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ORIGINAL PAPER

Detection and occurrence of microconstituents in reclaimed water used for irrigation – a potentially overlooked source

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Abstract An online SPE-HPLC-HESI-MS/MS method and an online SPE-HPLC-APPI-MS/MS method were developed to analyze 72 microconstituents in reclaimed water. In this study, 55 reclaimed water samples were collected from the sprinkler system for a year-long period at Florida International University Biscayne Bay Campus, where reclaimed water was reused for daily irrigation. Analysis results showed that several analytes were continuously detected in all reclaimed water samples and others will show rather transient signal increases. Coprostanol, bisphenol A, and DEET's maximum concentration exceeded 10,000 ng/L. The four most frequently detected compounds were diphenhydramine (100 %), DEET (98 %), atenolol (98 %) and carbamazepine (96 %).

Keywords Microcontituents · HESI · APPI · LC-MS/MS · Reclaimed water · Irrigation

Introduction

Water stress has become a serious problem worldwide because of the rapid population growth on the earth. Properly managed water resources are critical for sustainable development of water supply. In order to improve the management efficiency of water resources, wastewater treated to varying degrees are commonly reused worldwide for landscape, agriculture, irrigation, recharging, etc. In the US, treated water (reused or reclaimed) has been used in more than 3,000 application sites. Over $40\times10^6 m^3$ of reclaimed water is used in California every year [1, 2]. Nevertheless, potential adverse effects may still arise when reusing treated water. It is proved that current wastewater treatment plants (WWTPs) with primary treatment and secondary treatment processes could not remove PPCPs completely because PPCPs have been detected in the effluent of WWTPs [3–7]. Tertiary treatments such as granular active carbon adsorption, and advanced oxidation processes or micro- and ultra filtration or reverse osmosis have shown better removal efficiency but these treatments are not widely used in the majority of WWTPs [8–10]. Therefore, when reusing treated water, PPCPs will likely enter the natural ecosystem and distribute among environmental compartments where they may cause an effect. When treated water is used for irrigation, compounds with strong sorption and recalcitrant to degradation may remain on the surface of the soil and be taken by plants. Research about uptake of human pharmaceuticals in plants grown from soil suggested that compounds introduced by irrigation may be more available for plant uptake than those introduced by biosolid application [1]. In addition, microconstituents will enter surface water by direct irrigation or runoff not only exposing organism in the aqueous environment to rather complex mixtures [2, 11, 12], but creating a potential link to drinking water sources. When treated water is disposed by deep well injection practice, PPCPs could migrate through uncontained aquifers and contaminate ground water, this connection could then impact sources used for drinking water. The presence of microconstituents in surface samples is becoming ubiquitous and assessing differences between reclaimed water and

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surface waters will likely become more difficult [13] as treatment technology improves and more sensitive analytical methods are developed. Because of this, it is essential to create comprehensive monitoring methods to assess presence of PPCPs in treated wastewater and to follow their movement as they enter the natural ecosystem.

At Florida International University's Biscayne Bay Campus, treated wastewater from the North District WWTP is used for irrigation. The North District WWTP is located at NE 154 Street and is east of Biscayne Boulevard, and it receives wastewater from the North District of Miami-Dade County. The wastewater treatment plant was designed to have a flow of 454,249 m^3 per day with average daily flow around 425,858 m^3 per day. The facilities include screening, grit removal, primary sedimentation, activated sludge treatment by oxygenation and chlorination. Extra filtration (DynaSand Filtration, Leopold Filtration and Tetra filtration) and disinfection are applied to effluents before release to make the reclaimed water ready for use in irrigation. The present study monitored the reclaimed water stream collected between January 2011 and December 2011.

In this work, two methods including online SPE-HESI-MS/MS and online SPE-APPI-MS/MS were used to detect 72 microconstituents in the reclaimed water samples. This study provided key information about the chemical ecology of microconstituents in a typical treated wastewater stream that is routinely introduced to an environmental setting through water reuse.

Experimental

Chemicals

All the reference standards were >95 % purity and used as received. The identity of all analytes and surrogate standards and their origin are presented in the Electronic Supplementary Material. Basic chemical information and a description of the surrogate standards used for each analyte quantitation are shown in Electronic Supplementary Material, Table S1.

Sampling

Reclaimed water used for irrigation was collected directly from a sprinkler system using 500 mL polyethylene terephthalate bottles and was stored in the freezer at <10 °C until analysis time. The source of reclaimed water used in this study is the Miami-Dade Water and Sewer Department North District Wastewater Treatment Plant. Pure oxygen-activated sludge is used in the WWTP as the main secondary treatment process [14]. Extra filtration and disinfection are applied to effluents before release to make the reclaimed water ready for use in irrigation. The presence of chlorine was checked and controlled by aqua comparator test kit (Orbeco Hellige, Sarasota, USA) after sampling.

Sample preparation

Reclaimed water samples were filtered through glass fiber filters with a pore size of 0.45 μm. Samples were analyzed within 14 days in order to avoid potential degradation and transformation of analytes. One milliliter of reclaimed water samples were diluted to 5 mL before analysis. The pH of sample was adjusted to 2 using formic acid when sample was analyzed in the negative mode.

Online SPE concentration and HPLC separation

For pharmaceuticals (analytes 1 to 53), water samples (5 mL) were loaded to a Thermo Hypersil Gold aQ (20 mm×2.1 mm, 12 μm particle size) preconcentration column first. Then, the preconcentration column was washed with 1,000 μL of water at the same speed as loading speed and connected to the analytical column (Thermo Hypersil Gold aQ, 50 mm× 2.1 mm, 1.9 μm particle size) after the valve had switched to inject position. After the washing step, the loading column and analytical column underwent the same gradient in both positive and negative mode. The gradient programs of the loading and analytical pump are shown in Electronic Supplementary Material, Table S2. For hormones and sterols (analytes 54 to 72), the online SPE-APPI-MS/MS method followed the program described by Wang & Gardinali [15].

Mass spectrometry

Mass spectrometry analysis was performed using a TSQ Quantum Access triple quadrupole QqQ Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an Ion MAX source housing capable of operating heated electrospray ionization (HESI) and atmospheric pressure photoionization (APPI) mode. Quantitation for all sources was performed using selected reaction monitoring mode. Instrument control and data acquisition were performed using Xcalibur software (rev. 2.1, Thermo Fisher Scientific, San Jose, CA, USA). Source parameters for analytes were optimized using HESI using flow injection with a carrier stream of mobile phase. A mobile phase of 0.1 % formic acid in water/MeOH (50:50, *v*/*v*) was used for positive mode and water/MeOH (50:50, *v*/*v*) was used for negative mode. Each analyte and surrogate was injected to the ion source at a concentration of 10 μg/mL. Compound-dependent parameters such as tube lens and collision energy (CE) were optimized to obtain maximum signals in the QqQ system. The precursor ion, fragment ions and mass-dependent parameters are listed in Table 1.

Source-dependent parameters for optimal HESI detection were as follows: capillary temperature (350 °C), vaporizer

Table 1 Precursor ion, fragment ions and mass-dependent parameters of analytes

Compounds	RT (min)	HESI								
		Precursor ion	*m/z*	Tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3
Ketoprofen	14.35	[M−H]−	253.1	65	209.1	11				
Naproxen	14.50	[M−H]−	229.0	60	170.1	18	185.2	11		
Ibuprofen	15.12	[M−H]−	205.1	51	161.3	10				
Indomethacin	15.05	[M−H]−	356.1	65	312.1	12	297.1	21	282.1	32
Diclofenac	15.06	[M−H]−	294.0	60	250.0	14	214.0	22	178.1	28
Mefenamic acid	15.50	[M−H]−	240.1	65	196.1	19	192.1	28	180.1	29
Acetaminophen	NA	[M−H]−	150.0	49	107.2	22	118.1	33	132.1	24
Salicylic Acid	12.61	[M−H]−	137.1	48	93.1	19	65.2	31	75.2	37
Antipyrin	9.10	[M+H]+	189.1	72	77.2	36	56.3	36	131.1	22
Propyphenazone	10.59	[M+H]+	231.1	71	201.1	24	189.1	20	56.3	35
Phenylbutazone	11.60	[M+H]+	309.2	71	120.1	19	188.1	15	211.1	16
Codeine	6.98	[M+H]+	300.2	86	215.1	25	152.1	65	165.1	42
Clofibric Acid	14.44	[M−H]−	213.1	56	127.1	19	85.2	12	91.3	47
Gemfibrozil	15.50	[M−H]−	249.1	65	121.2	20	106.1	49	120.1	44
Bezafibrate	14.53	[M−H]−	360.1	63	274.1	20	154.0	32	85.2	19
Fenofibrate	12.68	[M+H]+	361.2	72	233.0	16	139.0	31	121.0	32
Atorvastatin	11.54	[M+H]+	559.3	88	440.3	21	250.0	42	276.1	40
Mevastatin	12.26	[M+H]+	391.3	74	185.1	14	229.1	13	159.1	26
Pravastatin	10.55	[M+Na]+	447.3	97	327.1	19	309.2	22		
Fluoxetine	10.92	[M+H]+	310.1	60	44.3	13	148.1	5	183.1	45
Paroxetine	10.62	[M+H]+	330.2	71	192.1	20	70.2	31	135.1	37
Diazepam	11.29	[M+H]+	285.1	77	193.1	32	154.1	26	222.1	26
Lorazepam	10.77	[M+H]+	321.1	74	275.0	22	303.0	15	229.1	31
Carbamazepine	10.48	[M+H]+	237.1	61	194.1	19	192.1	25		
Primidone	9.22	[M+H]+	219.1	68	91.2	28	162.2	12	117.2	23
Famotidine	5.81	[M+H]+	338.1	55	189.0	20	259.1	11	155.1	32
Ranitidine	5.92	[M+H]+	315.1	65	176.0	18	130.1	25	102.1	34
Cimetidine	5.83	[M+H]+	253.1	67	159.1	14	117.2	16	95.2	29
Loratadine	11.16	[M+H]+	383.1	82	337.1	23	267.1	31	259.1	30
Diphenhydramine	10.21	[M+H]+	256.2	54	167.1	14	165.2	37	152.1	37
Butalbital	13.72	[M−H]−	223.1	60	180.1	14	42.3	20	85.2	15
Phenobarbital	13.35	[M−H]−	231.1	61	188.2	14	42.5	15	85.3	15
Pentobarbital	13.99	[M−H]−	225.1	60	182.1	15	42.4	20	138.2	19
Atenolol	5.68	[M+H]+	267.2	78	145.1	26	190.1	18	133.1	31
Sotalol	5.76	[M+H]+	273.2	77	255.1	11	213.1	18	133.1	27
Metoprolol	9.30	[M+H]+	268.2	77	159.1	21	191.1	17	133.1	26
Propranolol	10.10	[M+H]+	260.2	78	183.1	17	155.1	25	157.1	20
Timolol	9.24	[M+H]+	317.2	84	261.1	16	244.1	21	188.0	25
Betaxolol	10.18	[M+H]+	308.2	82	121.1	26	133.1	26	91.1	39
Carazolol	9.80	[M+H]+	299.1	70	222.1	19	184.0	25	194.1	29
Pindolol	8.53	[M+H]+	249.2	72	116.1	17	172.1	17	144.1	25
Nadolol	8.63	[M+H]+	310.2	73	254.1	17	201.1	22	236.1	19
Salbutamol	5.52	[M+H]+	240.2	62	148.1	18	222.1	10	121.1	29
Clenbuterol	9.26	[M+H]+	277.1	71	203.0	16	259.1	10	132.1	30
Enalapril	9.97	[M+H]+	377.2	74	234.1	19	303.2	17	117.1	36
Hydrochlorothiazide	15.06	[M−H]−	296.0	78	269.0	20	205.0	23	126.1	33
Lisinopril	8.64	[M+H]+	406.2	88	84.2	33	246.1	22	309.2	18

Table 1 (continued)

Compounds	RT (min)	HESI								
		Precursor ion	*m/z*	Tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3
Furosemide	13.49	[M−H]−	328.9	75	285.0	17	204.9	23	126.0	36
Tamoxifen	1.91	[M+H]+	372.2	84	72.2	23	129.1	26	70.2	36
Metronidazole	5.98	[M+H]+	172.1	63	128.1	13	82.2	23	111.1	20
Clotrimazole	10.94	$[M-C_3H_3N_2]+$	277.1	60	165.1	27	199.0	31	242.1	20
Glibenclamide	11.62	[M+H]+	494.3	70	369.1	14	169.0	33	304.1	25

temperature (250 °C), sheath gas pressure (30 arbitrary units), aux gas pressure (20 arbitrary units), ion sweep gas pressure (5 arbitrary units) and spray voltage (4,000 V for positive polarity and 4,000 V for negative polarity). APPI parameters were previously described by Wang & Gardinali 2012 [15].

Method detection limit

Method detection limits (MDLs) were determined according to EPA guidelines [16]. Eight replicate tap water samples were spiked with all compounds at concentration ranging from one to eight times of the tested detection limit (DL) and analyzed using optimized conditions. MDLs were calculated from the calculated concentrations of the eight replicates for 5 mL sample size.

QA/QC

Blanks were run with each analytical batch to check for potential contamination and assess background levels of native analytes. Laboratory blank spike (LBS) and duplicate (LBSD) were also run with each analytical batch to check both accuracy and precision by evaluating the recovery of analytes. Isotopic dilution was used to increase the precision and accuracy of analysis. A five-point calibration curve was constructed with each batch to check for linearity (R^2>0.99) and analytical sensitivity. Method performance of analytes measured in APPI was verified and reported in Wang and Gardinali [15].

Result and discussion

Optimization of online SPE procedure

Water samples (5 mL) were loaded to the preconcentration column at different flow rates: 500, 1,000, 1,500, and 2,000 μL/min. Absolute recoveries of analytes (based on the response only) detected in the positive and negative ion modes are shown in Fig. 1a and b, respectively. Loading speed of 2,000 and 1,000 μL/min were chosen for positive mode and negative mode respectively because analytes were better recovered at these two flow rates. In the negative mode, the pH of samples was adjusted to 2 in order to increase the recovery of salicylic acid and clofibric acid on the loading column.

In order to reduce suppression by matrix coelution, a wash step was introduced after the samples were loaded to the preconcentration column. Only water was used to wash the preconcentration column because some analytes are rapidly affected by small portion of most organic solvent. Three different volumes of water (1, 2, and 3 mL) were tested in both positive and negative mode and results are shown in Fig. 2a and b, respectively. In the HESI+, recoveries of metronidazole, lisinopril, and primidone were significantly reduced when wash volume was above 1 mL. Similarly, in the HESI−, acetaminophen was not retained in the preconcentration column when the wash volume was more than 1 mL. This result is consistent with previous reported value [15]. Therefore, the Hypersil Gold aQ column is not a good choice to retain acetaminophen. Hydrochlorothiazide's recoveries are also severely affected when wash volume was more than 1 mL. Thus, 1 mL was also chosen as the wash volume in the negative mode. The same as acetaminophen, the Hypersil aQ column is not a good choice to retain hydrochlorothiazide.

Method detection limits

The calibration ranges, linearity, spike levels, MDLs, recovery and relative standard deviation (RSD) of analytes are shown in Table 2. The linearity of most analytes was more than 0.99. Only several of them were lower than 0.99 likely because of the lack of a proper surrogate for the quantitation. MDLs were calculated on the basis of 5 mL sample. Method detection limits were calculated from the standard deviation of eight replicated spiked water samples. MDLs of 14 analytes were below 5 ng/L, 12 analytes were less than 10 ng/L and 26 analytes were more than 10 ng/L. These MDLs are more than adequate for reclaimed water and can be improved by increasing the sample volume to 20 mL for cleaner matrices.

Application on reclaimed water samples

A total of 55 samples were analyzed over a year period. More than one compound was found in 100 % of the reclaimed water samples. The reason for the high detection frequency is that the treatment processes in the North District WWTP only include primary and secondary treatments that are not designed to remove microconstituents. Even though extra filtration and chlorination are applied to effluent, PPCPs and hormones are still not completely removed; 33 out of 72 target compounds were detected more than once during the sampling period. The total average concentration of analytes was 7,246 ng/L, which indicated that the reclaimed water carrying at least 7,246 ng/L of unregulated chemicals. The detected concentrations of all target compounds are shown in Fig. 3. About 15 % of the detections were more than 1,000 ng/L and 80 % of the high concentrations were derived from gemfibrozil, atenolol, caffeine, and bisphenol A.

Among the high concentration compounds, coprostanol, bisphenol A, and DEET are the three compounds maximum concentrations that exceeded 10,000 ng/L (Fig. 4). Bisphenol A is known to behave as a weak environmental estrogen, more recent research has demonstrated that bisphenol A

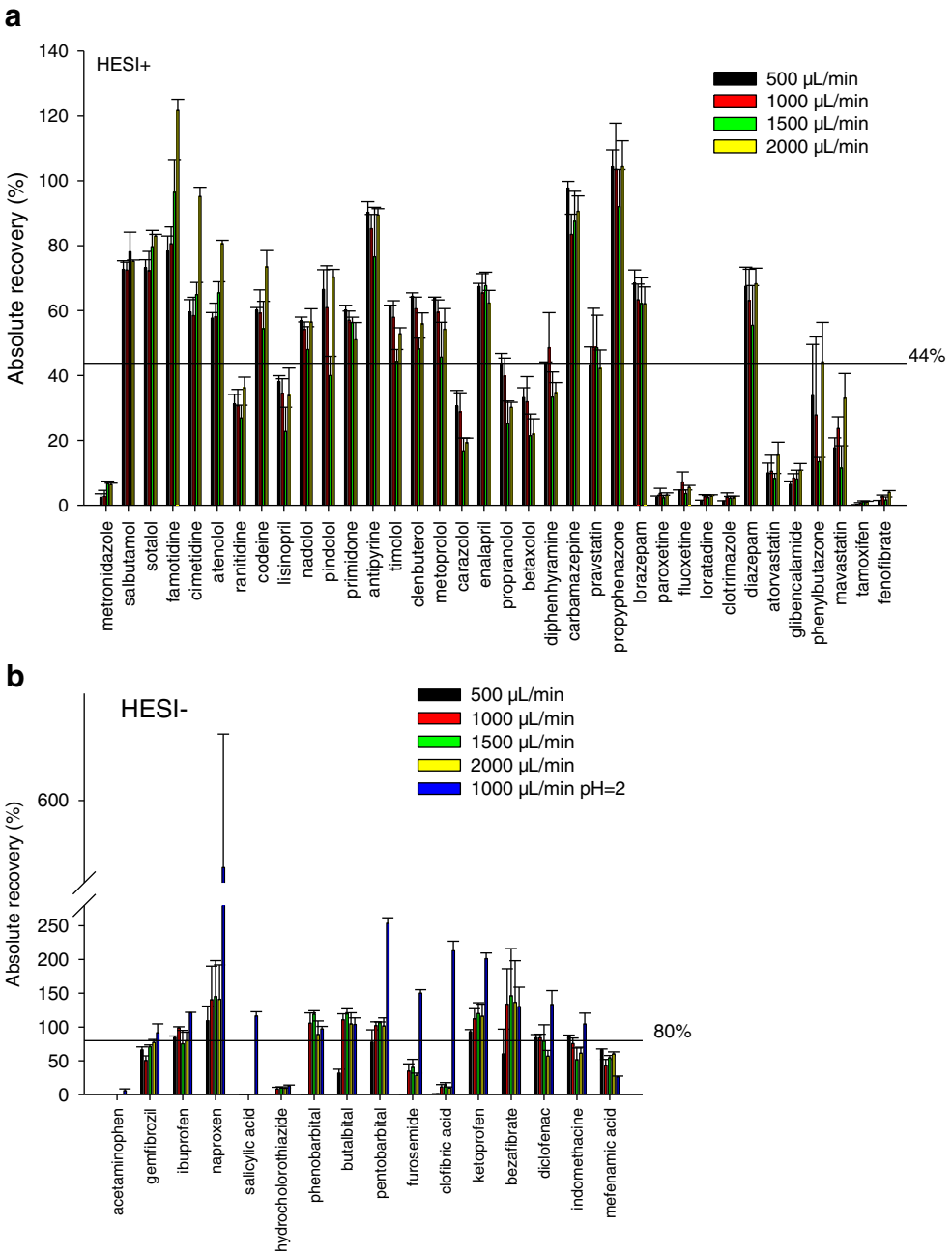

Fig. 1 (**a**) Absolute recovery of analytes at different load speeds in the HESI positive mode. (**b**) Absolute recovery of analytes at different load speeds in the HESI negative mode

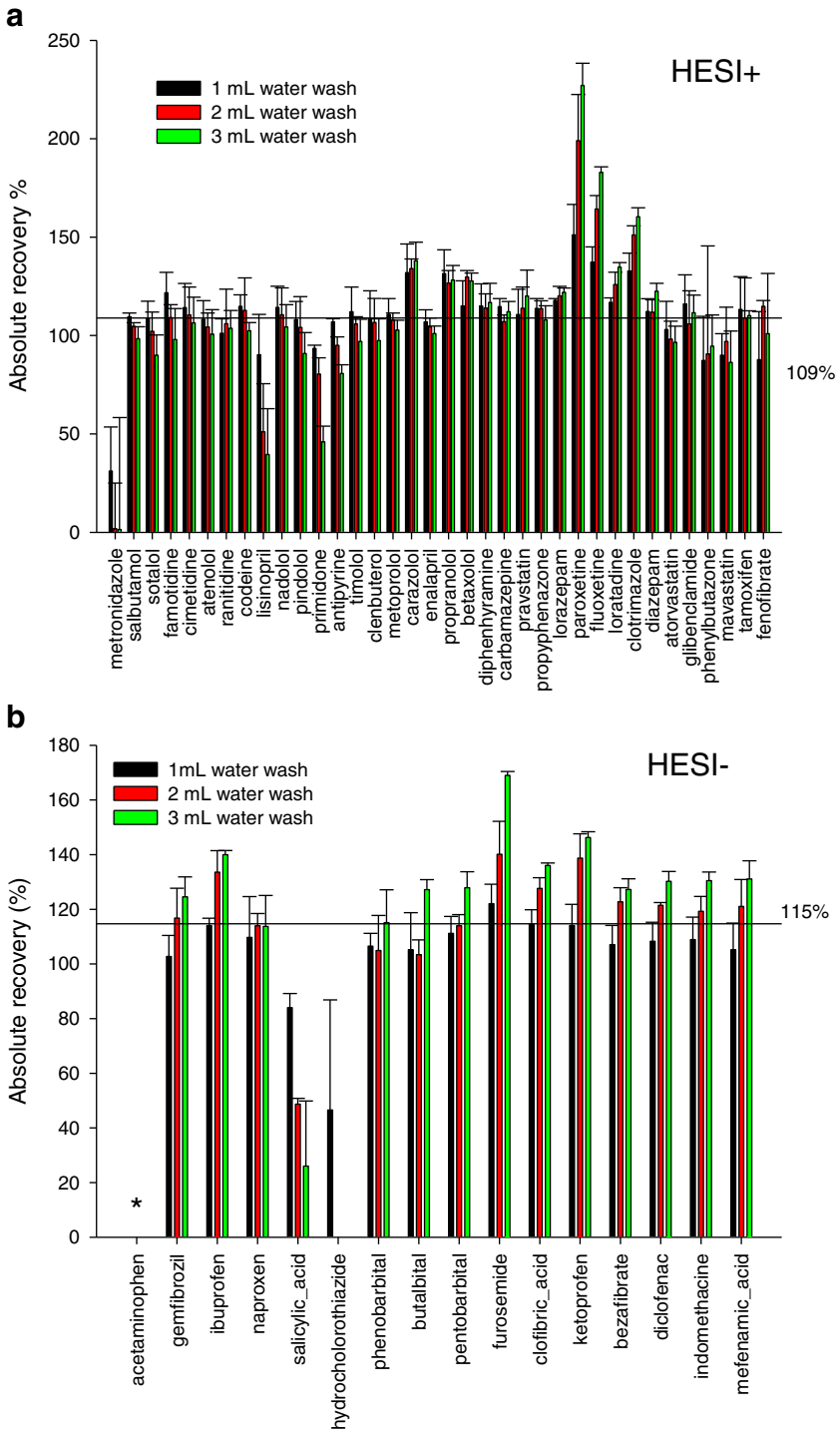

Fig. 2 (**a**) Absolute recovery of analytes in HESI positive mode with different wash volume. (**b**) Absolute recovery of analytes in HESI negative mode with different wash volume. Asterisk (*) indicates not recovered

may be similar to estradiol in stimulating adverse cellular responses [17]. DEET's chronic aquatic toxicity data for fish (8.42×10^6 ng/L), daphnia (5.13×10^6 ng/L), and algae (9.65×10^6 ng/L) [18] are all order of magnitude above the measured concentrations. Only two hormones (estrone and estradiol) were detected in the reclaimed water samples. The maximum detected concentrations of estrone (50.8 ng/L) and estradiol (58.5 ng/L) were relatively high compared to lowest observed effect concentration for fish (usually a few nanograms per liter) [19], but the detection frequency is only 2 %. Based on the concentrations detected, acute toxicity to aquatic organisms is unlikely to occur because most of the reported LC50 are 100–1,000 higher than concentrations presented in the reclaimed water.

In addition, the detection frequency is a critical factor since long-term chronic exposure to PPCPs, especially compounds with endocrine disruption effects, may cause problems even though their concentrations are low. The four

Table 2 Calibration range, linearity, spike level, recovery and RSD of analyte in HESI

		Calibration range (ng/L)	linearity	Spike level (ng/L)	MDLs (ng/L)	Recovery (%)	RSD (%)
1	Ketoprofen	5–500	0.9992	25	10	107	21
2	Naproxen	50–500	0.9981	250	74	98	16
3	Ibuprofen	5–500	0.9993	25	12	98	11
4	Indomethacine	5–500	0.9999	25	19	104	10
5	Diclofenac	5–500	0.9996	25	8.7	97	12
6	Mefenamic acid	5–500	0.9987	25	2.5	95	11
7	Acetaminophen	NR	NR	NR	NR	NR	NR
8	Salicylic acid	5–500	0.9994	25	24	106	9
9	Antipyrine	5–500	0.9993	10	4.5	112	25
10	Propyphenazone	5–500	0.9993	10	5.0	95	22
11	Phenylbutazone	25–500	0.9882	100	147	82	20
12	Codeine	5–500	0.9900	10	6.2	91	22
13	Clofibric acid	5–500	0.9976	25	24	100	8
14	Gemfibrozil	5–500	0.9986	25	14	102	14
15	Bezafibrate	5–500	0.9990	25	26	101	12
16	Fenofibrate	5–500	0.9841	10	14	82	18
17	Atorvastatin	5–500	0.9991	10	6.9	88	34
18	Mavastatin	10–500	0.9924	100	75	102	32
19	Pravstatin	5–500	0.9998	10	9.0	96	20
20	Fluoxetine	5–500	0.9938	10	12	81	27
21	Paroxetine	5–500	0.9998	10	12	85	11
22	Diazepam	5–500	0.9998	10	1.8	85	21
23	Lorazepam	5–500	0.9991	10	6.4	106	20
24	Carbamazepine	5–500	0.9997	10	2.8	100	26
25	Primidone	5–500	0.9966	10	29	95	14
26	Famotidine	5–500	0.9978	10	1.8	90	44
27	Ranitidine	5–500	0.9992	10	3.5	102	10
28	Cimetidine	5–500	0.9937	10	4.6	91	23
29	Loratadine	5–500	0.9984	10	5.6	89	21
30	Diphenhyramine	5–500	0.9931	10	4.3	107	33
31	Butalbital	25–500	0.9979	250	189	99	15
32	Phenobarbital	25–500	0.9983	250	40	78	33
33	Pentobarbital	25–500	0.9997	250	32	92	15
34	Atenolol	5–500	0.9947	10	7.0	96	17
35	Sotalol	5–500	0.9961	10	3.5	86	22
36	Metoprolol	10–500	0.9981	10	13	89	18
37	Propranolol	5–500	0.9941	10	14	89	21
38	Timolol	5–500	0.9983	10	3.9	99	17
39	Betaxolol	5–500	0.9971	10	19	89	28
40	Carazolol	5–500	0.9997	10	10	80	18
41	Pindolol	5–500	0.9889	10	10	99	23
42	Nadolol	5–500	0.9959	10	5.7	84	18
43	Salbutamol	5–500	0.9993	10	3.8	91	21
44	Clenbuterol	5–500	0.9920	10	4.1	97	32
45	Enalapril	5–500	0.9993	30	3.7	87	12
46	Hydrocholorothiazide	NR	NR	NR	NR	NR	NR
47	Lisinopril	50–500	0.9880	100	26	91	3
48	Furosemide	5–500	0.9994	25	8.2	94	11
49	Tamoxifen	10–500	0.9967	100	43	75	27

Table 2 (continued)

		Calibration range (ng/L)	linearity	Spike level (ng/L)	MDLs (ng/L)	Recovery (%)	RSD (%)
50	Metronidazole	25–500	0.9976	100	98	88	20
51	Clotrimazole	5–500	0.9847	10	6.3	89	20
52	Glibenclamide	25–500	0.9700	100	22	91	28

The MDLs for acetaminophen and hydrochlorothiazide is not available because they lost their recovery on the preconcentration column during wash step. *NR* means not recovered

most frequently detected compounds were diphenhydramine (100 %), DEET (98 %), atenolol (98 %), and carbamazepine (96 %). Diphenhydramine has been reported in water, sediment and fish, but the effects of diphenhydramine on aquatic organisms is still largely unknown. The reclaimed water samples showed chronic level of diphenhydramine all year long with a maximum concentration of 1,091 ng/L. A previous study indicated that the no-observed-effect concentration (NOEC) of diphenhydramine on reproduction of *D. magna* is 800 ng/L. As much as 17 % of the reclaimed water sampler exceeded this benchmark but there is no good way to assess its relevance. Atenolol was detected at a maximum concentration of 3,761 ng/L in 98 % of reclaimed water samples. Reproductive performance of *Daphnia magna*, the most sensitive organisms tested, showed a NOEC for atenolol at 1.8×10^6ng/L [20]. Winter and his coworker used fathead minnows as test species and undertook embryo-larval development assessment (early life stage or ELS) and short-term adult reproduction studies. The results of the ELS study showed that the $NOEC^{growth}$ and $LOEC^{growth}$ of atenolol were 3.2×10^6ng/L and 10×10^6ng/L, respectively. Short-term reproduction study, or $NOEC^{reproduction}$ and $LOEC^{reproduction}$ of atenolol were 10×10^6ng/L and $>10\times10^6$ng/L, respectively [21]. Compared to the toxicity test results, the detected concentrations of atenolol in reclaimed water samples are much lower than any of the concentration that will cause chronic effect to fish. Carbamazepine is an anticonvulsant pharmaceutical that is commonly found in effluent of WWTPs, surface water and drinking water [3, 22]. In this study, carbamazepine was detected in 96 % of reclaimed water samples with a maximum concentration of 173 ng/L. The chronic effect of carbamazepine to rainbow trout exposed carbamazepine for 42 days indicated that both physiological condition status and muscle-based biomarkers were significantly affected at levels above 2.0×10^6ng/L [23, 24]. By comparing with toxicity studies, the detected concentrations of compounds present in the reclaimed water were generally lower than the lowest-observed-effect concentrations for chronic effects, thus the risk associated with their occurrence is probably minimal.

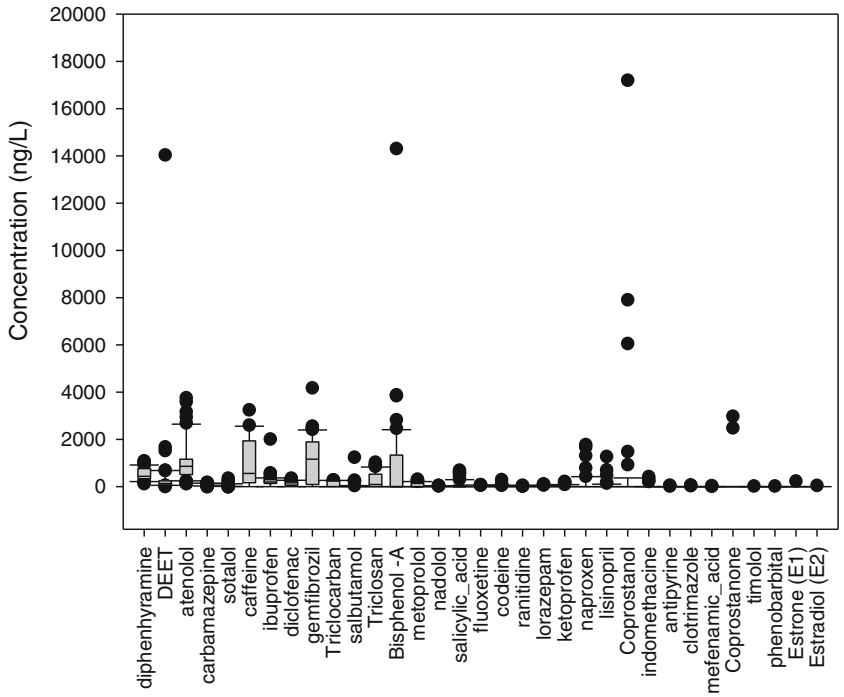

Fig. 3 Detected concentration ranges for all compounds in reclaimed water samples

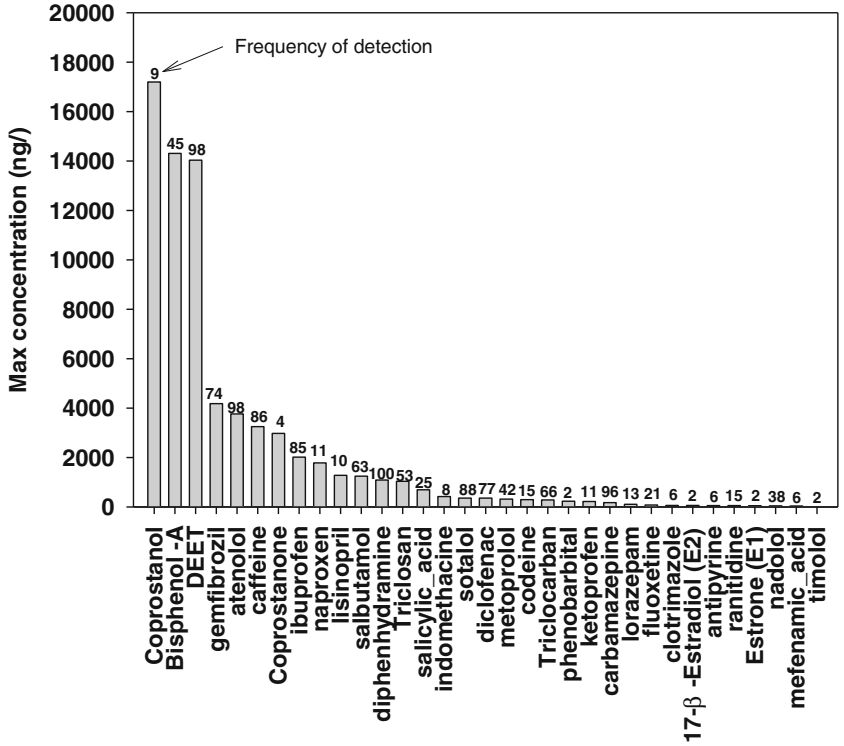

Fig. 4 Maximum concentration and detection frequency of compounds in reclaimed water samples

Although for a single compound, the detected concentrations were lower than the chronic NOECs, most of the time more than one analytes was found in the samples. The resulting additive effects of PPCP mixture could cause effects to organisms eventually. During the year study, multiple compounds were found in all reclaimed water samples and 13 % samples had total concentrations exceeded 10,000 ng/L. However, the effect and interactions of PPCP mixture in the

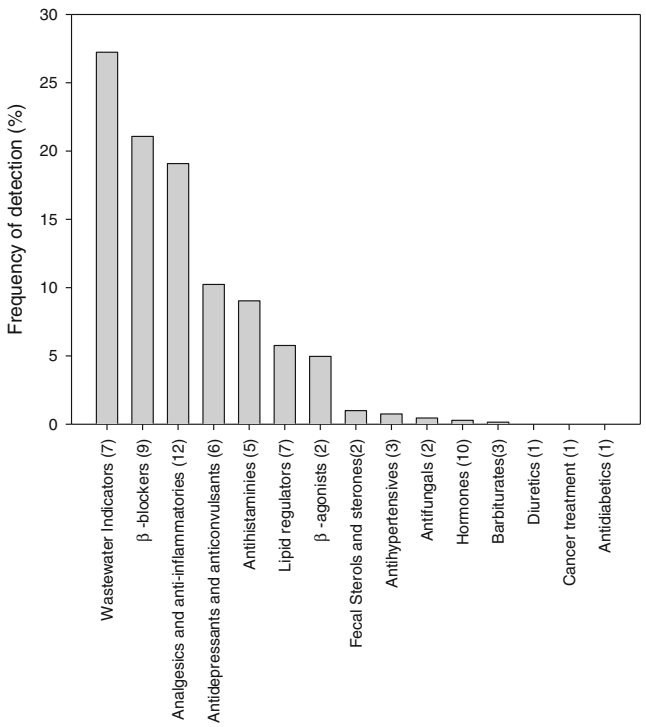

Fig. 5 Detection frequency as a percent of different classes in reclaimed water samples

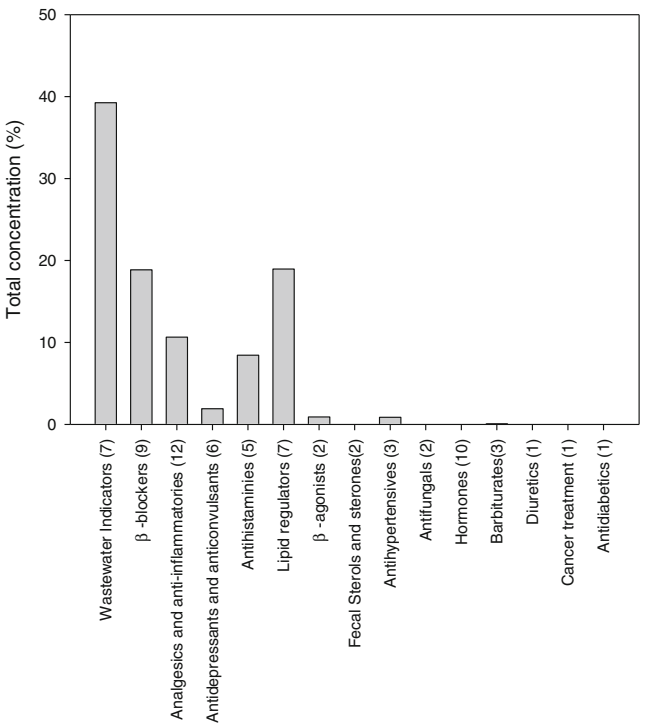

Fig. 6 Percent of total measured concentration for each group of compounds in reclaimed water samples

environmental samples is an area under development and further investigations are required to fully assess the potential implications. However, investigation has shown that biological resources inhabiting water bodies influenced by reclaimed water could accumulate PPCPs [11].

To obtain a broader view of the results, target compounds were divided into 15 groups on the basis of their general application or origins. The percent of detection frequencies of each group are shown in Fig. 5. The number of compounds in the group does not reflect the detection frequency. Detection frequency was influenced by the usage of compounds and their removal rate in the WWTPs. Wastewater indicators, β-blockers and analgesics/anti-inflammatories were the three most commonly detected groups, with a combined 67 % of detection frequency. The three groups also represented 69 % of the total measured concentrations, making them the most common and most abundant group of compounds present in the reclaimed water (Fig. 6). Another group that should be of concern is the lipid regulator group. Even though the percent of detection frequency is relatively low (6 %), compounds in the lipid regulator group accounts for as much as 20 % of the total concentration.

Average concentrations of analytes were added up monthly and results are shown in Fig. 7. The lowest total average concentration appeared in August, September and October. These three months are the wet season in South Florida. It is unlikely the rain diluted the sewage water and decreased the concentrations of target compounds. The three months with highest total average concentration were March, January, and May, which belong to the dry season in South Florida. Therefore, it is very likely that the weather changing has influence on the concentration of target compounds in the reclaimed water.

Conclusions

An online SPE-HPLC-HESI-MS/MS method and an online SPE-HPLC-APPI-MS/MS method were successful applied on simultaneously detection of 72 compounds in reclaimed water samples. The online SPE method was robust, sensitive, and reliable, making it suitable for routine analysis of environmental water samples. Reclaimed water samples were collected from the sprinkler system for a year-long period in Florida International University Biscayne Bay Campus, where it is routinely reused for irrigation. Analysis showed that multiple analytes were detected in all reclaimed water samples all the time. About 15 % of the detected compounds were above 1,000 ng/L. Among compounds with high concentrations (>1,000 ng/L), coprostanol, bisphenol A, and DEET's maximum concentrations exceeded 10,000 ng/L. The four most frequently detected compounds were diphenhydramine (100 %), DEET (98 %), atenolol (98 %), and carbamazepine (96 %). Wastewater indicators, β-blockers, and analgesics/anti-inflammatories were the three most detected groups and these three groups covered 67 % of detection frequency and 69 % of the total concentration. The 1-year study confirmed that current treatment in the North District WWTP does not effectively remove PPCPs from effluent. The microconstituents are continuously released to the environment through water reuse. This trend is not likely to change until effective treatment processes are incorporated into the WWTPs. Although no single compounds detected are above an acute or chronic benchmark to induce an effect, the implication of chronic exposure to multiple stressors is still largely unknown. In addition, parent compounds may degrade to thousands of metabolites, which are possibly more toxic

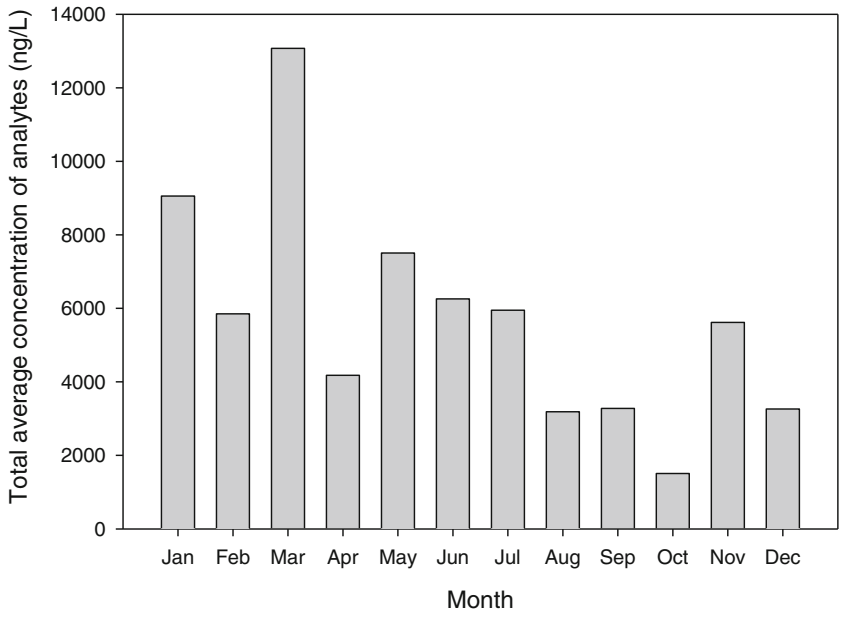

Fig. 7 Total average concentration of analytes in different month during the year

pollutants than parent compounds. Pollutants may accumulate in the biological resources through long-term exposure. Monitoring chemical will not solve the need of restoration and environmental quality management eventually, since they are not biological relevant. Thus, in the future, microbial assemblages may be a tool used to disclose chronic low level pollution, because they are highly response to environmental pressure and represent comprehensive environmental signals.

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