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RESEARCH PAPER

Simultaneous analysis of 45 pharmaceuticals and personal care products in sludge by matrix solid-phase dispersion and liquid chromatography tandem mass spectrometry

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Abstract Pharmaceuticals and personal care products (PPCPs) are a class of emerging contaminants widely distributed in the wastewater treatment system. The simultaneous analysis of multiple PPCPs in the sludge, which is a complex matrix, is still not fully studied. In this study, a procedure based on matrix solid-phase dispersion (MSPD) for the extraction of PPCPs from the sludge with determination by liquid chromatography tandem mass spectrometry (LC-MS/MS) was investigated. Forty-five PPCPs, including antibiotics, nonsteroidal anti-inflammatory drugs, β-blockers, antidepressants, antimicrobial agents, preservatives, UV filters, and so on, were studied. MSPD parameters, including the sorbent materials, the ratio of sample to sorbent, the eluent composition, and the elution volumes, were sequentially optimized. Best results were achieved by 0.1 g of sludge homogenized with 0.4 g of C18-bonded silica sorbent and elution by 6 mL methanol and 10 mL acetonitrile/5 % oxalic acid (8/2, *v*/*v*). The method quantification limits for the 45 PPCPs ranged 0.117–5.55 μg/kg. The PPCP recoveries ranged from 50.3 to 107 % with relative standard deviation lower than 15 %. The proposed method was applied to analyze PPCPs in the sludge collected from a domestic wastewater treatment plant over 1 year. Thirteen PPCPs were detected, with the concentrations of ofloxacin and triclocarban more than 1000 μg/kg. Temporal variations of the PPCP levels were observed. Thus, MSPD-LC-MS/MS method could achieve good sensitivity and recovery for the target PPCP analysis in the sludge samples, while MSPD provided one-step sample preparation which was easier and faster to perform compared to the commonly used methods.

Keywords PPCPs · Determination · Sludge · MSPD · LC-MS/MS

Introduction

Pharmaceuticals and personal care products (PPCPs) are a class of emerging contaminants and are used to prevent or treat human and animal diseases and improve the quality of daily life [1]. The detection of trace amounts of PPCPs in the environment is of great concern, as PPCPs are biologically active and could pose adverse impacts to human health and the ecosystems [2, 3]. For example, the wide application of antibiotics might lead to large-scale dissemination of antibiotic resistance gene [4]. The widely detected bisphenol A and triclocarban have been demonstrated to be the endocrine disruptors to fish in the surrounding aquatic system [5, 6]. Therefore, further understanding of PPCP occurrence and fate is necessary.

Previous studies indicated that the effluent from wastewater treatment plants (WWTPs) was one of the major pathways

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of PPCPs to the receiving water bodies [7]. In addition, PPCPs adsorbed onto the activated sludge could also be introduced to the environment through the sludge application [8]. Large number of studies investigated PPCP occurrence in the influent and effluent in WWTPs [9]. However, due to the limited analytical methods and the complicated matrix, few studies have been focused on the PPCPs in the sludge [10, 11]. Therefore, an easily performed method for determination of PPCPs in the sludge is still urgent.

PPCP separation was mainly achieved by gas chromatography (GC) and liquid chromatography (LC), with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) for detection [10]. Considering most PPCPs are polar substances at trace level, LC-MS/MS was applied in most studies to avoid the derivatization and to provide better sensitivity [12]. For the analysis of trace PPCPs in the sludge, it is challenging to develop the pretreatment method to extract the target analytes at low levels from the complex matrices. Ultrasonic extraction (USE) [13, 14], accelerated solvent extraction (ASE) [15], microwave assisted extraction (MAE) [16] were the frequently used pretreatments for PPCP extraction, followed by solid-phase extraction (SPE) of the raw extract with water dilution. This cleanup strategy might remove the highly polar interferences, which were not retained in the cartridge, or highly lipophilic interferences, which were not eluted by the eluents [17]. However, these two-step methods were complicated and time-consuming to operate. As an alternative, matrix solid-phase dispersion (MSPD), which could perform the extraction and cleanup in one step [18], provides a rapid pretreatment for the analysis of PPCPs in the solid samples. Recently, MSPD has been used for the extraction of antimycotic drugs [17], cardiac drugs [19], antibiotic and anti-parasitic drugs [12], and antimicrobial agents [20] in the sediment or sludge. However, only few targets were included in the previous reports. PPCPs comprise diverse chemical substances, including the antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), β-blockers, antidepressants, antimicrobial agents, preservatives, UV filters, and so on. Simultaneous determination of multiple PPCPs would provide more information of PPCP levels and make better evaluation on the occurrence, fate, and environmental risk of PPCPs. For example, in USEPA method 1694, more than 70 PPCPs were included [13]. Since the physicochemical properties of PPCPs are different, it is a challenge to simultaneously extract multiple targets efficiently. Some PPCPs are with high logKow and low water solubility (for example, triclosan and triclocarban), which have a tendency to partition onto the sludge [21], others are with low logKow and high water solubility, which have a tendency to partition in the water (for example, sulfamethoxazole) or to partition onto the sludge due to the ionic interactions (for example, oxytetracycline and ofloxacin) [22]. The diverse physicochemical properties make it difficult to efficiently extract all the targets. Therefore, development of an easily performed analytical method for simultaneous extraction and determination of multiple PPCPs at trace levels in the sludge is critically required.

In this study, we systematically investigated an MSPD-LC-MS/MS method for determination of 45 commonly used PPCPs in sludge. First, the LC-MS/MS parameters were optimized in multiple reaction monitoring (MRM) mode to get high sensitivity and selectivity. Secondly, MSPD operation conditions, including the sorbent materials, the ratio of sample to sorbent, the eluent composition, and the elution volumes, were sequentially investigated in relation to the extraction efficiencies of PPCPs. Thirdly, the method performance, including the target recoveries, method quantification limits (MQLs), and matrix effects, were evaluated. Finally, the proposed method was demonstrated by determining PPCPs in the sludge samples monthly taken from a domestic WWTP.

Experimental

Reagents and materials

Forty-five PPCPs were investigated based on their high usage and high detection frequencies [23, 24]. PPCPs were supplied by Sigma-Aldrich (USA), Fluka (USA), Dr. Ehrenstorfer GmbH (Germany), AccuStandard (USA), and Cambridge Isotope Laboratories (USA). Detailed information of the target PPCPs, including their commercial usage, is listed in Table S1 in the Electronic Supplementary Material (ESM). Standard mixtures of the 45 PPCPs each with the concentrations of 0.0500, 0.100, 0.200, 0.500, 2.00, 5.00, 20.0, 50.0, 200, and 500 μg/L were prepared in methanol.

The SPE empty reservoir (polypropylene, 3 mL), frits (20 μm polyethylene), and adsorbents (Supelclean ENVI-18 and LC-Florisil) were purchased from Supelco (USA). Methanol, acetonitrile, and acetone were HPLC grade and provided by Tedia (USA). The reagent water was obtained from a Milli-Q water purification system (Millipore, USA).

Sample collection

Dewatered sludge samples were collected monthly from December 2011 to December 2012 in a domestic WWTP in Fujian, China. This WWTP is equipped with a primary treatment process, an Orbal oxidation ditch process, and a UV disinfection process. The daily treatment capacity was about 45000 t in autumn and winter and 50000 t in spring and summer. Prior to MSPD treatment, the sewage sludge samples were subsequently freeze-dried, finely ground in a mortar, and kept at −20 °C in the dark. For the MSPD method development, dewatered sludge samples were collected in August 2013 in the same WWTP. The carbon content of the sludge

was 240 g/kg, which was determined by the total organic carbon analyzer (Shimadzu, Japan).

Sample preparation

Freeze-dried sludge samples were used for the MSPD method development. To evaluate the process extraction efficiencies, sludge samples were spiked with the PPCP standard at the concentration of 500 μg/kg, which were in the range of the target PPCP concentrations in the investigated sludge. The spiked mixture was aged over night, mechanically stirred, and air dried at room temperature before extraction. Sludge (0.1 g) was put into an agate mortar and mixed with 0.4 g C18 sorbent. The mixtures were blended by an agate pestle to get homogenized and packed into an empty polypropylene cartridge containing a polyethylene frit at the bottom. Another frit was placed on top of the mixture and compressed by a syringe plunger lightly. The packed cartridge was eluted by 6 mL methanol and 10 mL acetonitrile/5 % oxalic acid (8/2, *v*/*v*). The extracts were evaporated to dryness by a gentle stream of nitrogen in water bath at 40 °C and then dissolved in 1 mL of acetonitrile/water (1:1).

MSPD optimization

The sorbent material, the ratio of sample to sorbent, the eluent composition, and the elution volumes of the MSPD process were sequentially optimized based on the process extraction efficiencies [25], which were calculated as follows:

$$\mathrm{PE_p}(\%) = (C_1 - C_0) \times 100/C_\mathrm{s} \quad (1)$$

where $\mathrm{PE_p}$ is the process extraction of certain PPCP, C_1 is the average detected concentration of the spiked sludge (μg/kg), C_0 is the average detected concentration of original sludge without spike (μg/kg), and C_s is the spiked concentration.

Firstly, the sorbent materials were compared between the classic reversed-phase C18-bonded silica and the normal phase Florisil. Sample preparation was conducted based on Pavlovic's study [12] with modifications by mixing 0.100 g sludge with 0.200 g sorbent and eluting with 12 mL methanol, 6 mL methanol/acetone (1/1, *v*/*v*), and 10 mL acetonitrile/5 % oxalic acid (8/2, *v*/*v*). Secondly, the ratios of sample to sorbent at 1:2 and 1:4 were compared. Sludge (0.1 g) was taken considering that the PPCP contents were not too low to detect and the matrix effects were not too high to interfere the MS detection. The analyte elution was achieved by 12 mL methanol, 6 mL methanol/acetone (1/1, *v*/*v*), and 10 mL acetonitrile/5 % oxalic acid (8/2, *v*/*v*). Thirdly, five eluents (E1–E5), with different combination of the organic solvents and the acid modifiers, were evaluated for the selection of elution solvent. E1 was 12 mL methanol followed by 6 mL methanol/acetone (1/1, *v*/*v*), E2 was the mixture of 6 mL acetonitrile and 4 mL of 5 % oxalic acid water solution, E3 was the mixture of 8 mL acetonitrile and 2 mL 5 % oxalic acid, E4 was E1 followed by E2, and E5 was E1 followed by E3. Finally, the volume of elution solvent was minimized. The experiments of the MSPD optimization were performed in triplicate.

Determination conditions

Liquid chromatography triple quadropole mass spectrometry (LC-QqQ-MS) was applied to determine the PPCP concentrations. Shimadzu LC system (LC-20A, Shimadzu, Japan) equipped with a Kinetex C18 column (100 mm × 4.6 mm, 2.6 μm, Phenomenex, USA) was used to separate the analytes. A binary gradient with a flow rate of 0.5 mL/min was used. The electrospray ionization (ESI) in both positive and negative mode was applied for PPCP analysis (Table S1 in ESM). For the negative ESI, the mobile phase A contained 5 mmol/L ammonium acetate in water, while mobile phase B was methanol. For the positive ESI, the mobile phase A contained 0.1 % formic acid in water, while mobile phase B was methanol. The gradient elution program is shown in ESM Table S2. The sample injection volume was 10.0 μL. The mass spectrometric measurements were carried out on an ABI triple QqQ MS using MRM mode. Two transitions were monitored, and the most abundant transition was used for the quantification. The declustering potentials (DP), entrance potentials (EP), collision energies (CE), collision cell entrance (CEP), and collision cell exit potentials (CXP) were optimized by syringe pump infusion of individual standard solutions to obtain maximum sensitivity. The optimized MS parameters are provided in ESM Table S1. Figure S1 in the ESM shows the chromatograms of a 100-μg/L standard solution in methanol. Instrument quantification limits (IQLs) were evaluated based on the signal to noise ratio (*S*/*N*) of 10 using software Analyst 1.5 of AB Sciex. As shown in Table 1, IQLs were in the range of 3.00–465 pg for the 45 target PPCPs.

Matrix effect

In MS detection, matrix might enhance or suppress the MS response of PPCPs. Matrix effects were performed according to Hertzog's study, which was by comparing the slopes of the matrix-matched calibration solution in sludge extract and calibration solution prepared in solvent [26]. The matrix effects were evaluated using the following equation:

$$\mathrm{ME}(\%) = (S_\mathrm{m}/S_\mathrm{s} - 1) \times 100 \quad (2)$$

where S_m is the slope of the matrix, and S_s is the slope of the solvent. The positive values of ME indicate the enhancement of MS response by the matrix, and negative values indicate the suppression.

Table 1 Linearity range, correlation coefficients, recoveries, precision (RSD), matrix effects (%), IQLs, and MQLs of the MSPD-HPLC-MS/MS method

PPCPs	Commercial use	Linearity range (μg/L) In methanol	R^2	Linearity range (μg/L) In sludge extraction matrix	R^2	Recovery (%) ± RSD ($n = 4$)	Matrix effect (%)	IQLs (pg)	IQLMs (pg)	MQLs (μg/kg)
Sulfamerazine	Antibiotics	2–500	0.999	2–500	0.999	50.3 ± 4.5	18	60.1	88.5	1.76
Sulfameter	Antibiotics	2–500	0.999	2–500	0.998	50.7 ± 5.3	44	26.1	94.7	1.87
Sulfamethoxazole	Antibiotics	2–500	0.998	2–500	0.999	59.5 ± 5.5	32	11.9	36.1	0.607
Sulfadimethoxine	Antibiotics	0.5–500	1.00	0.5–500	0.999	52.8 ± 6.2	19	21.3	50.9	0.9637
Ofloxacin	Antibiotics	20–500	0.999	20–500	0.992	72.3 ± 6.7	−13	70.7	43.0	0.595
Sarafloxacin	Antibiotics	2–200	0.981	20–500	0.990	71.0 ± 14.9	–	18.8	43.3	0.610
Oxytetracycline	Antibiotics	5–500	0.998	20–500	0.998	86.6 ± 2.3	78	50.9	67.1	0.775
Tetracycline	Antibiotics	20–500	1.00	20–500	0.999	102.3 ± 14.4	90	41.5	435	4.25
Ketoprofen	NSAIDs	2–500	0.993	2–500	0.992	58.6 ± 7.7	−39	207	206	3.51
Naproxen	NSAIDs	5–500	0.998	5–500	0.999	60.8 ± 8.2	−41	110	44.4	0.731
Fenoprofen	NSAIDs	2–500	0.999	2–500	0.999	65.1 ± 3.2	−37	36.2	167	2.56
Diclofenac	NSAIDs	0.2–500	0.999	0.2–500	0.999	56.4 ± 8.3[a]	−2.0	123	39.4	0.699
Ibuprofen	NSAIDs	2–500	0.997	2–500	0.996	58.6 ± 6.2	−38	100	109	1.85
Codeine	NSAIDs	2–200	0.990	2–200	0.990	80.6 ± 7.4	–	18.6	20.0	0.248
Acetaminophen	NSAIDs	5–500	0.999	5–500	0.991	50.1 ± 4.3	−3.3	44.3	48.5	0.967
Antipyrine	NSAIDs	2–500	0.992	2–500	0.992	86.7 ± 5.0	−37	59.3	385	4.44
Propyphenazone	NSAIDs	0.1–500	0.995	0.1–500	0.994	54.8 ± 5.9	−26	20.3	15.8	0.288
Ethenzamide	NSAIDs	0.5–500	0.992	2–500	0.991	63.0 ± 8.5	−24	18.4	15.1	0.240
Indomethacin	NSAIDs	0.1–500	0.998	0.1–500	0.999	61.4 ± 6.6	−26	18.8	70.3	1.15
Crotamiton	NSAIDs	0.05–500	0.991	0.1–500	0.994	67.9 ± 5.2	−29	20.2	12.9	0.191
Clofibric acid	Lipid regulator	0.5–200	0.996	0.5–500	0.993	66.6 ± 7.1	1.4	26.8	59.5	0.894
Gemfibrozil	Lipid regulator	0.2–500	0.998	0.2–500	0.994	52.4 ± 4.5	34	53.9	25.2	0.481
Pirenzepine	Ulcer drug	0.1–500	0.996	0.2–500	0.995	71.9 ± 8.1	−38	13.4	26.2	0.365
Miconazole	Antifungal	0.1–500	0.991	0.1–500	0.990	53.5 ± 11.2[a]	−24	8.40	18.4	0.344
Carbamazepine	Anticonvulsant	0.1–500	0.996	0.2–200	0.995	53.8 ± 6.0	−17	4.30	16.6	0.308
Sildenafil	Sexual function agent	0.5–500	0.998	0.5–500	0.996	59.2 ± 8.6	3.9	39.3	52.6	0.889
Fluoxetine	Antidepressant	2–500	0.977	2–500	0.997	61.4 ± 16.0	77	465	164	2.67
Loratadine	Antiallergic agent	0.5–200	0.991	2–200	0.990	84.3 ± 2.1	–	6.90	9.90	0.117
Diazepam	Anxiolytic	0.5–500	0.995	0.5–500	0.994	65.8 ± 5.9	−32	11.8	19.2	0.291
Clenbuterol	ß-sympathomimetic	0.5–200	0.996	0.5–500	0.993	62.5 ± 8.7	2.9	5.3	53.8	0.861
Sotalol	ß-blockers	2–500	0.998	2–500	0.999	63.8 ± 2.4	−48	38.8	72.3	1.13
Metoprolol	ß-blockers	5–200	0.997	5–200	0.991	62.1 ± 5.9	−45	3.00	345	5.55
Atenolol	ß-blockers	0.5–50	0.996	2–200	0.991	90.3 ± 2.8	−47	82.0	164	1.82
Propranolol	ß-blockers	0.1–200	0.991	0.1–200	0.994	50.1 ± 3.9	−43	7.70	27.3	0.546
Methyl paraben	Preservative	2–500	0.999	2–500	0.999	50.1 ± 6.1	−33	84.2	240	4. 80
Propyl paraben	Preservative	2–500	0.999	2–500	0.999	64.1 ± 6.7	−30	21.9	67.1	1.05
Benzyl paraben	Preservative	0.2–500	0.998	0.2–500	0.994	55.2 ± 8.4	−49	10.4	13.2	0.239
Camphor	UV filters	2–500	0.999	2–500	0.999	80.6 ± 5.8	−14	28.9	69.0	0.856
Benzophenone-3	UV filters	0.5–500	0.998	2–500	0.998	54.1 ± 5.8	36	31.8	67.1	1.24
Octocrylene	UV filters	2–500	0.997	2–500	0.994	56.0 ± 15.1	−23	113	89.6	1.60
Triclocarban	Antimicrobials	0.2–500	0.996	0.2–500	0.998	65.2 ± 13.7	−8.2	21.4	49.6	0.760
Triclosan	Antimicrobials	5–500	0.992	5–500	0.991	107.0 ± 11.3	−7.1	161.8	64.8	0.605
Bisphenol A	Plasticizer	2–500	0.999	2–500	0.997	53.1 ± 13.5[a]	−42	70.2	238	4.48
Acetophenone	Fragrance	5–500	0.996	5–500	0.999	57.1 ± 12.4	−25	63.3	123	2.15
Thiabendazole	Fungicide	0.1–500	0.993	0.1–500	0.990	57.7 ± 4.6	−56	5.30	16.5	0.285

– data were not available due to the signals shifted to the next window

[a] $n = 7$

Quality assurance and quality control (QA/QC)

QA/QC was conducted to ensure the identification and quantification of the PPCPs. Identification of PPCPs was performed by LC-MS/MS with MRM, using the two highest characteristic precursor ion/product ion transition pairs (ESM Table S1). The ratios of product transitions were calculated to ensure correct identification with the acceptance criteria within 20 %. An instrumental blank, procedural blank, sample duplicate, and matrix spike were applied for each batch for the analysis of sludge samples [13].

Statistical analysis

Pair *t* test was used to determine any significant differences of the process extraction efficiencies during the optimization of the sorbent materials and the ratio of sample to sorbent. One-way ANOVA was applied to compare the process extraction

efficiencies among five elution solvents. Friedman test was applied to evaluate the seasonal variation of PPCP concentrations. All the statistical analysis was conducted by PAST v 2.17.

Results and discussion

MSPD optimization

Sorbent materials

C18 sorbent provided significantly better extraction efficiencies compared to Florisil (pair *t* test, $p < 0.001$). Specifically, 37 out of 45 PPCPs showed higher recoveries using C18 sorbent compared to Florisil (Fig. 1). Of particular note, the recoveries of ofloxacin and sarafloxacin using C18 as the sorbent were 87.0 and 69.7 % while reduced to 11.8 and 10.7 % using Florisil, respectively. The high recoveries of these quinolone antibiotics using C18 were probably because C18 is believed to improve the disruption and dispersion of the sludge due to their lipophilic character [18, 27] and released the targets from the matrix. In addition, the sludge and C18 sorbent might form a layered structure, and sample-associated PPCPs might distribute in and on the multi-layered structure based on their polarities [28], which might make PPCPs easily be eluted. Therefore, C18 was chosen as the dispersant sorbent and used for the further optimization.

Ratio of sample to sorbent

As shown in Fig. 2, the process extraction efficiencies of PPCPs were significantly higher using 0.4 g C18 sorbent compared to 0.2 g sorbent (pair *t* test, $p = 0.001$).The differences of

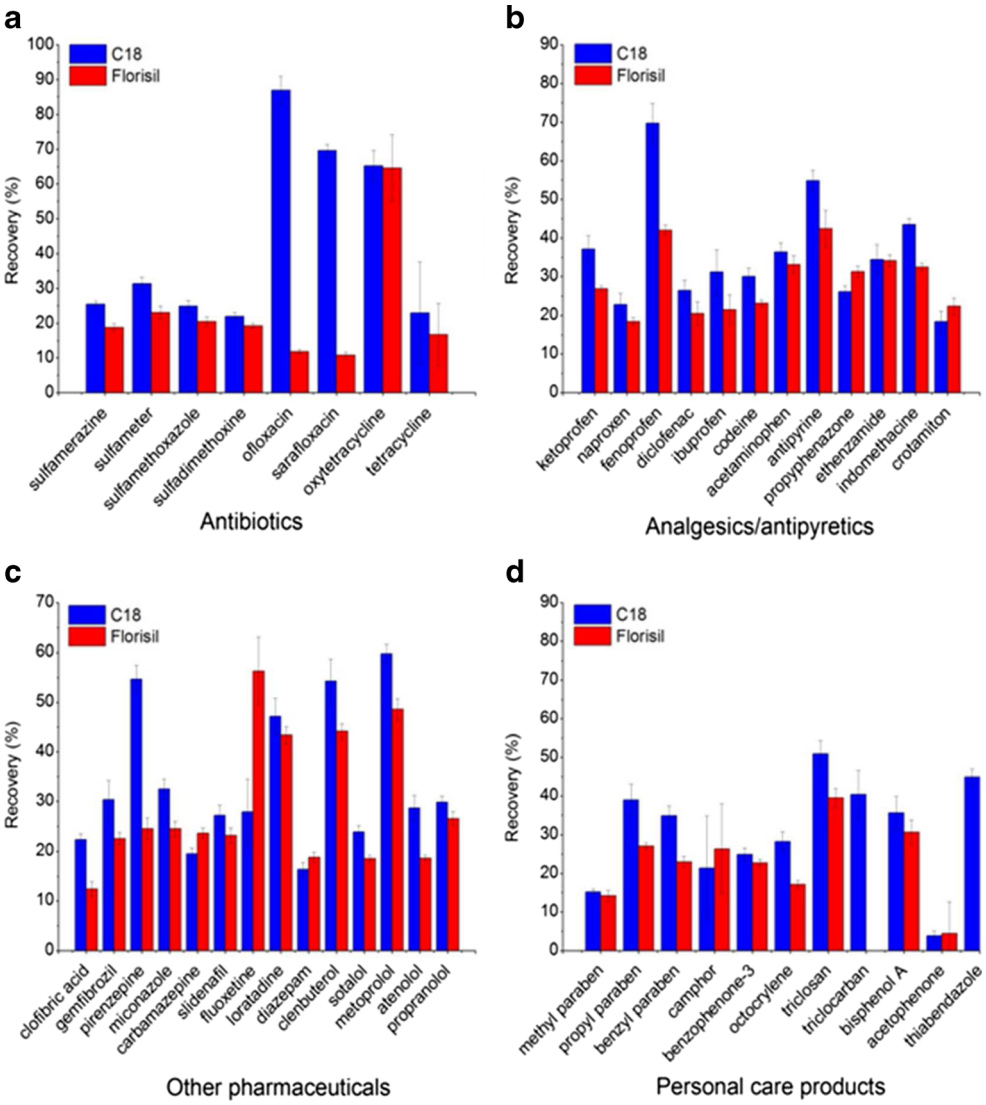

Fig. 1 **a**–**d** Process extraction efficiencies of PPCPs using C18 and Florisil as the sorbent materials (*error bars* indicate RSD, $n = 3$). These results were obtained from the analysis of the sludge spiked with PPCP standards at 500 μg/kg. MSPD parameters were 0.100 g sludge mixed with 0.200 g Florisil or C18 sorbent using the elution solvent of 12 mL methanol, 6 mL methanol/acetone (1/1, *v*/*v*), and 10 mL acetonitrile/5 % oxalic acid (8/2, *v*/*v*)

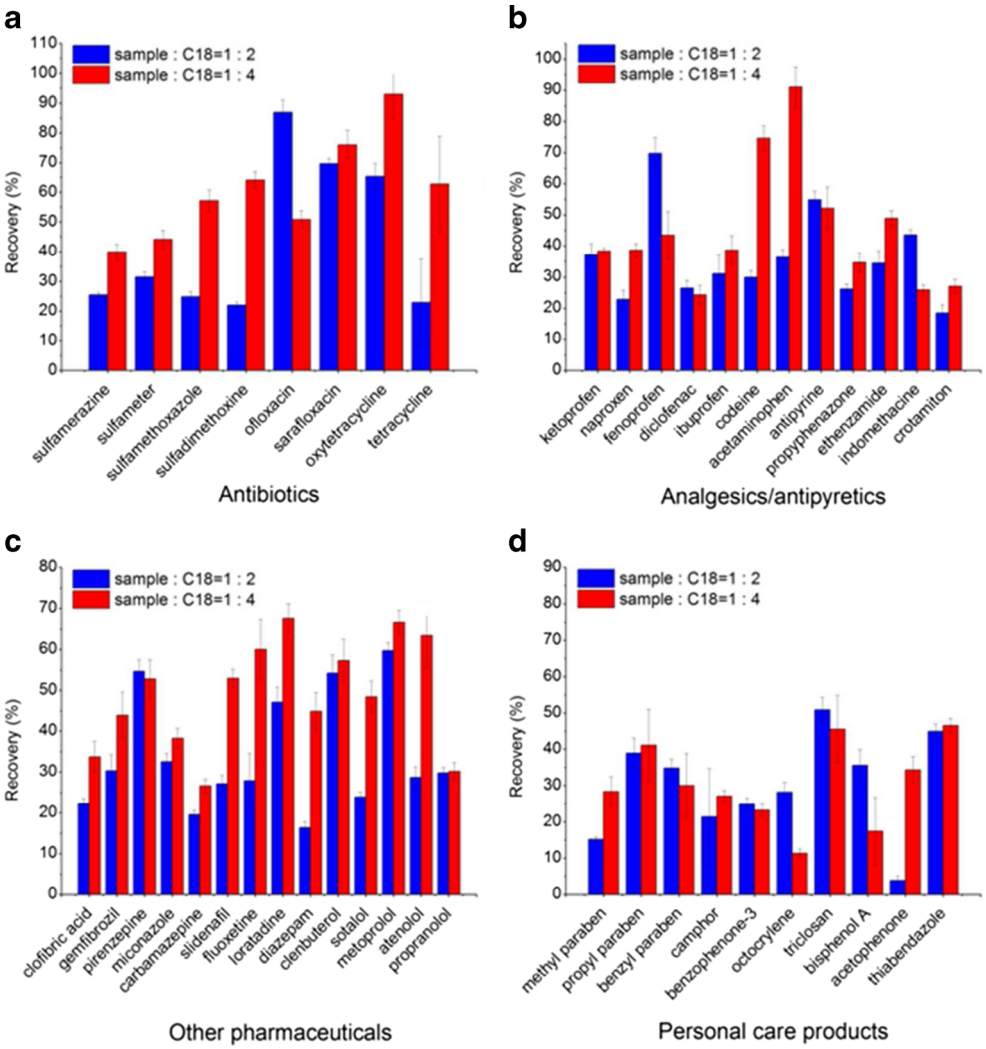

Fig. 2 **a**–**d** Process extraction efficiencies of PPCPs under different ratios of sample to sorbent (*error bars* indicate RSD, $n = 3$). These results were obtained from the analysis of the sludge spiked with PPCP standards at 500 μg/kg. MSPD parameters were 0.100 g sludge mixed with 0.200 or 0.400 g C18 sorbent using the elution solvent of 12 mL methanol, 6 mL methanol/acetone (1/1, *v*/*v*), and 10 mL acetonitrile/5 % oxalic acid (8/2, *v*/*v*)

the process extraction efficiencies might be due to redistribution of the target PPCPs in the sludge and sorbent. For PPCPs with low logKow (ESM Table S1) and high water solubility, including sulfamethoxazole, sulfadimethoxine, tetracycline, codeine, acetaminophen, acetophenone, and sotalol, the recoveries were more than two times when 0.4 g C18 was applied (Fig. 2). The adsorption onto the C18 might help the subsequent elution by the organic solvent. In contrast, for PPCPs with high logKow, including octocrylene, indomethacine, and bisphenol A, the extraction efficiencies decreased when the sorbent amount increased, which might be due to the tight bond between the PPCPs and sorbent and consequently incomplete elution. Therefore, the ratio of sample to sorbent at 1:4 was used for the following optimization.

Elution solvent

The elution solvent is important as the analytes should be efficiently eluted while the matrix components should be retained in the cartridge [18]. Generally, organic solvents are employed as the eluent. Acids or bases in the organic or inorganic forms were used to modify the elution pH in order to change the ionization form of the analytes and thereby affect the extractability and MS response [29, 30]. As shown in Fig. 3, E1 provided best recoveries for the sulfonamide antibiotics. However, the eluents modified by acid (E2–5) provided better recoveries for quinolones and tetracyclines, which might be due to the higher solubility of quinolones and tetracyclines in the acidic media [31]. For the NSAIDs, the best recoveries were achieved by E1 followed by E5, which was in the range of 52.0–107.8 and 47.6–86.7 %, respectively. For the other pharmaceuticals and PCPs, the recoveries were in the range of 0–116.9, 11.3–80.1, 24.0–130.0, 15.5–87.3, and 50.7–107.0 % by E1–5, respectively. One-way ANOVA was applied to compare the extraction efficiencies, and results showed that E3 and E5 provided significantly higher extraction efficiencies compared to E1, E2, and E4 ($p < 0.01$), while E5 provided marginal better extraction efficiencies compared to E3 for the PCP group ($p = 0.089$). Thus, E5 was selected as the elution solvent.

Volume of elution solvent

Further study was performed to minimize the volume of the elution solvent. E5 was divided into five portions. PPCPs were sequentially eluted, collected, concentrated, and detected separately by the five eluent portions. The ratios of the MS response of each portion to the sum are present in Fig. 4. In the first portion (6 mL methanol), 21 PPCPs were completely eluted, and 14 PPCPs were eluted more than 90 %. The second (6 mL methanol) and third portion (6 mL methanol/acetone (1/1, *v*/*v*)) contributed to less than 10 % of the total elution, which might be covered by the following elution. Thus, the second and third eluent portions were not included in the following studies. The fourth portion (5 mL acetonitrile/5 % of oxalic acid (8/2, *v*/*v*)) played an important role in the elution of quinolone and tetracycline antibiotics. In addition, the fifth portion (5 mL acetonitrile/5 % of oxalic acid (8/2, *v*/*v*)) also contributed for 5.6–10 % of the elution of quinolones and tetracyclines. Therefore, 6 mL methanol and 10 mL acetonitrile/5 % oxalic acid (8/2, *v*/*v*) were applied as the elution solvent.

Performance of the method

Analytical performance characteristics of the proposed method are summarized in Table 1. Standard calibration of the 45 PPCPs each with the concentrations in the range of 0.05–500 μg/L was prepared to test the instrumental linearity. Calibration curves for quantification were obtained by plotting mass response versus the concentration of the corresponding target species. As shown in Table 1, most PPCPs have a good linearity range of 2–500 μg/L, with the correlation coefficients (R^2) more than 0.990. Procedural blanks demonstrated the absence of the contamination for any of the target PPCPs. Recoveries were calculated as the difference between the concentrations measured for the spiked ($n = 4$) and non-spiked sludge ($n = 4$) divided by the added level of each analyte. Obtained recoveries ranged from 50.3 to 107 %, with the

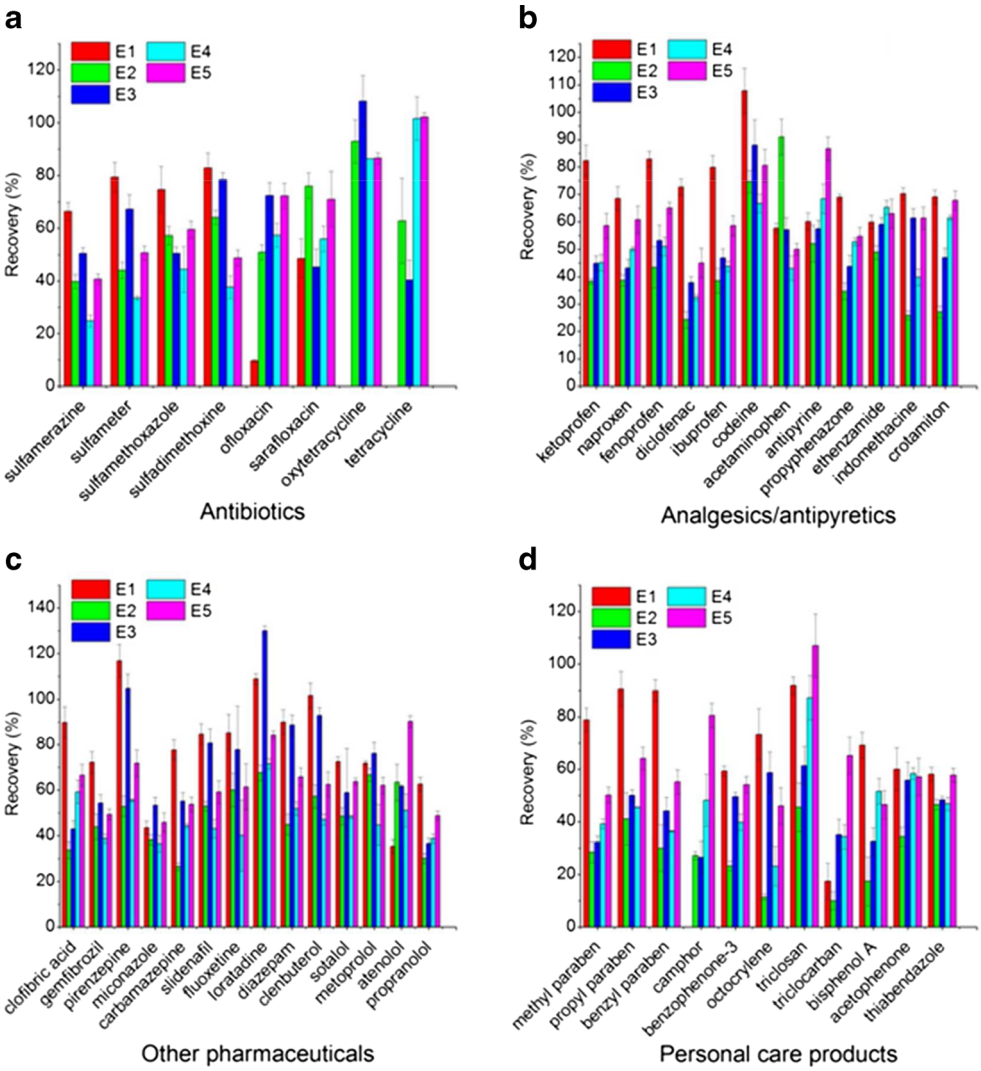

Fig. 3 **a**–**d** Process extraction efficiencies of PPCPs using different elution solvents (*error bars* indicate RSD, $n = 3$). These results were obtained from the analysis of the sludge spiked with PPCP standards at 500 μg/kg. MSPD parameters were 0.100 g sludge mixed with 0.400 g C18 sorbent using E1–E5 as the elution solvent. E1 was 12 mL methanol followed by 6 mL methanol/acetone (1/1, *v*/*v*), E2 was the mixture of 6 mL acetonitrile and 4 mL 5 % of oxalic acid water solution, E3 was the mixture of 8 mL acetonitrile and 2 mL 5 % oxalic acid, E4 was E1 followed by E2, and E5 was E1 followed by E3

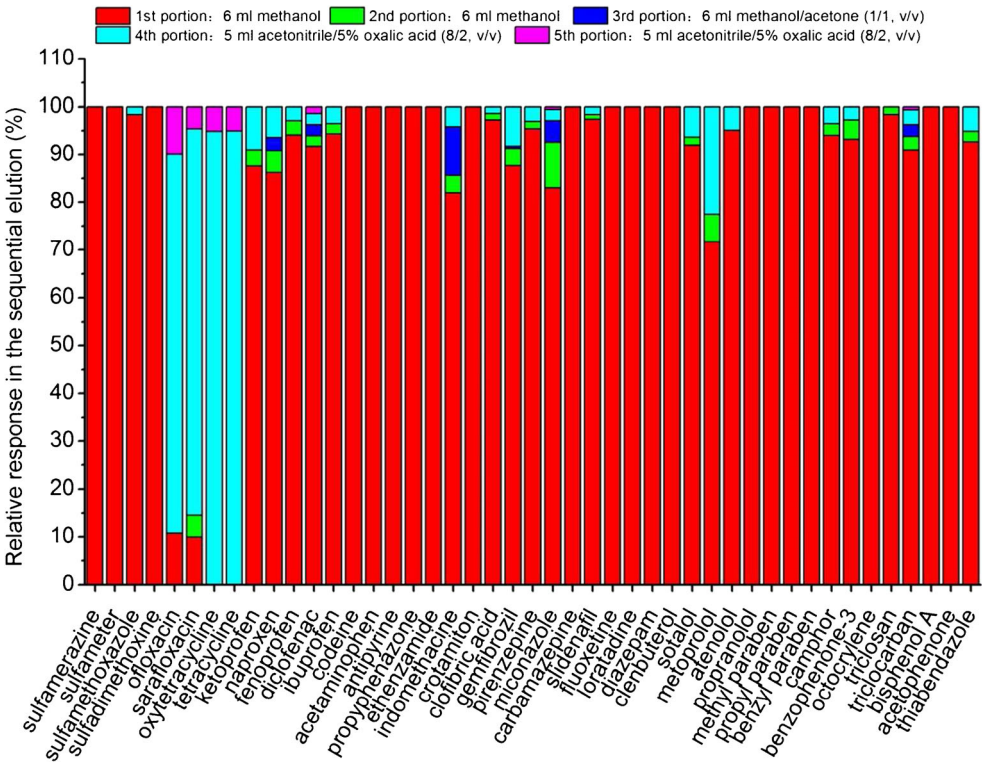

Fig. 4 Sequential elution of PPCPs by five eluent portions. These results were obtained from the analysis of the sludge spiked with PPCP standards at 500 μg/kg. MSPD parameters were 0.100 g sludge mixed with 0.400 g C18 sorbent using the elution solvent of 12 mL methanol, 6 mL methanol/acetone (1/1, *v*/*v*), and 10 mL acetonitrile /5 % oxalic acid (8/2, *v*/*v*). Eluent portions: first portion, 6 mL methanol; second portion, 6 mL methanol; third portion, 6 mL methanol/acetone (1/1, *v*/*v*); fourth and fifth portion, 5 mL acetonitrile/5 % of oxalic acid (8/2, *v*/*v*)

relative standard deviation (RSD) less than 15 %. However, due to the relatively low recoveries of a few PPCPs and the substantial matrix effects, the isotope labeled internal surrogates were suggested to employ in the future study. The matrix effect (Table 1) in this method ranged from −56 %, the highest suppression (thiabendazole), to 90 %, the highest enhancement (tetracycline). The high matrix effect for the sludge samples was also observed in the previous studies [10, 17]. Considering the high matrix effect, the standard addition method was recommended for the quantification of the PPCP analysis in the sludge to minimize the analytical error due to the matrix effect. The standard addition calibration of the 45 PPCPs each with the concentrations in the range of 0.05–500 μg/L was applied to test the linearity. As shown in Table 1, most PPCPs have a good linearity range of 2–500 μg/L, with R^2 more than 0.990. The instrument quantification limits of the matrix (sludge extract) spiked standard (IQLMs) were evaluated based on the *S*/*N* ratio of 10. As shown in Table 1, IQLMs were in the range of 9.90–345 pg for the 45 target PPCPs.

MQLs were calculated using the following equation:

$$\mathrm{MQLs} = \frac{\mathrm{IQLMs}}{\mathrm{R} \times m \times 10} \quad (3)$$

where *R* is the recovery for each PPCP, and *m* is the sludge amount. The MQLs for the 45 PPCPs were in the range of 0.117–5.55 μg/kg.

Table 2 describes the comparison of different extraction methods with our method in terms of extraction time, extraction and cleanup procedure, recovery percent, matrix effect, method quantification limits, and number of analytes. Compared to the commonly used method [13, 14], the proposed method could achieve similar sensitivity and recovery for the target PPCPs. However, the extraction and cleanup procedure of MSPD were conducted in only one step. It is simpler and faster to operate compared to the USE [13, 14, 32], ASE [33], or MAE [34, 35] processes for the PPCP extraction, which were followed by the SPE for the cleanup. In addition, a large number of PPCPs were analyzed simultaneously, which would provide more information and better understanding on the PPCP contamination in the sludge. Thus, MSPD-LC-MS/MS is an attractive and alternative method to determine a variety of PPCPs in sludge samples.

Application to sludge samples

The proposed method was applied to detect PPCPs in sewage sludge collected from a local WWTP over 1 year. Solvent blank and method blank were conducted in each batch, and results showed that no target was detected in the blank. Standard spike was conducted in each batch, and the recoveries of most PPCPs were in

Table 2 Comparison of different extraction methods for PPCPs in solid samples

Method	Extraction type	Extraction time	Cleanup procedure	Recoveries (%)	Matrix effect (%)	MQL (ng/g)	Number of analytes
USEPA [13]	Ultrasonication and centrifugation followed by SPE	More than 4 h	SPE	50	Not mentioned	Not mentioned	50
Yu et al. [14]	Ultrasonication and centrifugation followed by SPE	More than 4 h	SPE	65–125	−21 to 76	0.1–3.0	20
Ding et al. [33]	Accelerated solvent extraction followed by SPE	More than 12 h	SPE	49–95	Not calculated	1.9–488	15
Montesdeoca-Esponda et al. [34]	Microwave-assisted micellar extraction	30 min	No cleaning procedure	73	Not calculated	0.49–1.85	5
Our method	MSPD	30 min	Extraction and cleanup in single step	50–107	−56 to 90	0.117–5.55	45

the range of 50–120 % (less than three targets were outside the range for each batch). Among the 45 target PPCPs, 13 PPCPs were detected (ESM Fig. S2a, b). The highest concentrations were observed for ofloxacin, with the average concentration of 2270 μg/kg, followed by triclocarban, with the average concentration of 1440 μg/kg. In addition, tetracycline, oxytetracycline, triclosan, fenoprofen, and miconazole also showed higher levels, with the average concentrations more than 100 μg/kg. The antibiotics (including ofloxacin, oxytetracycline, and tetracycline) and NSAIDs (including diclofenac) were lower compared to the sewage sludge collected in 45 WWTPs throughout China [36]. However, higher concentrations of triclocarban were observed in this study [14, 37].

Seasonal variations of PPCPs in the sludge were studied. Generally, lower PPCP concentrations (Friedman's test, $p = 0.031$) were observed in summer (June, July, and August) compared to winter (December, January, and February). As shown in Fig. 5, the NSAID and antibiotic levels were higher in cold seasons compared to the hot seasons. Previous studies showed that, due to the larger consumption [38], NSAID and antibiotic concentrations in the wastewater were higher in the cold seasons [23], which might lead to the higher amount of PPCPs absorbed onto the sludge. Moreover, the higher temperature in the hot seasons probably decreased the PPCP levels in sludge by increasing the microbial activity via the enzymatically catalyzed reaction and PPCP diffusion to the cells [39]. On the contrary, octocrylene was only detected during August to October, while higher concentrations of camphor were observed in the hot seasons, which might be due to the high consumption of the UV filters in the hot seasons. In addition, the concentrations of the antimicrobial agents and preservatives fluctuated during the 12-month monitoring. The temporal variations of the PPCP levels indicated the sampling over an extended period was necessary for understanding the PPCP contamination in the sludge [40].

Correlations were found among PPCPs, especially for the PPCPs with similar usages. Spearman's correlation test was carried out with the detected concentrations by the 12-month sludge. Results showed that positive correlation was observed within the antibiotics, for example, oxytetracycline and tetracycline ($p = 0.050$), and ofloxacin and oxytetracycline ($p = 0.045$). In addition, the two antimicrobial agents and the two preservatives also showed marginal positive correlations, with p value of 0.094 and 0.075. This is probably due to their coexistence in the medicine or goods for their similar usages [24] and close environmental behavior due to similar physicochemical properties.

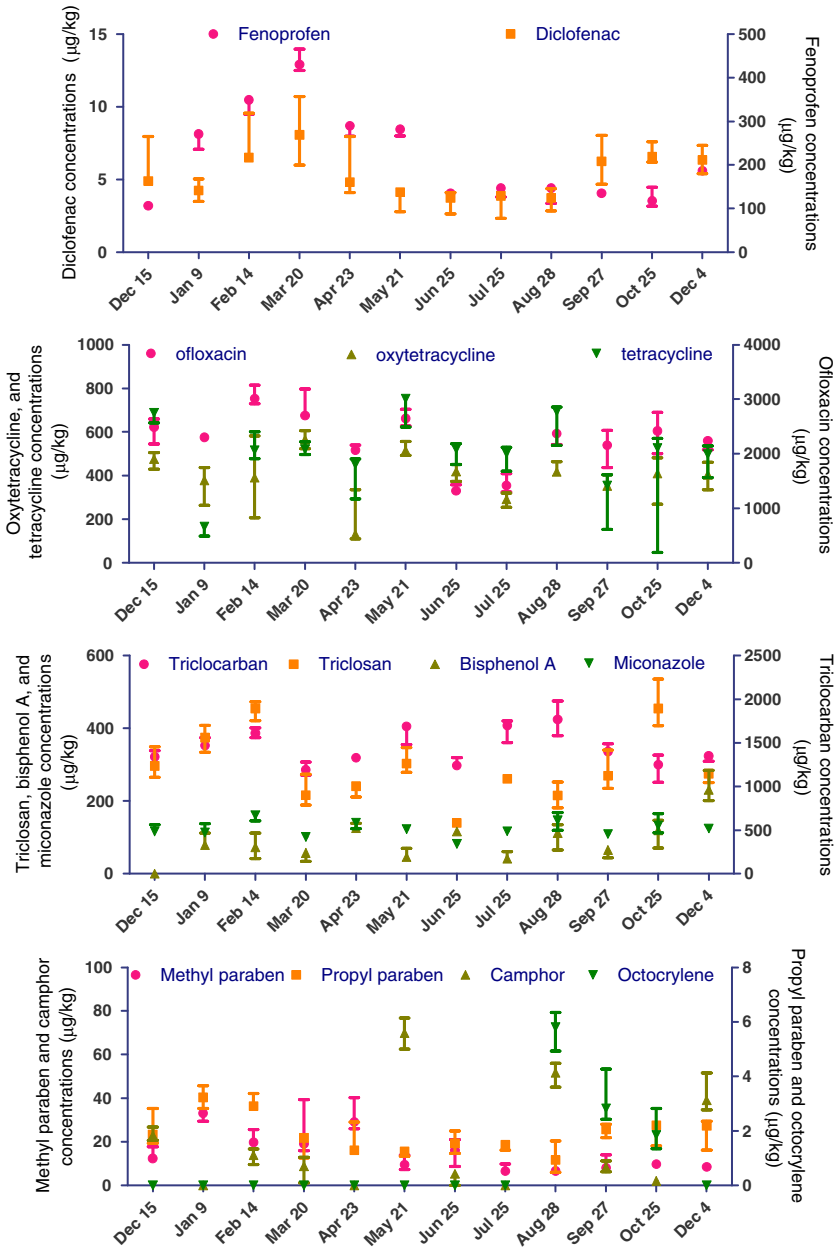

Fig. 5 Seasonal variations of PPCP concentrations in the sludge (*symbols* represent the mean concentration, and *error bars* represent value range)

Conclusions

An MSPD-HPLC-MS/MS method was developed for the simultaneous determination of 45 PPCPs with different physicochemical properties in the sewage sludge. The application of MSPD as the sample preparation process provides quantitative recoveries with moderate consumption of organic solvents. The MSPD sample preparation process was easy to operate in terms of the extraction and cleanup procedure in one step. The proposed method was sensitive with the MQL values in the range of 0.117–5.55 μg/kg. Sludge samples, collected from a domestic WWTP over 12 months, were analyzed by the developed method. Among the 45 PPCPs, 13 targets were detected, and the highest concentrations observed were for ofloxacin and triclocarban with the average concentrations more than 1000 μg/kg. Temporal variations were observed indicating that the sampling over an extended period was necessary for better understanding the PPCP contamination in the sludge. In conclusion, MSPD is an attractive and alternative to other preparation techniques to extract a variety of PPCPs in sludge samples for LC-MS/MS analysis.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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