Multi-residue determination of pharmaceutical and personal care products in vegetables

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ABSTRACT

Treated wastewater irrigation and biosolid amendment are increasingly practiced worldwide and contamination of plants, especially produces that may be consumed raw by humans, by pharmaceutical and personal care products (PPCPs), is an emerging concern. A sensitive method was developed for the simultaneous measurement of 19 frequently-occurring PPCPs in vegetables using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS) for detection, combined with ultrasonic extraction and solid phase extraction (SPE) cleanup for sample preparation. Deuterated standards were used as surrogates to quantify corresponding analytes. The corrected recoveries ranged between 87.1 and 123.5% for iceberg lettuce, with intra- and inter-day variations less than 20%, and the method detection limits (MDLs) in the range of 0.04–3.0 ng g^{-1} dry weight (dw). The corrected recoveries were equally good when the method was used on celery, tomato, carrot, broccoli, bell pepper and spinach. The method was further applied to examine uptake of PPCPs by iceberg lettuce and spinach grown in hydroponic solutions containing each PPCP at 500 ng L^{-1}. Twelve PPCPs were detected in lettuce leaves with concentrations from 0.2 to 28.7 ng g^{-1} dw, while 11 PPCPs were detected in spinach leaves at 0.04–34.0 ng g^{-1} dw. Given the diverse chemical structures of PPCPs considered in this study, this method may be used for screening PPCP residues in vegetables and other plants impacted by treated wastewater or biosolids, and to estimate potential human exposure via dietary uptake.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are a diverse group of chemicals used extensively in medicines and personal hygiene products. Environmental contamination of PPCPs is an increasing public concern due to their ubiquitous occurrence [1–3] and potential adverse effects on nontarget organisms and humans [4]. Due to the incomplete removal of PPCPs during treatment at wastewater treatment plants (WWTPs), considerable amounts of PPCPs remain in WWTP effluents (also termed treated wastewater) and biosolids and then enter the environment through the disposal or reuse of treated wastewater and biosolids [5–7].

The use of treated wastewater for agricultural irrigation has been practiced for decades in some regions such as Israel [8] and is expected to increase rapidly in many arid and semi-arid areas around the world due to the increasing water scarcity caused by urbanization and climate change [9]. The land application of biosolids as soil amendment is also practiced worldwide, both as a source of plant nutrients and a way for waste disposal [10,11]. Many

studies have documented the presence of various PPCPs in soils irrigated with treated wastewater or amended with biosolids [12–14]. Once in soil, PPCPs may enter plant tissues via root uptake [15–17]. For instance, a recent study showed 5 PPCPs at 13.9–532 ng g^{-1} (fresh weight) in apple tree leaves and alfalfa receiving treated wastewater irrigation [18].

So far only a few PPCPs have been considered for their plant uptake potential, leaving the majority of PPCPs unexplored. A critical hurdle to the broad assessment of PPCP residues in plants is the lack of sensitive analytical methods for simultaneous determination of compounds from multiple chemical classes. Trace analysis of PPCPs in various environmental matrices is achieved with high-end instrumentation such as high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS) [19,20].

Compared to analysis of PPCPs in water or soil, determination of trace levels of PPCPs in plants presents greater challenges due to high contents of pigments, and fatty or waxy materials which may induce severe matrix interferences [21]. Therefore, a prerequisite for analysis of PPCPs in plant samples is an effective sample preparation procedure that eliminates potential interferences while ensuring maximum recovery. Several extraction techniques have been tested for plant tissue samples, including accelerated solvent extraction (ASE) [15], pressurized liquid extraction (PLE) [21,22],

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sonication [23], sea sand disruption method (SSDM) [24] and matrix solid-phase dispersion (MSPD) in combination with pressurized fluid extraction (PFE) [25]. Solid-phase extraction (SPE) [15,16,23] and hexane washing [21] have been tested for sample cleanup. However, these methods either lack good sensitivity or have considered just a few PPCPs as target analytes. Thus a method for multi-residue determination of a broad range of PPCPs in plant tissues at environmentally relevant levels is necessary, especially for edible plants such as vegetables since the potential human exposure is expected to be the greatest from vegetables that are consumed raw.

In this study, we developed a sensitive method for analyzing trace levels of PPCPs in vegetables by using an optimized protocol coupling ultrasonic extraction, SPE cleanup and HPLC–electrospray ionization (ESI)–MS/MS detection. A total of 19 PPCPs from different chemical classes, most of which commonly occurring in treated wastewater and/or biosolids [6,26], were used as the target analytes. The method, initially developed using iceberg lettuce (*Lactuca sativa* L.), was validated using six other common vegetables, and further applied to evaluate PPCP accumulation in iceberg lettuce and spinach (*Spinacia oleracea*) grown hydroponically under greenhouse conditions.

2. Experimental

2.1. Chemicals and materials

Acetaminophen, caffeine, meprobamate, atenolol, trimethoprim, carbamazepine, diazepam, gemfibrozil and N,N-diethyl-*meta*-toluamide (DEET) were purchased from Sigma–Aldrich (St. Louis, MO). Primidone was obtained from Spectrum Chemical (Gardena, CA). Sulfamethoxazole was provided by MP Biomedicals (Solon, OH). Dilantin, diclofenac and triclocarban were from TCI America (Portland, OR). Naproxen, ibuprofen and triclosan were from Alfa Aesar (Ward Hill, MA). (3S,5S)-Atorvastatin sodium salt was from Santa Cruz Biotechnology (Santa Cruz, CA). Fluoxetine HCl, fluoxetine-d_6 oxalate and diazepam-d_5 were from Cerilliant (Round Rock, TX). All other deuterated standards were purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). The structures of target compounds, along with their pK_a and log K_{ow} values, are shown in Table 1.

The solvents used in this study were from Fisher (Fair Lawn, NJ). Ultrapure water was produced using a Barnstead E-Pure water purification system (Thermo Scientific, Dubuque, IA). Individual stock solution of each PPCP was prepared in methanol and stored in an amber glass vial at −20 °C. The polytetrafluoroethylene (PTFE) filters (13 mm, 0.2 μm) were purchased from Millipore (Carrigtwohill, Cork, Ireland).

2.2. Optimization of sample preparation

Fresh vegetables were cut into small pieces and freeze-dried for 3 d or longer to remove residual water using a freeze dry system (Labconco, Kansas City, MO). The dried samples were then ground to powder using a coffee grinder with stainless steel grinding chamber (Hamilton Beach, Picton, Ontario, Canada) and stored at −20 °C until extraction.

Iceberg lettuce was used in method development involving selection and optimization of conditions for extraction, sample cleanup and instrumentation. Two extraction methods, ASE and ultrasonic extraction, were evaluated using freeze-dried lettuce samples spiked with mixed standards and deuterated compounds. For ASE, a Dionex ASE350 system (Sunnyvale, CA) was used under the following operating conditions: 10 ml extraction cells with glass fiber filters at both ends, 80–130 °C equilibration temperature,

1500 psi equilibration pressure, 5 min heat time, 5 min static equilibration, 2 cycles, 60% flush volume and 100 s purge time. Several extraction solvents were tested, including water, methanol, acetonitrile, methylene chloride, methyl tert-butyl ether (MTBE) and 0.35% phosphoric acid/acetonitrile (1:1, v/v) mixture [27]. For sonication, the sample was mixed with 20 ml solvent in a 50 ml glass centrifuge tube and then sonicated in a Fisher Scientific FS110H ultrasonic water bath (50/60 Hz, Pittsburgh, PA) for 20 min, followed by centrifugation at 1000 × g for 20 min. The supernatant was decanted into a 40 ml vial and the residue was extracted one more time using fresh solvent. The tested solvents for sonication included methanol, acetonitrile, acetone, ethyl acetate and MTBE.

Different SPE cartridges and operating conditions were systematically evaluated using lettuce extract spiked with mixed standards and deuterated compounds in developing the sample cleanup procedure. The four tested cartridges were: 150 mg Oasis HLB (Waters, Milford, MA) containing hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene, 150 mg Oasis MCX (Waters) containing a strong cation-exchange mixed-mode polymeric sorbent built upon the HLB copolymer, 150 mg Oasis MAX (Waters) containing a strong anion-exchange mixed-mode polymeric sorbent built upon the HLB copolymer, and 200 mg Evolute ABN (Biotage, Charlottesville, VA) containing a polymer-based sorbent with a balanced combination of polar (hydrophilic) and non-polar (hydrophobic) interactions. For the HLB and ABN cartridges, the lettuce extract was re-dissolved in 1 ml methanol followed by addition of 20 ml ultrapure water before loading onto the SPE cartridge and the analytes were eluted under gravity with 7 ml methanol. For the MCX cartridge, the loading solution was adjusted to pH 3, and then the cartridge was washed with 2 ml 2% formic acid and eluted with 4 ml methanol followed by 4 ml 5% NH$_4$OH in methanol. For the MAX cartridge, the loading solution was the same as HLB, while the washing solvent was 2 ml 5% NH$_4$OH and the eluting solvent was 4 ml methanol followed by 4 ml 2% formic acid in methanol. The cleaned extract was dried under a gentle nitrogen stream and reconstituted with 0.5 ml methanol. To remove possible solid particles, all samples were filtered through PTFE filters before analysis by HPLC–MS/MS.

2.3. Instrumental analysis and optimization

In this study, an ACQUITY ultra-performance liquid chromatography (UPLC^TM) system (Waters, Milford, MA) consisting of binary solvent manager and sample manager was used. Chromatographic separation of compounds was performed at 40 °C, using ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size, Waters). In the preliminary experiments, several mobile phases including water, methanol and acetonitrile, and various mobile phase modifiers including ammonium acetate and formic acid, were tested for improving analyte resolution and ESI performance. Water (acidified with formic acid) and methanol were finally chosen as the mobile phases, with 0.001% formic acid in water/methanol (95/5, v/v) as mobile phase A and pure methanol as mobile phase B. The following gradient program (with respect to mobile phase B) was used: 0–0.5 min, 5–50% B; 0.5–9 min, 50–100% B; 9–10 min, 100% B; 10–12 min, re-equilibrate with 5% B. The flow rate was 0.2 ml/min, and the sample injection volume was 5 μl.

Analytes were determined using a Waters Micromass triple quadrupole detector equipped with an ESI source. Data acquisition was performed in both positive and negative ESI modes and the optimized MS parameters were as follows: source temperature, 120 °C; desolvation temperature, 350 °C; capillary voltage, 3.2 kV; cone voltage, 30 V; desolvation gas flow, 600 L h^{-1} and cone gas flow, 50 L h^{-1}. The collision gas (Argon, 99.999%) flow in the collision cell was kept at 0.2 ml/min. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode and the

Table 1
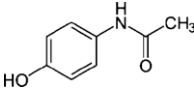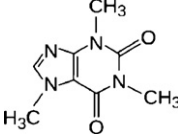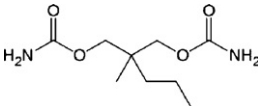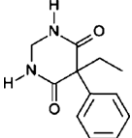Structures and selected physico-chemical properties of target compounds.

Compound	CAS number	Structure	pK_a [a]	$\log K_{ow}$ [a]
Pharmaceuticals (16)				
Acetaminophen	103-90-2		9.38	0.46
Caffeine	58-08-2		10.4	−0.07
Meprobamate	57-53-4			0.70
Primidone	125-33-7			0.91
Sulfamethoxazole	723-46-6		1.85 [22]	0.89
Atenolol	29122-68-7		9.6	0.16
Trimethoprim	738-70-5		7.12	0.91
Carbamazepine	298-46-4		2.3 [15]	2.45
Dilantin	57-41-0		8.33	2.47
Naproxen	22204-53-1		4.15	3.18
Diazepam	439-14-5		3.4	2.82
Fluoxetine	54910-89-3		10.09 [15]	4.05; 1.25–4.3 (pH 2–11) [34]

Table 1 (*Continued*)

Compound	CAS number	Structure	pK_a[a]	log K_{ow}[a]
Atorvastatin	134523-00-5		4.5 [35]	6.36
Ibuprofen	15687-27-1		4.91	3.97
Gemfibrozil	25812-30-0			4.77
Diclofenac	15307-86-5		4.15	4.51
Personal care products (3)				
Triclosan	3380-34-5		7.9 [15]	4.76
DEET	134-62-3		0.67 [36]	2.18
Triclocarban	101-20-2		12.7 [15]	4.90

[a] From database provided by Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm.

optimized parameters are listed in Table 2. A dwell time of 0.02 s per ion pair was used. All data were acquired and processed using the MassLynx 4.1 software. The choice of product ion for quantification and qualification of each analyte was based on the most intense fragmentation signal and the optimization of cone voltages, energy collisions and other instrument parameters was made individually for each compound through the direct infusion of standard solutions into the stream of mobile phase.

2.4. Method performance evaluation

Confirmation of the target analytes in plant samples was based on the MRM ion transitions as well as comparing the retention time of each peak to the corresponding standard. The performance of the optimized method was evaluated by considering response linearity, instrument detection limits (IDLs), method detection limits (MDLs), recoveries, and repeatability (intra- or inter-day variations) using iceberg lettuce samples. For calibration standards, appropriate dilutions were made from working standard solutions in the concentration range of 0.25–2500 μg L^{-1} for triclosan and 0.01–100 μg L^{-1} for the other PPCPs. The IDLs were estimated using diluted standards by reaching a signal to noise (S/N) ratio of 3. The MDLs were determined by adding small amounts of mixed standards to the lettuce sample, analyzing with the optimized method and then calculating the lowest concentration for each analyte at

which a signal with an S/N ratio of 3 was generated. The absolute recovery for each analyte was calculated as the amount detected over that spiked. The corrected recovery was calculated as the amount detected, after correction with the corresponding deuterated surrogate, over that spiked. Repeatability of the method was estimated from the standard deviation (STD) of three replicates at each spiking concentrations (10 and 100 ng g^{-1} dry weight (dw)) analyzed during the same day (intra-day precision) and across three days (inter-day precision), respectively. Further method validation was achieved by applying the optimized method to the analysis of an additional six vegetables (spinach, green bell pepper, tomato, carrot, broccoli and celery) that may be consumed raw.

2.5. Method application

The optimized method was further validated by applying to study the accumulation of PPCPs in young plants of iceberg lettuce and spinach grown hydroponically under greenhouse conditions. Seedlings of iceberg lettuce and spinach with 3–4 leaves were purchased from Certified Plant Growers (Temecula, CA) through a local nursery. Each plant was carefully rinsed off the growth media and then transplanted into a 450 ml glass jar containing nutrient solution spiked with mixed PPCPs standards, with each PPCP at 500 ng L^{-1}, a concentration within the range seen for some target PPCPs in treated wastewater [6,26]. The nutrient solution

Table 2
Optimized MRM parameters for the analysis of PPCPs and their deuterated compounds by HPLC–MS/MS.

Compound	MRM (m/z)			
	Quantification	CV[a]/CE[b]	Qualification	CV/CE
ESI+				
Acetaminophen	152 > 110	25/15	152 > 92.5	25/25
Acetaminophen-d_4	156 > 114	28/17		
Caffeine	195 > 138	45/15		
Caffeine-d_9	204 > 144	45/15		
Meprobamate	219 > 158	15/10	219 > 97	15/10
Meprobamate-d_3	222 > 161	15/10		
Primidone	219 > 162	30/15		
Primidone-d_5	224 > 167	25/10		
Sulfamethoxazole	254 > 156	26/15	254 > 108	26/21
Sulfamethoxazole-d_4	258 > 160	30/15		
Atenolol	267 > 145	35/30		
Atenolol-d_7	274 > 145	35/30		
Trimethoprim	291 > 230	50/25	291 > 123	50/25
Trimethoprim-d_9	300 > 234	50/25		
DEET	192 > 119	36/15	192 > 91	36/25
DEET-d_7	199 > 126	36/19		
Carbamazepine	237 > 194	30/20	237 > 192	30/20
Carbamazepine-d_{10}	247 > 204	35/20		
Dilantin	253 > 182	30/20		
Dilantin-d_{10}	263 > 192	30/20		
Diazepam	285 > 154	45/25	285 > 193	45/25
Diazepam-d_5	290 > 154	45/25		
Fluoxetine	310 > 44	25/10	310 > 148	25/15
Fluoxetine-d_6	316 > 44	25/15		
Atorvastatin	559 > 440	35/25		
Atorvastatin-d_5	564 > 445	35/25		
ESI−				
Naproxen	229 > 170	20/15	229 > 185	20/15
Naproxen-d_3	232 > 173	20/15		
Ibuprofen	205 > 161	20/5		
Ibuprofen-d_3	208 > 164	20/5		
Gemfibrozil	249 > 121	25/15		
Gemfibrozil-d_6	255 > 121	25/15		
Triclosan	287 > 35	30/8	289 > 35	30/8
Triclosan-d_3	290 > 35	25/10		
Diclofenac	294 > 250	25/10	294 > 214	25/20
Diclofenac-d_4	298 > 254	25/10		
Triclocarban	313 > 160	30/20	315 > 162	30/10
Triclocarban-d_4	317 > 160	30/10		

[a] Cone voltage (V).
[b] Collision energy (eV).

was made according to Pedler et al. [28]. Three replicates were used for each vegetable type. The experiment was carried out in a green house, with temperature ranging from 12 to 32 °C and the air humidity ranging from 40 to 90%. The PPCP-spiked nutrient solution was exchanged every 3 d to avoid nutrient depletion and to limit algal growth. After 21 d of growth, new plant leaves were harvested and analyzed using the optimized method as described above.

2.6. Statistical analysis

All samples were prepared and analyzed as triplicates to provide a robust statistical analysis. Statistical comparison was performed by one-way analysis of variance (ANOVA) with a p-value lower than 0.05 using SPSS software, version 19 for Windows (SPSS, Inc., Chicago, IL).

3. Results and discussion

3.1. Mobile phase selection

In this study, a series of preliminary experiments were performed testing different mobile phases in order to achieve good separation (i.e., reduction of peak tailing and better resolution) and high sensitivity in a run containing ESI+ and ESI−. Use of methanol as the organic phase was found to increase the sensitivity for most compounds as compared to acetonitrile, and sharp symmetrical peaks were observed after addition of formic acid or ammonium acetate to water as the aqueous phase. Although 0.1% formic acid in water was used as the mobile phase for simultaneous determination of both acidic and basic PPCPs in previous studies [15,29], it was observed that the sensitivity was improved by 4–14 times when the concentration of formic acid was decreased from 0.1 to 0.001% for some acidic PPCPs detected in the ESI− mode, including naproxen, ibuprofen, gemfibrozil and diclofenac, while the change had little effect for PPCPs detected in the ESI+ mode. Ammonium acetate did not result in a sensitivity enhancement as formic acid. Therefore, methanol and water/methanol (95/5) containing formic acid at 0.001% were finally chosen as the binary components of mobile phase in this study.

3.2. Extraction and SPE cleanup conditions

Comparison of recoveries showed that ultrasonic extraction consistently resulted in better recoveries than ASE extraction for most of the target PPCPs. A further evaluation was subsequently made to select the best solvent for sonication extraction (without cleanup procedure). Both methanol and acetone completely failed to recover acetaminophen from the spiked lettuce sample (Fig. 1), likely due to the co-extracted matrix that induced severe signal suppression in the ESI mode. Sonication extraction using ethyl
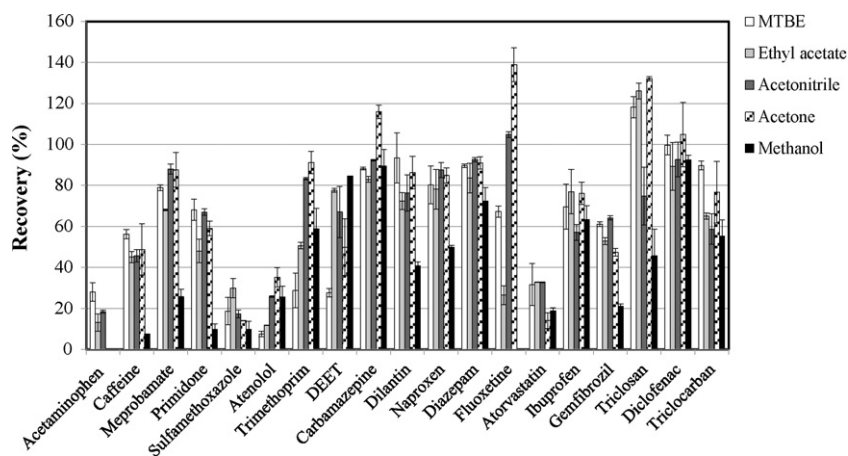

Fig. 1. Comparison of extraction solvent used in ultrasonic extraction.
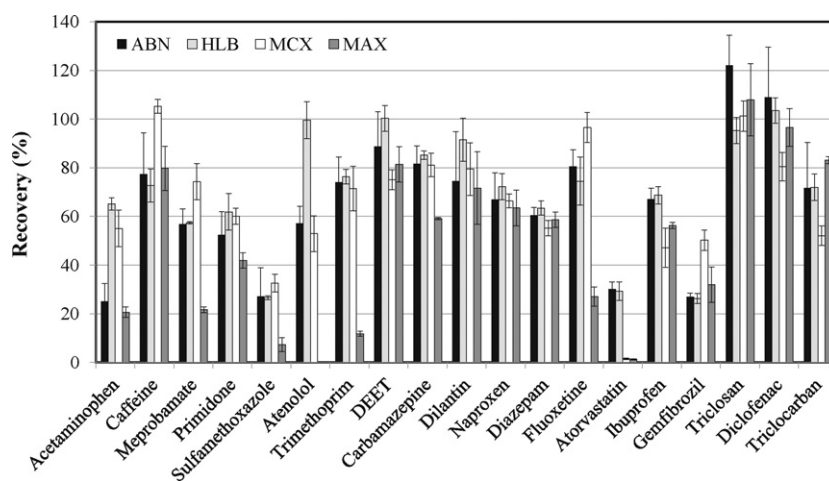
Fig. 2. Comparison of SPE cartridges in cleanup procedure.

acetate generally did not improve recoveries than those with either MTBE or acetonitrile ($p > 0.05$). Between acetonitrile and MTBE, acetonitrile extraction resulted in a higher extraction efficiency for atenolol, trimethoprim and fluoxetine than MTBE ($p < 0.01$), while MTBE extraction led to significantly better recoveries for caffeine, triclosan, and triclocarban ($p < 0.05$). Therefore, the optimized sonication procedure included two steps of extraction for all PPCP analytes, with MTBE as the solvent for the first extraction, and acetonitrile as the solvent for the second extraction.

It has been shown that ESI is susceptible to matrix components [30,31], which may result in signal suppression or isobaric interference and therefore decrease the sensitivity of assay. To reduce matrix effects, an additional step involving SPE cartridge cleanup was applied. The Oasis HLB and Evolute ABN cartridge have been employed previously to clean up plant material extracts for the determination of trimethoprim, sulfamethoxazole, carbamazepine, fluoxetine, triclosan and triclocarban [15,16,32]. Redshaw et al. proposed the use of two cartridges (strong-anion exchange cartridge and polymeric phase cartridge) placed in tandem as the cleanup step to analyze fluoxetine in cauliflower extract [23]. In this study, recoveries of the four different types of SPE cartridges, including Oasis HLB, MCX, MAX and Evolute ABN, are shown in Fig. 2. While there was no significant difference ($p > 0.05$) between ABN and HLB cartridges for most compounds, the recoveries for acetaminophen

and atenolol with ABN were significantly lower ($p < 0.05$) than that with HLB. For some compounds (i.e., caffeine, meprobamate, fluoxetine and gemfibrozil) MCX showed a better recovery ($p < 0.01$) than HLB, while for atenolol, DEET, atorvastatin and ibuprofen, HLB performed better than MCX ($p < 0.01$), especially for atorvastatin, for which the recovery using MCX was only $1.5 \pm 0.3\%$. The MAX cartridge generally displayed recoveries similar or lower than HLB. For instance, recoveries using MAX were substantially lower ($p < 0.01$) than those using HLB for acetaminophen, meprobamate, primidone, sulfamethoxazole, atenolol, trimethoprim, carbamazepine, fluoxetine and atorvastatin. Given its consistently higher recoveries for most of the target PPCPs over the other three SPE cartridges, HLB was finally selected as the optimal SPE cartridge for sample cleanup.

A further evaluation was made to determine the effect of loading solution pH and eluting solvents when using HLB cartridges. The recoveries of atenolol (0%) and atorvastatin (1.8%) were very low when the extract solution was acidified, suggesting pH manipulation was unlikely to improve HLB performance for these compounds. A variety of eluting solvents, including methylene chloride, ethyl acetate, methanol and acetonitrile were tested. Methyl chloride and ethyl acetate showed a stronger tendency to elute plant hydrophobic compounds (e.g., carotene) from the HLB cartridge as compared to methanol. Therefore, the final method

Table 3
Validation parameters for determination of PPCPs in plant tissue samples (iceberg lettuce leaves).

Compound	Linearity (R^2)	IDL (pg)	MDL (ng g^{-1})	Absolute recovery (corrected recovery, %)		Intra-day (% STD)		Inter-day (% STD)	
				10 ng g^{-1}	100 ng g^{-1}	10 ng g^{-1}	100 ng g^{-1}	10 ng g^{-1}	100 ng g^{-1}
Acetaminophen	0.9982	0.5	0.5	61.9 (103.9)	58.3 (100.8)	4.0	2.8	17.4	7.6
Caffeine	0.9994	1.0	1.0	66.1 (121.0)	54.2 (97.1)	7.1	2.3	10.1	2.9
Meprobamate	0.9992	0.07	0.06	68.9 (99.6)	60.4 (98.3)	7.5	1.3	15.0	7.0
Primidone	0.9989	2.0	1.6	62.8 (97.0)	66.7 (102.7)	8.9	3.4	16.9	12.9
Sulfamethoxazole	0.9986	0.1	0.5	11.2 (87.1)	16.4 (102.7)	4.1	4.4	1.2	2.4
Atenolol	0.9983	1.2	1.4	44.8 (102.1)	48.6 (103.0)	3.8	2.2	7.8	1.3
Trimethoprim	0.9993	0.25	0.3	57.6 (94.9)	57.1 (98.7)	4.1	3.4	9.1	6.4
DEET	0.9992	0.1	0.1	72.5 (123.4)	65.6 (102.1)	10.4	7.6	6.0	1.5
Carbamazepine	0.9996	0.05	0.05	79.7 (102.6)	71.1 (94.9)	4.4	1.1	7.9	6.0
Dilantin	0.9998	1.6	1.2	75.5 (110.5)	87.5 (111.0)	17.0	5.1	18.2	2.5
Naproxen	0.9999	0.1	0.1	69.3 (94.8)	64.7 (98.2)	7.1	4.1	18.2	8.7
Diazepam	0.9996	0.05	0.05	60.4 (102.6)	60.3 (102.3)	2.5	1.7	9.5	4.1
Fluoxetine	0.9997	0.02	0.04	41.5 (93.3)	37.9 (105.1)	3.9	5.3	0.3	8.6
Atorvastatin	0.9998	0.1	1.1	4.9 (107.4)	4.8 (95.8)	1.0	2.0	3.2	3.5
Ibuprofen	0.9998	0.5	0.5	63.0 (112.1)	58.8 (103.7)	5.3	1.7	16.5	8.5
Gemfibrozil	0.9989	0.1	0.3	23.0 (91.6)	26.2 (103.3)	2.9	1.5	3.1	0.2
Triclosan	0.9991	6.25	3.0	111.1 (123.5)	112.6 (119.8)	8.5	14.8	12.6	17.0
Diclofenac	1.0000	0.25	0.2	90.8 (97.0)	88.5 (101.6)	5.4	3.9	7.5	4.8
Triclocarban	0.9987	0.05	0.04	78.4 (109.3)	65.8 (103.1)	5.1	3.1	4.6	5.4

included a simple dilution of the sample extract with water without pH adjustment, and elution with methanol.

3.3. Method performance and application

Through the above incremental optimization, the final method is suggested to consist of the following steps:

(1) Freeze-dry fresh plant tissues and grind into fine powder;
(2) Place 0.2 g aliquots in a centrifuge tube, spike with deuterated standards as recovery surrogates, and extract in a sonication bath with 20 ml MTBE, followed by 20 ml acetonitrile;
(3) Dry the combined extract under nitrogen at 40 °C, recover with 1 ml methanol, and dilute with 20 ml ultrapure water;
(4) Load the aqueous sample solution onto an HLB cartridge (pre-conditioned with 6 ml methanol and 6 ml ultrapure water) which is then dried under nitrogen gas, and elute with 7 ml methanol;
(5) Dry the cleaned extract under nitrogen, recover in 0.5 ml methanol, filter through a syringe PTFE filter and inject into a HPLC–MS/MS for detection.

The performance and rigorousness of the optimized method were evaluated using a range of parameters. Under the used instrumental conditions, the calibration curves showed excellent linearity in the concentration ranges with $R^2 \geq 0.998$ (Table 3) and the IDLs of the 19 target PPCPs were between 0.02 (fluoxetine) and 6.25 (triclosan) pg. The calculated MDLs ranged from 0.04 to 3.0 ng g^{-1} dw for iceberg lettuce, which were significantly lower than those reported previously [15,22,25]. For example, in the method reported by Wu et al. for analysis of selected PPCPs in soybean, the limits of detections (LODs) were 0.1–4.89 ng g^{-1} dw [15]. The MDLs for carbamazepine, fluoxetine, and triclocarban in the current method were 3.5–20 times lower than those reported by Wu et al., while the MDL for triclosan (3.0 ng g^{-1} dw) was comparable with their reported value (4.89 ng g^{-1} dw). The relatively low MDLs of the optimized method, and the inclusion of a large number of PPCPs, together suggest that this method may be used for screening the occurrence of PPCPs in vegetables and other plant tissues that are impacted by the use of treated wastewater or biosolids.

The average recoveries of PPCPs in iceberg lettuce spiked at two concentrations (10 and 100 ng g^{-1} dw) are shown in Table 3. The absolute recoveries were >60% for most PPCPs. For some PPCPs, including sulfamethoxazole, fluoxetine, atorvastatin and gemfibrozil, the average compound recoveries were low (4.8–41.5%). Hu et al. used MeOH:HCl (1:1,v:v) and acetone to extract antibiotics from vegetables (radish, rape, celery and coriander) combined with HLB cleanup, which resulted in higher recoveries for sulfamethoxazole (71%) [32]. The absolute recovery of fluoxetine was generally low, but had a high variability. This has also been observed in previous studies (15–59%) [15,23]. In this study, deuterated standards of the target PPCPs were used as recovery surrogates to account for the matrix-induced signal suppression or enhancement in ionization, analyte loss during sample preparation, and variations in the instrumental response from injection to injection. The corrected recoveries were found in the range of 87.1–123.5% (Table 3), indicating that the deuterated analogs provided good quality control in the simultaneous analysis of a broad range of PPCPs that encompass different structures and properties. The intra- and inter-day precision of the method was between 0.2 and 18.2% (Table 3), suggesting good method repeatability.

In addition to iceberg lettuce, 6 other vegetable species were also included for validation of method recoveries (Table 4). The absolute recoveries of PPCPs were apparently matrix-dependent. For example, the recovery of atorvastatin in celery leaf was low (7.1%), while in bell pepper it increased to 76.3%. Some compounds,

Table 4
Absolute and corrected recoveries (in parenthesis) of PPCPs in different plant tissues.[a]

	Celery leaf	Tomato	Carrot root	Broccoli	Bell pepper	Spinach leaf
Acetaminophen	59.5 ± 3.2 (105.7 ± 3.4)	32.2 ± 2.4 (107.6 ± 8.6)	61.5 ± 1.8 (127.8 ± 13.9)	19.7 ± 6.9 (94.6 ± 18.3)	51.4 ± 4.7 (116.9 ± 18.2)	34.1 ± 2.3 (128.6 ± 17.9)
Caffeine	76.6 ± 2.7 (123.3 ± 16.2)	22.9 ± 3.0 (56.8 ± 9.0)	50.3 ± 1.2 (106.9 ± 14.9)	35.0 ± 2.4 (56.3 ± 5.9)	47.6 ± 1.9 (85.1 ± 10.3)	37.1 ± 1.6 (61.9 ± 13.8)
Meprobamate	60.8 ± 0.7 (117.4 ± 1.8)	33.7 ± 1.9 (82.8 ± 6.3)	53.9 ± 3.6 (89.3 ± 18.1)	26.2 ± 5.3 (126.4 ± 12.5)	54.8 ± 1.4 (86.0 ± 9.0)	43.2 ± 7.0 (109.1 ± 10.2)
Primidone	62.1 ± 6.4 (72.4 ± 14.7)	22.8 ± 1.6 (93.9 ± 22.5)	47.8 ± 2.8 (97.4 ± 11.3)	31.6 ± 1.7 (129.3 ± 12.7)	39.8 ± 1.9 (101.7 ± 18.7)	20.7 ± 3.3 (103.8 ± 18.7)
Sulfamethoxazole	10.9 ± 0.9 (100.0 ± 5.7)	9.7 ± 0.6 (105.9 ± 12.6)	18.2 ± 2.9 (105.9 ± 17.6)	17.2 ± 1.4 (116.2 ± 17.1)	19.1 ± 2.6 (123.4 ± 12.3)	19.7 ± 4.3 (118.2 ± 3.7)
Atenolol	29.1 ± 1.9 (129.6 ± 6.1)	2.5 ± 2.2 (121.7 ± 14.7)	24.2 ± 5.3 (106.9 ± 16.4)	26.4 ± 2.5 (100.2 ± 18.9)	5.4 ± 0.6 (104.8 ± 13.0)	28.6 ± 5.0 (106.0 ± 8.4)
Trimethoprim	50.6 ± 2.7 (93.7 ± 3.6)	17.9 ± 1.8 (91.8 ± 12.1)	42.1 ± 5.3 (101.8 ± 4.3)	50.9 ± 2.7 (90.1 ± 3.2)	42.7 ± 4.8 (122.8 ± 12.2)	43.9 ± 4.8 (101.8 ± 4.2)
DEET	67.2 ± 10.5 (108.5 ± 6.5)	87.8 ± 2.7 (120.3 ± 4.1)	42.8 ± 2.2 (103.8 ± 2.7)	67.1 ± 1.0 (115.9 ± 5.3)	59.6 ± 0.8 (109.8 ± 5.4)	75.3 ± 6.5 (115.6 ± 3.9)
Carbamazepine	55.7 ± 1.6 (66.9 ± 0.5)	59.4 ± 5.1 (106.7 ± 4.3)	72.4 ± 4.6 (104.7 ± 2.4)	49.8 ± 2.0 (102.3 ± 4.0)	50.7 ± 7.9 (74.9 ± 10.1)	59.2 ± 5.0 (114.6 ± 10.1)
Dilantin	80.7 ± 5.3 (124.5 ± 9.2)	63.4 ± 13.1 (124.7 ± 17.5)	57.9 ± 11.6 (81.4 ± 11.4)	23.7 ± 6.7 (113.7 ± 15.8)	55.1 ± 4.0 (85.9 ± 5.6)	18.4 ± 4.0 (99.7 ± 19.2)
Naproxen	81.6 ± 2.7 (122.1 ± 7.6)	69.7 ± 3.0 (93.9 ± 11.8)	70.2 ± 5.1 (112.5 ± 18.0)	50.5 ± 3.9 (123.9 ± 12.1)	56.2 ± 2.9 (96.4 ± 12.5)	57.3 ± 3.7 (85.5 ± 12.3)
Diazepam	74.1 ± 2.6 (102.3 ± 3.9)	58.2 ± 1.3 (109.8 ± 0.4)	50.8 ± 0.1 (98.2 ± 8.4)	35.2 ± 1.5 (100.1 ± 3.2)	62.9 ± 11.6 (106.9 ± 7.2)	44.1 ± 4.4 (105.3 ± 3.2)
Fluoxetine	59.5 ± 6.7 (99.9 ± 9.5)	51.8 ± 1.2 (98.9 ± 1.3)	39.3 ± 5.1 (102.1 ± 0.8)	8.7 ± 0.6 (112.4 ± 4.7)	10.5 ± 3.9 (108.2 ± 10.2)	7.4 ± 3.8 (110.4 ± 10.7)
Atorvastatin	7.1 ± 5.1 (105.7 ± 3.4)	37.9 ± 3.1 (107.6 ± 8.6)	64.9 ± 10.6 (91.1 ± 6.8)	27.6 ± 3.3 (113.9 ± 12.3)	76.3 ± 13.7 (98.2 ± 5.5)	29.6 ± 3.3 (100.5 ± 14.8)
Ibuprofen	92.7 ± 3.7 (95.0 ± 6.1)	74.0 ± 10.6 (121.2 ± 17.3)	72.9 ± 12.4 (71.6 ± 14.7)	34.5 ± 10.2 (106.0 ± 7.4)	89.6 ± 5.5 (105.9 ± 6.9)	52.7 ± 11.9 (100.5 ± 13.9)
Gemfibrozil	55.0 ± 0.5 (93.9 ± 0.9)	65.9 ± 2.7 (102.3 ± 8.3)	92.3 ± 9.0 (103.1 ± 10.2)	4.3 ± 1.9 (128.3 ± 10.3)	71.5 ± 4.8 (119.3 ± 8.9)	15.5 ± 1.7 (75.4 ± 8.6)
Triclosan	107.9 ± 8.0 (114.6 ± 12.7)	103.7 ± 8.0 (114.6 ± 12.8)	104.1 ± 10.2 (103.8 ± 9.5)	28.4 ± 11.0 (102.9 ± 13.5)	95.5 ± 12.0 (119.7 ± 12.8)	56.5 ± 7.5 (111.9 ± 15.0)
Diclofenac	95.9 ± 3.2 (111.3 ± 5.0)	84.5 ± 8.1 (103.6 ± 14.1)	72.5 ± 11.6 (94.5 ± 10.7)	84.5 ± 5.8 (104.6 ± 10.7)	107.1 ± 1.2 (117.2 ± 7.8)	78.6 ± 15.0 (94.1 ± 8.3)
Triclocarban	97.4 ± 2.0 (105.0 ± 3.0)	75.8 ± 0.8 (111.7 ± 1.7)	81.7 ± 3.6 (94.9 ± 3.4)	20.6 ± 10.0 (103.4 ± 11.6)	71.3 ± 7.0 (95.3 ± 3.0)	60.5 ± 9.0 (114.7 ± 8.3)

[a] Spiked concentration: 10 ng g^{-1} dw; n = 3.

Table 5
Concentrations of PPCPs in leaves of iceberg lettuce and spinach grown hydroponically in nutrient solutions containing each compound at 500 ng L^{-1} ($n = 3$).

	Concentrations (ng g^{-1} dw)	
	Iceberg lettuce	Spinach
Acetaminophen	ND[a]	ND
Caffeine	3.2 ± 0.7	1.8 ± 0.4
Meprobamate	5.0 ± 1.6	0.9 ± 0.3
Primidone	8.5 ± 1.7	ND
Sulfamethoxazole	ND	ND
Atenolol	ND	1.1 ± 0.03
Trimethoprim	1.1 ± 0.4	1.1 ± 0.2
DEET	2.8 ± 0.9	ND
Carbamazepine	28.7 ± 1.8	2.9 ± 1.3
Dilantin	9.2 ± 2.5	2.1 ± 0.9
Naproxen	ND	0.04 ± 0.06
Diazepam	17.8 ± 2.9	ND
Fluoxetine	21.6 ± 2.3	34.0 ± 10.4
Atorvastatin	1.6 ± 1.7	2.3 ± 3.1
Ibuprofen	ND	ND
Gemfibrozil	0.2 ± 0.2	0.2 ± 0.2
Triclosan	ND	ND
Diclofenac	ND	ND
Triclocarban	0.3 ± 0.1	2.5 ± 0.9

[a] ND, not detected.

including acetaminophen, ibuprofen, gemfibrozil, triclosan and triclocarban, appeared to have lower absolute recoveries in broccoli than in the other vegetables (Table 4). However, the use of deuterated standards as recovery surrogates again greatly minimized the plant species-specific matrix effect. After correcting the recoveries using the isotope labeled analogs, the recoveries for all vegetables were in the range of 56.3–129.6% with STD less than 20% (Table 4). The vegetables tested in this study represented some of the most common types that were consumed raw or with minimal processing. Therefore, the above validations suggested that the developed method may be used on a wide range of vegetables and likely other plants as well.

As an ultimate test, the developed method was used to analyze accumulation of the target PPCPs into live plants grown under hydroponic conditions, as a simulation of the practice of treated wastewater irrigation. The concentrations found in the leaves of the iceberg lettuce and spinach are summarized in Table 5. In the lettuce leaves, a total of 15 PPCPs were detected above the MDLs, with concentrations from 0.2 to 28.7 ng g^{-1} dw. In spinach, a total of 16 PPCPs were detected with concentrations of 0.04–34.0 ng g^{-1} dw. Five PPCPs, including acetaminophen, sulfamethoxazole, ibuprofen, triclosan and diclofenac, were not detected above the MDLs in either plant species. Nine PPCPs were taken up by both vegetables, including caffeine, meprobamate, trimethoprim, carbamazepine, dilantin, fluoxetine, atorvastatin, gemfibrozil and triclocarban. In lettuce, the highest concentrations of PPCPs were carbamazepine (28.7 ng g^{-1} dw), fluoxetine (21.6 ng g^{-1} dw) and diazepam (17.8 ng g^{-1} dw), while in spinach the detected PPCPs were lower than 5 ng g^{-1} dw except for fluoxetine (34.0 ng g^{-1} dw). Previous studies have reported the uptake and accumulation of some PPCPs in plant tissue, including carbamazepine, sulfamethoxazole, fluoxetine, triclosan, and triclocarban [15,18,22,23,33]. The greenhouse experiment in the current study demonstrated for the first time the plant uptake of meprobamate, primidone, atenolol, DEET, dilantin, diazepam, atorvastatin and gemfibrozil, indicating their potential for plant accumulation from soil irrigated with treated wastewater. Although the exposure concentrations in this study were within the frequently detected ranges in treated wastewater for some PPCPs, future studies using soil or field conditions are necessary to evaluate the potential for plant accumulation of PPCPs and the consequent implications for human and animal exposure through dietary uptake.

4. Conclusion

A reliable and sensitive method was established through stepwise optimization for simultaneous analysis of a broad range of PPCPs in common vegetables. This method includes the use of ultrasonic water bath for extraction, SPE (using HLB cartridge) for cleanup, deuterated standards for recovery calibration, and HPLC–ESI–MS/MS for detection. The MDLs of the optimized method for the 19 PPCPs, most of which are frequently found in treated wastewater, were in the range of 0.04–3.0 ng g^{-1} dw for iceberg lettuce samples. When deuterated standards were used as recovery surrogates, the corrected recoveries in various vegetables were in a range of 56.3–129.6%, with intra- and inter-day variations less than 20%. A greenhouse simulation experiment showed that lettuce and spinach were capable of taking up many PPCPs when exposed to environmentally relevant concentrations of these trace contaminants. Although the human risk from dietary uptake of such impacted vegetables may be small when only a single PPCP is considered, given that many PPCPs are simultaneously present in treated wastewater or biosolids, and that there could be hyposensitive populations, more research is clearly needed to quantitatively evaluate the occurrence of PPCPs in human edible plants such as vegetables, especially under representative field conditions.

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