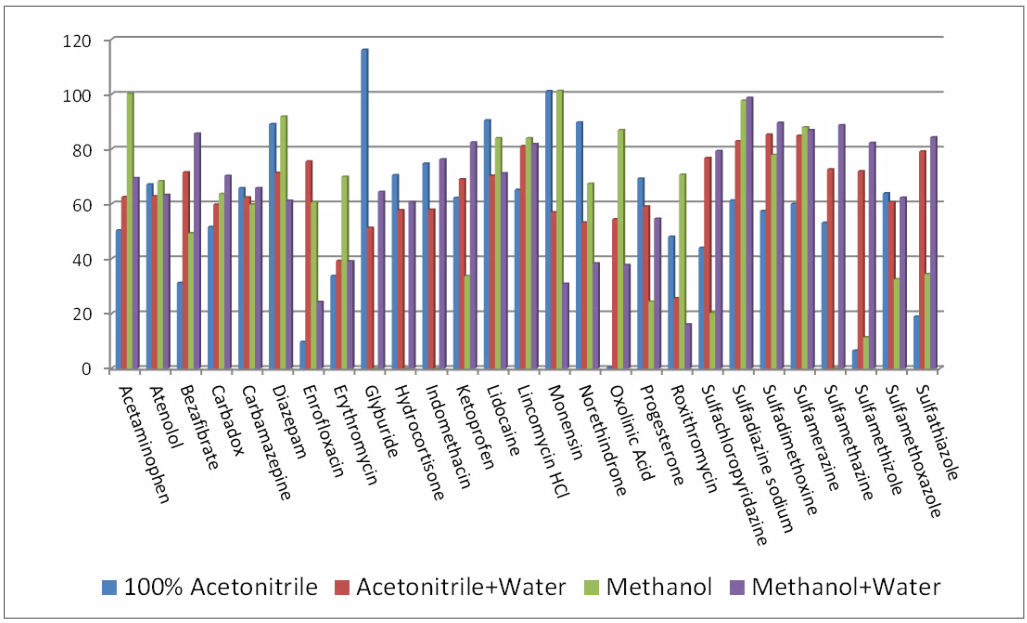

Figure 1. Optimization of extraction solvent.

The extraction procedure has been evaluated for the analysis of 49 targeted compounds. Table 2 shows 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 using calculated standard deviation and a student $t_{0.01} = 2.998$. (RSD: relative standard deviation, %; REC: recovery, %, calculated at a spiking concentration of 10–12x of the MDL).

Compound	RSD (%)	MDL (ng/g)	REC (%)
19-Norethisterone	10	27	75
Acetamidophenol	2.4	21	57
α-Estradiol	13	572	112
α-Ethynyl Estradiol	3.9	68	97
Atenolol	4.7	39	91
β-Estradiol	3	121	98
Bisphenol A	20	135	76
Caffeine	9.9	26	72
Carbadox	16	99	88
Carbamazepine	8.2	6	80
Chloramphenicol	5.6	7	73
Chlorotetracycline	9.3	110	132
Ciprofloxacin	5.6	35	88
Clofibric acid	1.9	7	94
DEET	16	10	67
Diazepam	8	33	57
Diclofenac sodium	6.6	16	88
Doxycycline HCl	15	94	87
Enrofloxacin	10	56	78
Equilin	3.9	20	98
Esterone	2.8	23	93
Estriol	9.6	81	94
Gemfibrozil	12	15	116
Glipizide	7.7	9	78

Compound	RSD (%)	MDL (ng/g)	REC (%)
Hydrocortisone	4.1	42	56
Ibuprofen	3.7	51	114
Indomethacin	4.6	15	92
Ketoprofen	16	18	64
Lidocaine	8.4	6	73
Lincomycin HCl	7.4	11	80
Naproxen	13	44	95
Norfloxacin	9.9	27	76
Ofloxacin	6.1	39	89
Oxolinic acid	8.7	63	100
Oxybenzone	14	14	54
Oxytetracycline HCl	8.3	57	128
Progesterone	5.9	20	96
Roxithromycin	13	65	141
Sulfachloropyridazine	10	14	76
Sulfadiazine sodium	15	269	50
Sulfadimethoxine	9.4	11	66
Sulfamerazine	17	22	73
Sulfamethazine	7.1	9	74
Sulfamethizole	6.7	9	74
sulfamethoxazole	7.1	12	91
Sulfathiazole	9.4	13	80
Trimethoprim	20	70	98
Tylosin	9.9	287	97

Quantitative Determination of PPCPs in Biosolids Samples

Quantitative determination of targeted PPCPs in biosolids is shown in Table 3. Five compounds [bisphenol A, caffeine, carbamazepine (CBZ), triclocarbon (TCC), and triclosan (TCS)] were found in all six samples at the high ppb range. Concentrations of TCC and TCS found were out of the range of the highest calibration level (1000 ng/mL) and should be treated as semi-quantitative values.

Targeted screening results from the same sample set are shown in Table 4. The compounds listed were detected in all of the samples. These include known treatment by-products of CBZ, TCC, and TCS. Artificial sweeteners, surfactants, and 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
	ng/g					
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. Compounds identified in different biosolids using criteria described in Data Analysis section for targeted screening.

Compound Name	RT (Min.)
Ethofumesate	1.6
Fenofibric acid	3.8
Metoprolol	3.9
Neotame	2.5
Spiroxamine	10.9
Sucralose	2
4-Chloro-2-(2,4-dichloro-phenoxyl)-phenol	10.6
4- & 6-Chloro-triclosan	10.9
Acridine	3.1
Acridone-N-carbaldehyde	5.8
Benzotriazol	3.4
Benzyldimethyldodecylammonium	10.4
Benzyldimethylhexadecylammonium	10.9
Benzyl-dimethyl-tetradecylammonium	10.7
Carbamazepin-10,11-dihydroxy	5.3
Carbamazepine-10,11-epoxid	5.4
Dibutyl phthalate	11.1
Didecyldimethylammonium	10.8
Diethyl phthalate	9.3
Diethylhexyl phthalate	12.8

Compound Name	RT (Min.)
Dihexadecyldimethylammonium	11.8
Dodecyltrimethylammonium	10.1
Galaxolide	11.7
Galaxolidone	11.2
Hexadecyltrimethylammonium	10.8
Isoproturon	2.5
Mefenamic acid	9.2
Methyl-Benzotriazol	5.1
Metoprolol	3.8
Myristyltrimethylammonium	10.6
N-Desvenlafaxine	3.5
Nonylphenol diethoxylate	11.6
Nonylphenol monoethoxylate	9.2
O-Desvenlafaxine	3.5
Phenazon (Antipyrine)	7.5
Primidon	3.5
Tonalide	11.7
Tramadol	3.5
Tributyl phosphate	11.1

Conclusion

- A rapid dilute and shoot method for the quantitative determination of targeted CECs, such as 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.
- 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.

References

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