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Determination of pharmaceuticals in solid samples in municipal wastewater treatment plants by online SPE LC–MS/MS using QuEChERS extraction

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Abstract In this study, a pretreatment method based on the QuEChERS method has been applied for simultaneously extracting 27 residual pharmaceuticals from wastewater solids. The extracted compounds have been analyzed using online solid-phase extraction (SPE) coupled to liquid chromatography with tandem mass spectrometry (LC–MS/MS). A recovery test was conducted according to the absorbent type, and buffers were added in the sample extraction step. The highest recovery efficiency could be observed when Na_2SO_4 was used as an absorbent and Na_2EDTA was injected during the extraction process; the recovery efficiencies of the proposed method for the target compounds ranged from 61.3 to 137.2%, and the repeatability was 6.8%. These recovery and repeatability data showed that the proposed method could reliably analyze the 27 target residual pharmaceuticals. The concentrations of the target compounds were all below the limits of quantification: 830 ng g^{-1} for the target compounds in suspended solids, 2353 ng g^{-1} in activated sludge, and 1929 ng g^{-1} in waste sludge. The analytical method established in this study can be applied to quantify residual pharmaceuticals in solid samples and to investigate their behaviors in a municipal wastewater treatment plant.

Keywords Method validation · QuEChERS extraction · Online SPE · Pharmaceuticals · Municipal wastewater

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Introduction

With the improvement in living standards, an increasing number of newly developed chemicals, and with the advancement of medical technology, emerging micropollutants, such as pharmaceuticals, personal care products, and endocrine disruptors, have been continuously detected in aquatic environments (Carballa et al., 2004; Daughton & Ternes, 1999; De Oliveira et al., 2020; Sui et al., 2015). Pharmaceuticals are used to treat human and animal diseases, to promote the growth

of agricultural and livestock industries, and to improve immunity; however, they are discharged from the body without being completely metabolized (Dorne et al., 2007; Han & Lee, 2017). Pharmaceuticals detected in aquatic environments originate from the effluent of a municipal wastewater treatment plant (WWTP) (Joss et al., 2006; Luo et al., 2014; Vieno et al., 2007). The presence of these compounds in aquatic environments, even in trace amounts (in ng L^{-1} to μg L^{-1}), may harm aquatic ecosystems because of their bioaccumulation and toxic properties (Afonso-Olivares et al., 2017; Behera et al., 2011; Gabet-Giraud et al., 2010; Park et al., 2018). In South Korea, the occurrence and behavior of pharmaceuticals in the environment have been investigated for 5 years since 2008. Nationwide efforts have revealed that the effluents from livestock manure treatment facilities and municipal WWTPs would account for the largest proportion among the emission sources of the environmental pharmaceuticals (Kim et al., 2020; NIER, 2012).

Pharmaceuticals are typically analyzed using liquid chromatography with tandem mass spectrometry (LC–MS/MS) after manual solid-phase extraction (SPE) with pre-selected cartridges according to the characteristics of the target compounds. However, this process is time-consuming and requires complex pretreatment processes such as extraction, purification, and concentration. The matrix effect which may occur when samples are loaded also needs attention (Hwang et al., 2013; Trenholm et al., 2009). On the contrary, online SPE LC–MS/MS can shorten the analytical time by combining and automating the sample pretreatment processes while more reliably producing reproducible analytical results (López-Serna et al., 2010). It can also reduce the overall analytical cost because a small number of samples are required for the analysis, and online SPE cartridges can be cleaned and reused (Goh et al., 2016; Trenholm et al., 2009). Due to these benefits, the studies on the analysis of pharmaceuticals in surface water, groundwater, drinking water, and influent and effluent of municipal WWTPs have been actively conducted using online SPE (Khan et al., 2012; López-Serna et al., 2010; Panditi et al., 2013; Trenholm et al., 2009). However, it is difficult to quantitatively evaluate the compounds adsorbed on the surface of solids from municipal WWTPs, such as suspended solids and sludge, because many analytical methods have been developed mainly to investigate detectable concentrations in the liquid samples.

Among the pretreatment methods for solid samples, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is used to analyze multi-component residual pesticides and antibiotics in fruits and vegetables. It was developed by Anastassiades et al. (2003) and certified by the Association of Analytical Communities (AOAC) and European Committee for Standardization (CEN) (AOAC, 2011; CEN, 2008). Its application has been expanded to the analysis of a variety of micropollutants adsorbed on soil, manure, and sewage sludge. The target micropollutants include antibiotics, pesticides, non-steroidal anti-inflammatory drugs (NSAIDs), and metabolites (Guo et al., 2016; Park et al., 2020; Ponce-Robles et al., 2017; Rossini et al., 2016). The extraction step of the QuEChERS method is based on partitioning through salting-out extraction. Acetonitrile is used as the extraction solvent, and the interfering compounds included in the extract are removed in the purification step after extraction. In previous studies, the QuEChERS method was used to extract compounds absorbed on solid samples. However, there is limited research analyzing residual pharmaceuticals in sewage samples, such as suspended solids in the influent and sludge of municipal WWTPs. There are only a few previous cases where the combination of the QuEChERS method and online SPE LC–MS/MS has been used for analysis. The QuEChERS method can improve the extraction efficiency through partial modifications in the extraction step, such as the absorbent type, pH adjustment, and inhibition of the interference of polar components. However, it is difficult to apply the same method when the target compounds and media are different. Therefore, it is necessary to establish an analytical approach so that target compounds with different physicochemical properties (e.g., chemical structure, molecular weight, polarity, and solubility) can be simultaneously analyzed.

The objectives of this study are (1) to evaluate the modified QuEChERS-based pretreatment method coupled to online SPE LC–MS/MS in the analysis of pharmaceuticals in wastewater solids and (2) to simultaneously analyze 27 residual pharmaceuticals (e.g., antibiotics, NSAIDs, stimulants, and hormones) in WWTP solids including suspended solids, activated sludge, and waste sludge.

Material and methods

Target compounds

In this study, 27 target residual pharmaceuticals were selected, including 11 antibiotics, 6 NSAIDs, 2 antiarrhythmic agents, and 8 other compounds. Their physicochemical properties are shown in Table 1. High-purity (>98%) reagents were purchased from Sigma-Aldrich and Fluka and used as standard materials. After selecting the solvents (methanol, water, and 0.1 N HCl) for each material according to its dissolution characteristics, a 10-mg L^{-1} standard solution was prepared and stored in a freezer at −20 °C or less and a refrigerator at 4 °C. As for the surrogate, a standard solution was prepared using methanol and three compounds (acetaminophen-D4, ciprofloxacin-D8, and sulfadimethoxine-(phenyl-13C6)) diluted and used on the day of the analysis.

Table 1 Chemical properties of target compounds

No.	Compounds	Molecular formula	Molecular weight (g/mol)	p*K*a	Water solubility (mg mL^{-1})	Log K_{ow}
	Analgesics/non-steroidal anti-inflammatory drugs (NSAIDs)					
1	Acetaminophen	$C_8H_9NO_2$	151.2	9.4	30.4	0.5
2	Acetylsalicylic acid	$C_9H_8O_4$	180.2	3.5	4.6	1.2
3	Diclofenac	$C_{14}H_{11}C_{l2}NO_2$	296.2	4.2	4.5×10^{-3}	3.9
4	Ibuprofen	$C_{13}H_{18}O_2$	206.3	4.9	4.1×10^{-2}	3.6
5	Ketoprofen	$C_{16}H_{14}O_3$	254.3	4.5	0.1	3.1
6	Naproxen	$C_{14}H_{14}O_3$	230.3	4.2	0.1	3.2
	Antibiotics					
7	Cefradine	$C_{16}H_{19}N_3O_4S$	349.4	2.6/7.3	2.8	−0.3
8	Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.4	6.1/8.7	11.5	0.3
9	Clarithromycin	$C_{38}H_{69}NO_{13}$	748	9	3.4×10^{-4}	3.2
10	Erythromycin	$C_{37}H_{67}NO_{13}$	734	8.9	5.2×10^{-4}	3.1
11	Ofloxacin	$C_{18}H_{20}FN_3O_4$	361.4	6.3/7.9	28.3	−0.4
12	Oxolinic acid	$C_{13}H_{11}NO_5$	261.2	6.9	8	0.9
13	Roxithromycin	$C_{41}H_{76}N_2O_{15}$	837.1	9.2	1.9×10^{-5}	2.8
14	Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	310.3	2.1/6.1	0.4	1.6
15	Sulfamethazine	$C_{12}H_{14}N_4O_2S$	278.3	2.1/7.5	11.3	0.6
16	Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.3	1.6/5.7	3.9	0.9
17	Trimethoprim	$C_{14}H_{18}N_4O_3$	290.3	7.1	2.3	0.9
	Antiarrhythmic agents					
18	Atenolol	$C_{14}H_{22}N_2O_3$	266.3	9.6	0.7	0.2
19	Propranolol	$C_{16}H_{21}NO_2$	259.4	9.4	0.2	0.7
	Antihistamines					
20	Cimetidine	$C_{10}H_{16}N_6S$	252.3	6.8	10.5	0.4
21	Diphenhydramine	$C_{17}H_{21}NO$	255.4	9	0.4	3.3
	Hormone					
22	Testosterone	$C_{19}H2_8O_2$	288.4	–	0.1	3.3
	Stimulant					
23	Caffeine	$C_8H_{10}N_4O_2$	194.2	14	2.6	−0.1
	Others					
24	Carbamazepine	$C_{15}H_{12}N_2O$	236.3	13.9	1.8×10^{-2}	2.5
25	Gemfibrozil	$C_{15}H_{22}O_3$	250.3	4.5	5.0×10^{-3}	4.8
26	Iopromide	$C_{18}H_{24}I_3N_3O_8$	791.1	10.6	2.4×10^{-2}	−2.1
27	Sildenafil	$C_{22}H_{30}N_6O_4S$	474.6	5.9	3.5	2.8

Pretreatment and analysis

The suspended solids from the influent were filtered out using a 0.45-μm glass fiber filter paper (47 mm; Hawach Scientific, Xi'an, China), and the filter paper with solids was placed in a 50-mL glass centrifuge tube. Afterward, 1% acetic acid (5 mL; Sigma-Aldrich, Saint Louis, MO, USA), acetonitrile (10 mL; J.T. Baker, Phillipsburg, NJ, USA), Na_2SO_4 (2 g; Wako Pure Chemical, Osaka, Japan), 40 mg mL^{-1} Na_2EDTA (0.2 mL; Sigma-Aldrich, Saint Louis, MO, USA), and 100 μg L^{-1} surrogate (100 μL) were added and mixed using a vortex mixer for 1 min. The homogenized sample was subjected to solid–liquid separation using a centrifugal separator at 4000 rpm for 5 min. A layer of acetonitrile of 1 mL was placed in a glass tube and concentrated using a nitrogen concentrator, and then it was dissolved using a solvent prepared by mixing methanol (J.T. Baker, Phillipsburg, NJ, USA) and 0.1% formic acid (Wako Pure Chemical, Osaka, Japan) at a 1:9 ratio. Subsequently, it was filtered using a 0.45-μm syringe filter made of polyvinylidene fluoride (PVDF) (Advantec, Tokyo, Japan) to prevent the impurities and particulate matter generated during the extraction process from flowing into the LC column, and then used as the final sample. The sludge sample was subjected to solid–liquid separation using a centrifugal separator at 3000 rpm for 5 min. The supernatant liquid was filtered using a 0.45-μm syringe filter made of PVDF and analyzed in the same way as the liquid sample. One gram (wet mass) of the sludge left in the glass centrifuge tube after centrifugation was analyzed in the same sequence as the suspended solid extraction method (Fig. 1).

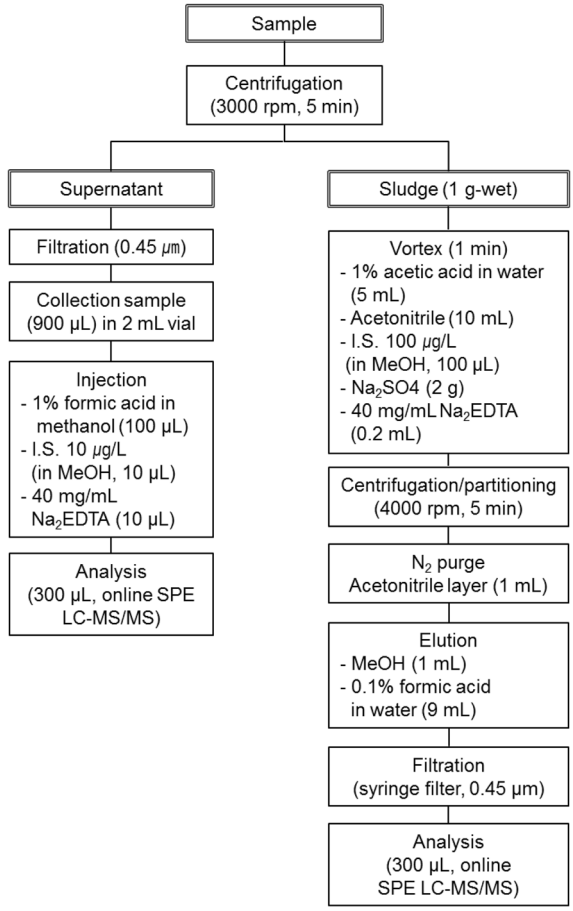

Fig. 1 Schematic of the pharmaceutical analysis of solid samples

Analytical conditions

High-performance liquid chromatographic instrument (HPLC; Nexera X2, Shimadzu, Japan) was used as the analyzer, and the separated peaks were identified and quantified using a mass spectrometer (LCMS-8050, Shimadzu, Japan). As mobile-phase solutions, the flow rate of 0.1% formic acid (A) and acetonitrile (B) was set to 0.2 mL min^{-1}. The composition change of the mobile phase was set to 10% B (0–2.5 min)-100% B (13.0–17.0 min)-10% B (17.1–20.0 min). The sample injection volume was set to 300 μL, and ACE 5 C18-PFP (150 × 2.1 mm) and MAYI-ODS(G) (2.0 × 10 mm) were used for the HPLC column and online SPE column, respectively. As for the MS analysis, the negative electrospray ionization (ESI) mode was used for four compounds (acetylsalicylic acid, diclofenac, ibuprofen, and gemfibrozil), and the other compounds were ionized in the positive ESI mode.

Solid sample recovery test

The QuEChERS method was used to extract the residual pharmaceuticals adsorbed on the solid samples (suspended solids and sludge), and the recovery test was conducted under the experimental conditions presented in Table 2. First, as $MgSO_4$ and Na_2SO_4 are mainly used as absorbents in the extraction step of the QuEChERS method (Anastassiades et al., 2003; Bourdat-Deschamps et al., 2014; Rossini et al., 2016), the recovery was

Table 2 Experimental conditions for the recovery test

No.	Salts for extraction	Buffers	Na_2EDTA injection
E1	$MgSO_4$	Na acetate	During extraction
E2	$MgSO_4$	Na acetate	After extraction
E3	$MgSO_4$		During extraction
E4	$MgSO_4$		After extraction
E5	Na_2SO_4	Na acetate	During extraction
E6	Na_2SO_4	Na acetate	After extraction
E7	Na_2SO_4		During extraction
E8	Na_2SO_4		After extraction

evaluated according to the addition of the two absorbents. The pH acts as an important factor to improve the extraction efficiency in the pretreatment process; thus, recovery by the addition of a buffer (Na acetate) to adjust the pH lowered by the injection of acetic acid and by the injection of Na_2EDTA to inhibit chelate formation and remove interfering compounds (e.g., heavy metals) was compared in the analysis process.

Sludge samples collected from the aerobic tanks of the bioreactors of the WWTPs were used. The supernatant liquid was removed through centrifugation at 3000 rpm for 5 min. Concentrations were measured three times under each experimental condition without the standard solution, and the standard solution was added to make the final concentration reach 100 ng g^{-1}. Based on the average concentrations, the recovery was calculated as follows:

$$\text{Recovery } (\%) = \frac{C_{\text{spiked}} - C_{\text{unspikded}}}{C_{\text{known}}} \times 100 \quad (1)$$

where

C_{spiked}: concentration measured after adding the standard solution to the sample

$C_{unspiked}$: concentration measured after not adding the standard solution to the sample

C_{known}: concentration of the standard solution added to the sample

Target municipal wastewater treatment plants and sample collection

The capacity of municipal WWTP A (plant A) is 42,500 m^3 day^{-1}, and it employs a membrane bioreactor for the treatment process. The bioreactor is composed of an anaerobic tank, an anoxic tank, and an aerobic tank. In the aerobic tank, a submerged membrane with a 0.04 μm pore size made of PVDF is in operation, and municipal wastewater is released to the ocean after biological treatment. The capacity of municipal WWTP B (plant B) is 47,000 m^3 day^{-1}, and a moving-bed biofilm reactor is used for the treatment process. Fluidized media are placed in the aerobic tank of the bioreactor to maximize the activity of the microorganisms, and then post-denitrification is applied. Municipal wastewater subjected to biological treatment is used as the municipal reuse water through high-speed coagulative precipitation and activated carbon treatment. Target samples of the influent, activated sludge, and waste sludge were collected from the two municipal WWTPs using stainless steel containers and brown glass bottles. Samples were collected three times from June 2018 to February 2019. Influent samples were collected via 24 h composite sampling at 4-h intervals, and sludge samples were collected via grab sampling. Upon collection, the samples were sealed using a polytetrafluoroethylene stopper and moved to an ice box for storage at 4 °C. Pretreatment was performed within 4 days after sample collection, and analysis was completed within 72 h after pretreatment.

Results

Recovery evaluation of solid sample

The recovery rates of the solid samples were evaluated by partially modifying the QuEChERS method according to the experimental conditions presented in Table 2, and the results are shown in Fig. 2. First, the recoveries were compared after adding $MgSO_4$ (E1–4) and Na_2SO_4 (E5–8) to evaluate the extraction efficiency according to the absorbent type. Among the 27 compounds, 11–14 were included in the 70–130% recovery range when $MgSO_4$ was added. Diclofenac (80.3–111.1%), erythromycin (72.8–103.4%), roxithromycin (75.5–84.4%), atenolol (105.6–116.0%), and oxolinic acid (75.5–102.2%) exhibited a high recovery. Some compounds, such as ciprofloxacin (16.9–58.3%), cimetidine (22.6–59.8%), sulfamethoxazole (32.2–55.9%), and cefradine (5.7–53.5%), exhibited an average recovery of less than 50%. When Na_2SO_4 was added, 12–18 compounds were

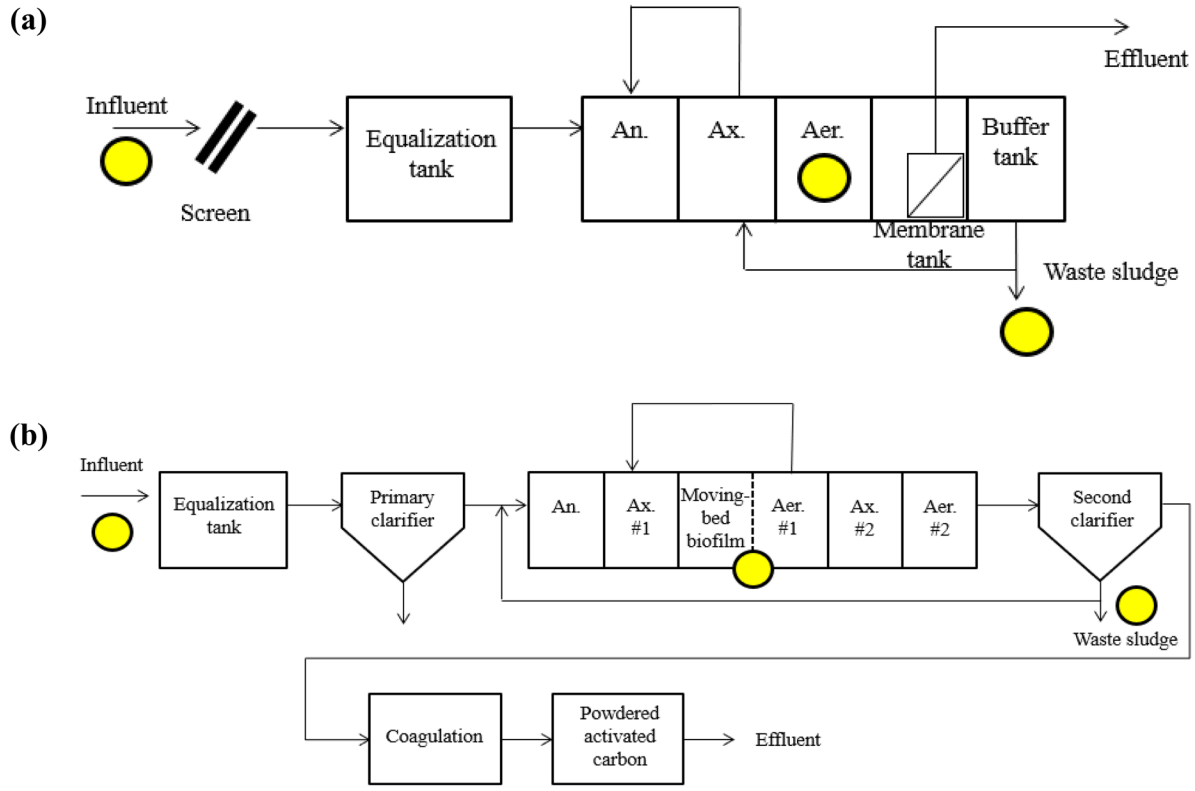

Fig. 2 Process flow diagrams and sampling sites at target municipal wastewater treatment plants. **(a)** Plant A, **(b)** Plant B. An. anaerobic; Ax. anoxic; Aer. aerobic

included in the 70–130% recovery range. Naproxen (83.1–97.9%), acetaminophen (83.6–92.5%), erythromycin (81.3–104.6%), carbamazepine (84.4–99.6%), and sildenafil (80.7–88.0%) exhibited high recovery rates.

Additionally, of the 27 compounds, when Na acetate was not added as a buffer, 25 (E3), 19 (E4), 26 (E7), and 24 (E8) compounds exhibited recoveries in the range 50–130%, regardless of the absorbent type, and 18 (E1), 17 (E2), 22 (E5), and 19 (E6) compounds showed similar recoveries when the buffer was added. Acetylsalicylic acid, propranolol, and atenolol exhibited an average recovery of 90% or more regardless of the addition of the buffer. Caffeine, roxithromycin, naproxen, diclofenac, and carbamazepine showed an average recovery of 80% or more. In addition, the average recoveries of ciprofloxacin, cimetidine, and cefradine were 56.4%, 53.5%, and 57.5%, respectively, when the buffer was not added, but were 29.0%, 35.4%, and 20.5% when the buffer was added.

Na_2EDTA is commonly used in the pretreatment process of residual pharmaceuticals adsorbed on solid samples because it improves the extraction efficiency by preventing the chelate formation of fluoroquinolone and tetracycline compounds (Lindsey et al., 2001; Pailler et al., 2009). When the recovery was evaluated according to the injection sequence of Na_2EDTA during pretreatment, out of the 27 compounds, 18 (E1), 25 (E3), 22 (E5), and 26 (E7) exhibited recoveries in the range 50–130% when Na_2EDTA was injected during the extraction process, whereas 17 (E2), 19 (E4), 19 (E6), and 24 (E8) compounds showed similar recoveries when Na_2EDTA was injected after the extraction process. When the recoveries were compared under the same conditions except for the injection sequence of Na_2EDTA, the results showed that the Na_2EDTA injection during the extraction process resulted in high recoveries (Fig. 3).

Quality control

A calibration curve was created by sequentially diluting the 10 mg L^{-1} standard solution and quantified using the absolute calibration curve method. The

calibration curve linearity evaluation results show that the coefficient of determination (R^2) was 0.99 or higher for all the compounds (Table 3). The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.2–1.3 and 0.6–4.0 ng g^{-1} on the chromatogram when the signal-to-noise (S/N) ratios were higher than 3 and 10, respectively (Shrivastava & Gupta, 2011). The seven samples were prepared by adding the standard materials to each compound using the pretreatment method described in "Recovery evaluation of solid sample" so that the concentrations could be 10 ng g^{-1} (low level), 50 ng g^{-1} (middle level), and 100 ng g^{-1} (high level), and the recovery was obtained using Eq. (1). As shown in Table 4, the ranges of the low-level, middle-level, and high-level recoveries were 61.3–120.6%, 67.2–119.3%, and 64.0–137.2%, respectively. As constant recovery rates were observed regardless of the added volume of the standard solution, it was deemed possible to extract residual pharmaceuticals present in solid samples in various concentrations. In addition, the low level showed a relative standard deviation of 6.8%, thereby exhibiting a relatively lower precision than the middle level (3.7%) and high level (3.5%).

Analysis of pharmaceuticals in solid samples from the municipal wastewater treatment plants

Suspended solids

The analysis results showed the detection of 19 compounds in the suspended solids of the influent of plant A. Caffeine, diphenhydramine, naproxen, ofloxacin, and roxithromycin exhibited a 100% detection frequency, whereas eight compounds, including carbamazepine, erythromycin, propranolol, and trimethoprim, were not detected. In the influent of plant A, acetylsalicylic acid (830 ng g^{-1}) exhibited the highest average concentration in suspended solids, followed by ibuprofen (731 ng g^{-1}), acetaminophen (264 ng g^{-1}), atenolol (211 ng g^{-1}), and caffeine (201 ng g^{-1}). Sulfadimethoxine (1 ng g^{-1}), gemfibrozil (1 ng g^{-1}), and iopromide (7 ng g^{-1}) were detected in significantly lower concentrations than the other compounds.

Thirteen compounds were detected in the suspended solids of the influent of plant B. Acetaminophen, caffeine, naproxen, and testosterone exhibited a 100% detection frequency, and the eight compounds that were

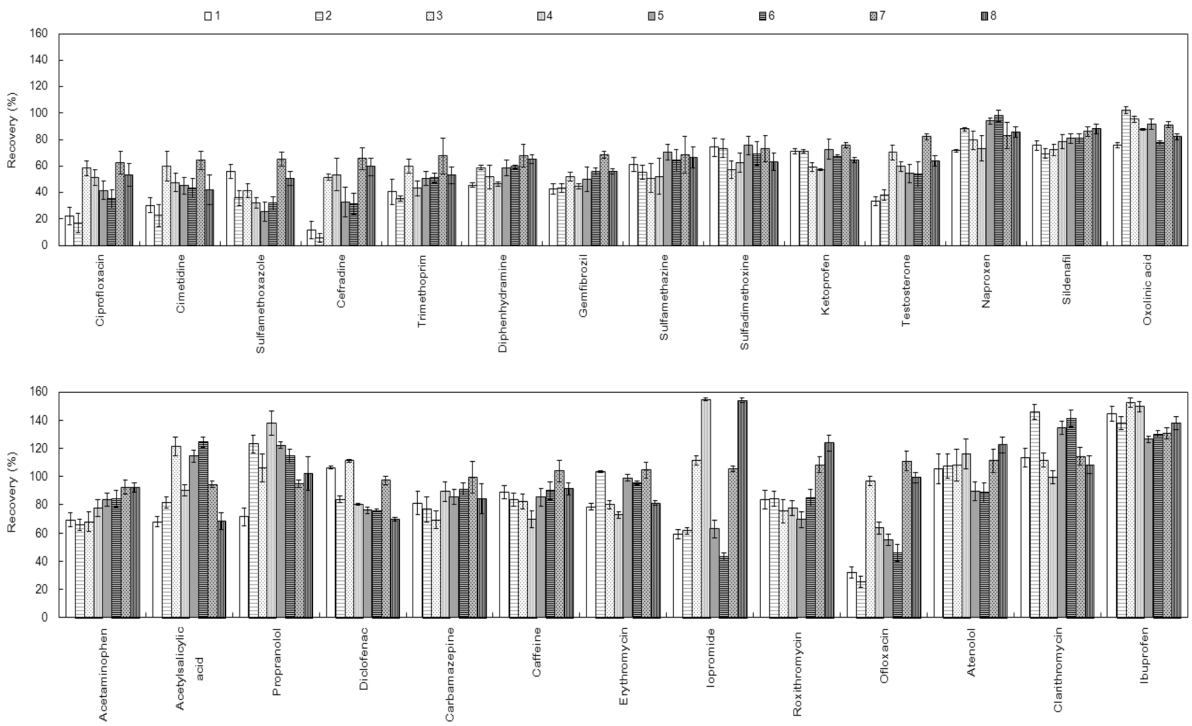

Fig. 3 Recoveries of pharmaceuticals under each experimental condition

Table 3 Linearity and detection limits for target compounds

Compounds	R^2	LOD (ng L^{-1})	LOQ (ng L^{-1})
Acetaminophen	0.999	1.1	3.3
Acetylsalicylic acid	0.991	1.2	3.5
Atenolol	0.994	0.7	2.0
Caffeine	0.999	0.9	2.7
Carbamazepine	0.998	0.6	1.8
Cefradine	0.995	1.3	4.0
Cimetidine	0.999	0.2	0.7
Ciprofloxacin	0.998	0.7	2.1
Clarithromycin	0.994	0.6	1.7
Diclofenac	0.996	0.3	1.0
Diphenhydramine	0.990	0.6	1.8
Erythromycin	0.994	0.4	1.3
Gemfibrozil	0.991	0.4	1.3
Ibuprofen	0.996	0.9	2.6
Iopromide	0.998	0.9	2.6
Ketoprofen	0.998	0.4	1.1
Naproxen	0.999	0.5	1.4
Ofloxacin	0.991	1.0	3.1
Oxolinic acid	0.994	0.5	1.6
Propranolol	0.998	0.5	1.4
Roxithromycin	0.997	0.4	1.3
Sildenafil	0.995	0.6	1.8
Sulfadimethoxine	0.998	0.2	0.6
Sulfamethazine	0.998	0.4	1.2
Sulfamethoxazole	0.999	0.3	1.0
Testosterone	0.999	0.3	0.9
Trimethoprim	0.998	0.2	0.7

LOD limit of detection, *LOQ* limit of quantification

not detected in plant A were also not detected in plant B. In the influent of plant B, caffeine (675 ng g^{-1}) exhibited the highest average concentration in suspended solids, followed by acetylsalicylic acid (649 ng g^{-1}), ibuprofen (623 ng g^{-1}), acetaminophen (304 ng g^{-1}), and clarithromycin (160 ng g^{-1}). Testosterone (5 ng g^{-1}) and ciprofloxacin (12 ng g^{-1}) were detected in low concentrations (Table 5). Some compounds, such as atenolol, ketoprofen, and diclofenac, were not detected in plant B even though they were detected with the concentrations in the range of 35–211 ng g^{-1} in plant A.

Sludge

Seventeen compounds were detected in the activated sludge that originated from plant A. Nine compounds, including diphenhydramine, roxithromycin, and iopromide, exhibited 100% detection frequency, whereas 10 others, including acetaminophen, atenolol, and cimetidine, were not detected. Further, in plant A, the concentration range of the compounds in the activated sludge was < LOQ–2353 ng g^{-1}, with diphenhydramine (2353 ng g^{-1}) exhibiting the highest average concentration, followed by erythromycin (1013 ng g^{-1}), gemfibrozil (877 ng g^{-1}), ibuprofen (767 ng g^{-1}), and iopromide (628 ng g^{-1}). Trimethoprim (8 ng g^{-1}), testosterone (9 ng g^{-1}), and sulfamethoxazole (15 ng g^{-1}) showed lower concentrations than the other compounds.

Further, 19 compounds were detected in the waste sludge originating from plant A. Twelve of these compounds, including clarithromycin, ketoprofen, and erythromycin, exhibited 100% detection frequency, whereas eight others, including acetaminophen, acetylsalicylic acid, and cimetidine, were not detected. The concentration range of these compounds in this waste sludge originating from plant A was <LOQ–1929 ng g^{-1}, with clarithromycin (1929 ng g^{-1}) exhibiting the highest average concentration, followed by diclofenac (1900 ng g^{-1}), diphenhydramine (1374 ng g^{-1}), and erythromycin (1071 ng g^{-1}). Conversely, the concentrations of naproxen, ketoprofen, iopromide, and ibuprofen (100–500 ng g^{-1}) as well as those of trimethoprim, testosterone, and sulfamethoxazole (≤ 10 ng g^{-1}) were relatively low.

Furthermore, 21 compounds were detected in the activated sludge originating from plant B. Specifically, eight compounds, including erythromycin, propranolol, and roxithromycin, exhibited 100% detection frequency. However, six other compounds, including atenolol, carbamazepine, and diclofenac, were not detected. The concentrations of these compounds in this activated sludge from plant B were in the range <LOQ–852 ng g^{-1}, with gemfibrozil (852 ng g^{-1}) exhibiting the highest average concentration, followed by diphenhydramine (686 ng g^{-1}), oxolinic acid (398 ng g^{-1}), and ibuprofen (373 ng g^{-1}). The concentrations of these compounds in this activated sludge from plant B tended to be lower than those in the activated sludge from plant A. However, the concentrations of trimethoprim (8 ng g^{-1}), testosterone (23 ng g^{-1}), and sulfamethoxazole (29 ng g^{-1}) in the activated sludge from plant B were low and similar to those in the activated sludge from plant A.

Twenty-one compounds were detected in the waste sludge originating from plant B. Eleven of these

Table 4 Method validation for pharmaceuticals in the solid phase

Compounds	Solid phase (n=7)					
	Low level (10 ng g^{-1})		Middle level (50 ng g^{-1})		High level (100 ng g^{-1})	
	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
Acetaminophen	88.9	11.9	102.3	7.9	90.5	4.5
Acetylsalicylic acid	87.2	12.8	93.0	11.3	92.0	5.0
Atenolol	85.5	7.4	103.3	3.0	115.1	8.1
Caffeine	101.0	8.4	112.1	5.2	99.1	3.1
Carbamazepine	90.9	6.2	96.5	1.9	97.9	1.6
Cefradine	71.0	17.9	69.1	6.2	68.7	2.9
Cimetidine	65.8	3.3	77.5	3.1	69.1	0.8
Ciprofloxacin	61.3	12.6	68.3	6.2	64.0	4.5
Clarithromycin	115.9	4.8	119.3	3.7	118.9	4.4
Diclofenac	94.9	3.2	105.1	0.7	96.1	1.6
Diphenhydramine	70.7	8.0	70.3	1.7	68.1	2.0
Erythromycin	96.2	4.3	104.7	1.8	101.7	1.8
Gemfibrozil	67.5	6.3	67.2	4.9	67.4	1.4
Ibuprofen	111.8	7.5	118.8	7.0	137.2	3.1
Iopromide	115.0	7.3	109.6	6.5	99.5	7.8
Ketoprofen	77.0	4.7	74.1	2.8	71.7	3.8
Naproxen	85.3	5.4	84.9	1.2	82.4	3.9
Ofloxacin	101.6	9.8	113.7	3.0	114.0	3.6
Oxolinic acid	120.6	4.3	105.7	6.7	96.2	4.2
Propranolol	96.7	4.6	101.3	2.5	97.0	1.2
Roxithromycin	91.9	4.4	96.2	2.5	100.3	4.5
Sildenafil	83.7	6.7	87.4	1.7	90.4	1.3
Sulfadimethoxine	68.7	2.7	73.0	1.1	67.4	1.7
Sulfamethazine	71.1	5.5	78.2	1.3	70.2	7.1
Sulfamethoxazole	61.3	5.4	71.8	1.4	66.3	6.7
Testosterone	77.1	3.8	85.5	1.6	86.7	2.0
Trimethoprim	72.6	3.2	70.6	2.7	71.6	1.7

[Surrogate recoveries in the solid phase] acetaminophen-D4: 74.3(± 8.6)%, sulfadimethoxine-13C6: 92.1(±9.2)%, ciprofloxacin-d8: 78.4(±7.6)%

compounds, including clarithromycin, ofloxacin, and diclofenac showed 100% detection frequency, whereas six others, including cefradine, acetaminophen, and ibuprofen, were not detected. The concentrations of these compounds in this waste sludge from plant B were in the range <LOQ–1096 ng g^{-1}. Specifically, naproxen (1096 ng g^{-1}) exhibited the highest average concentration, followed by diphenhydramine (1000 ng g^{-1}), gemfibrozil (870 ng g^{-1}), and erythromycin (410 ng g^{-1}). Some of the compounds that were not detected in the waste sludge from plant A, such as atenolol, caffeine, and acetylsalicylic acid, were detected in the waste sludge from plant B, even though their concentrations were relatively low.

Discussion

When the recoveries of solid samples were evaluated according to the absorbent type by partially modifying the QuEChERS method, compounds such as atenolol, erythromycin, naproxen, oxolinic acid, and caffeine exhibited high recoveries (70% or more on average) regardless of the absorbent type. However,

Table 5 Concentration of pharmaceuticals in suspended solids, activated sludge, and waste sludge in municipal wastewater treatment plants

	Suspended solids (ng g^{-1})						Activated sludge (ng g^{-1})						Waste sludge (ng g^{-1})					
	Plant A ($n = 3$)			Plant B ($n = 3$)			Plant A ($n = 3$)			Plant B ($n = 3$)			Plant A ($n = 3$)			Plant B ($n = 3$)		
	Mean	SD	D.F. (%)	Mean	SD	D.F. (%)	Mean	SD	D.F. (%)	Mean	SD	D.F. (%)	Mean	SD	D.F. (%)	Mean	SD	D.F. (%)
Acetaminophen	264	244	67	304	122	100	–	–	0	112	–	33	–	–	0	–	–	0
Acetylsalicylic acid	830	492	100	649	108	67	–	–	0	91	–	33	–	–	0	31	6	100
Atenolol	211	288	67	–	–	0	–	–	0	–	–	0	–	–	0	14	–	33
Caffeine	201	181	100	675	381	100	–	–	0	–	–	0	–	–	0	21	7	100
Carbamazepine	–	–	0	–	–	0	–	–	0	–	–	0	–	–	0	89	69	67
Cefradine	23	–	33	–	–	0	–	–	0	19	–	33	–	–	0	–	–	0
Cimetidine	102	–	33	26	–	33	–	–	0	35	8	67	–	–	0	32	8	100
Ciprofloxacin	33	24	67	12	1	67	–	–	0	–	–	0	–	–	0	–	–	0
Clarithromycin	80	30	67	160	141	67	–	–	0	19	–	33	1929	2743	100	199	112	100
Diclofenac	35	37	67	–	–	0	–	–	0	–	–	0	1900	2479	100	171	43	100
Diphenhydramine	23	13	100	19	22	67	2353	2511	100	686	220	67	1374	951	100	1000	182	67
Erythromycin	–	–	0	–	–	0	1013	1243	67	125	97	100	1071	1360	100	410	378	100
Gemfibrozil	1	–	33	–	–	0	877	736	100	852	1241	100	996	448	100	870	278	67
Ibuprofen	731	–	33	623	–	33	767	181	100	373	62	67	398	–	33	–	–	0
Iopromide	7	–	33	62	–	33	628	610	100	334	279	100	144	91	100	369	104	67
Ketoprofen	72	63	67	–	–	0	469	–	33	19	9	67	109	158	100	383	263	67
Naproxen	70	97	100	28	13	100	102	40	100	91	27	67	105	128	67	1096	682	67
Ofloxacin	75	69	100	36	10	67	85	67	100	263	240	67	85	27	100	40	38	100
Oxolinic acid	–	–	0	–	–	0	79	45	67	398	228	67	72	43	100	107	46	100
Propranolol	–	–	0	–	–	0	58	77	100	56	24	100	63	6	100	265	137	100
Roxithromycin	98	58	100	68	59	67	48	28	100	84	37	100	56	27	100	57	18	67
Sildenafil	–	–	0	–	–	0	46	21	67	–	–	0	34	–	33	27	10	100
Sulfadimethoxine	1	–	33	–	–	0	39	26	67	14	5	100	34	–	33	–	–	0
Sulfamethazine	–	–	0	–	–	0	20	6	100	23	0	67	34	16	100	89	5	67
Sulfamethoxazole	–	–	0	–	–	0	15	–	33	29	–	33	10	–	33	38	9	67
Testosterone	24	–	33	5	1	100	9	–	33	23	7	100	7	2	100	29	18	100
Trimethoprim	–	–	0	–	–	0	8	5	67	8	5	100	5	–	33	–	–	0

SD standard deviation, *DF* detection frequency

some compounds, such as trimethoprim, diphenhydramine, gemfibrozil, and testosterone, exhibited higher recoveries with the addition of Na_2SO_4 compared to that of $MgSO_4$. The average recoveries of ciprofloxacin and ofloxacin were 37.3% and 54.5%, respectively, when $MgSO_4$ was added; however, they increased to 48.2% and 77.9% when Na_2SO_4 was added because fluoroquinolones form chelates with divalent magnesium contained in $MgSO_4$ (Marshall & Piddock, 1994). The recoveries of fluoroquinolone and tetracycline compounds, such as ofloxacin, ciprofloxacin, and chlortetracycline, were improved using Na_2SO_4 as an absorbent instead of $MgSO_4$ (Bourdat-Deschamps et al., 2014).

In the experiment, the pH ranged from 5.3 to 6.1 when with the addition of Na acetate and from 2.8 to 3.7 without it. The recoveries of macrolide antibiotics, such as clarithromycin and roxithromycin, were high without the addition of Na acetate because these compounds tend to decompose when the pH increases (Ternes & Joss, 2006). In addition, residual pharmaceuticals show a difference in the degree of ionization depending on the pH of the extract because they have different acid dissociation constants (p*K*a) (Table 1), and this changes the extraction efficiency in the pretreatment process. Sulfamethoxazole, sulfamethazine, and sulfadimethoxine exhibited 5–30% higher recoveries without the addition of Na acetate than when it was added because sulfonamide antibiotics have two p*K*a values, and thus, protonation and deprotonation occur under strong acid and base conditions, respectively. This indicates that maintaining acidic conditions by not injecting Na acetate can improve the recoveries in the simultaneous analysis of multiple components rather than adjusting the pH by injecting Na acetate during pretreatment. In a previous study, the recoveries of various residual pharmaceuticals improved under acidic conditions (pH 2) during the extraction of solid samples, such as soil and sludge (US EPA, 2007).

The recovery evaluation results of solid samples show that the average recovery was 88.5% when Na_2SO_4 was used as an absorbent during pretreatment without the addition of Na acetate, and Na_2EDTA was injected during the extraction process as in the experimental conditions of E7. In this case, oxolinic acid (91.2%), acetaminophen (92.5%), acetylsalicylic acid (94.3%), propranolol (95.0%), diclofenac (97.2%), carbamazepine (99.6%), caffeine (104.0%), erythromycin (104.6%), and iopromide (105.5%) exhibited high recoveries. The recoveries of the 27 compounds ranged from 62.6 to 130.5%, which were higher than the results of the tests conducted under different conditions, indicating that residual pharmaceuticals adsorbed on solid samples can be most effectively extracted. The relationships between the physicochemical properties of target compounds, such as water solubility, p*K*a, the octanol/water partition coefficient (log K_{ow}), and their recovery rates based on each pretreatment method, were also evaluated. However, no statistically significant relations were observed between the different variables.

The results of the analysis of the quality control samples established through the recovery test (recovery: 61.3–137.2%; relative standard deviation: 6.8% on average) confirmed the possibility of an accurate and reproducible analysis. Previous research developed an analytical method using online SPE LC–MS/MS to identify the concentrations of NSAIDs and metabolites in sewage sludge and reported a 37–101% recovery of 13 compounds (Rossini et al., 2016). Bourdat-Deschamps et al. (2014) reported a 70–127% recovery of 13 residual pharmaceuticals adsorbed on solid samples (e.g., sludge and slurry supernatants) using online SPE LC–MS/MS. This study evaluated the pretreatment conditions and presented the optimal extraction method.

It is difficult to compare the concentrations detected in this study with those in other studies because studies that have analyzed residual pharmaceuticals in the suspended solid samples from WWTPs are lacking. However, in a study conducted by Wang et al. (2018), pharmaceuticals in suspended solid in WWTP influents were analyzed after extraction using phosphate buffer and acetonitrile and clean-up via SPE using traditional cartridges. They observed that the concentration of ofloxacin (2530 ng g^{-1}) was higher than that detected in this study; however, the concentrations of caffeine and acetaminophen they reported (137 and 16 ng g^{-1}, respectively) were lower than those detected in this study. Further, Ashfaq et al. (2017) analyzed the residual pharmaceuticals in solid samples from municipal WWTPs after sample extraction following the guidelines of the EPA method 1694 (US EPA, 2007) as well as SPE procedures. They reported the concentrations of ofloxacin (activated sludge: 4870 ng g^{-1}, suspended solids: 4680 ng g^{-1}), ciprofloxacin (313 ng g^{-1}, 391 ng g^{-1}), diclofenac

(19 ng g^{-1}, 30 ng g^{-1}), ketoprofen (46 ng g^{-1}, 59 ng g^{-1}), and sulfamethoxazole (3 ng g^{-1}, 2 ng g^{-1}), and their study also showed concentrations corresponding to quinolone compounds that were 10–100 times higher than those detected in the activated sludge and suspended solids in this study. Moreover, Radjenovic et al. (2009) analyzed 20 residual pharmaceuticals, including acetaminophen (primary settling sludge: 150 ng g^{-1}, activated sludge: 90 ng g^{-1}), ketoprofen (200 ng g^{-1}, 50 ng g^{-1}), ibuprofen (530 ng g^{-1}, 60 ng g^{-1}), and diclofenac (210 ng g^{-1}, 150 ng g^{-1}) in solid samples collected from municipal WWTPs using the pressurized liquid extraction method and the SPE clean-up step. They detected different concentrations depending on the sludge characteristics. The amount adsorbed on the solid samples is related to the physicochemical properties of the target compound, such as log K_{ow} and p*K*a. Log K_{ow} has been used as an index to evaluate the number of residual pharmaceuticals adsorbed on solid samples because it can identify the hydrophilicity and hydrophobicity of the target compound. In recent years, absorption properties have been evaluated by not only using the previously reported log K_{ow} value but also by deriving the soil/water partition coefficient through the quantitative analysis of liquid and solid samples (Narumiya et al., 2013; Park et al., 2017a, b). Future research should compare the amount of the target compounds adsorbed on solid samples detected through the physicochemical properties of the compounds with the experimentally derived values.

This study confirmed that the concentrations of adsorbed compounds vary depending on the solid sample type. Errors may occur if only liquid samples are analyzed to evaluate the behavior and removal efficiency of residual pharmaceuticals in municipal WWTPs, because some compounds were detected in high concentrations in influent suspended solids. Accurate evaluation will be possible if the liquid and solid samples of municipal WWTPs are analyzed using the pretreatment method established in this study. Moreover, it will be possible to identify the behavior of residual pharmaceuticals in the biological process of municipal WWTPs through further research because it is possible to quantitatively detect the concentrations in activated sludge and waste sludge. The results of this study are expected to be used to develop treatment processes that can efficiently remove residual pharmaceuticals using biological sludge and to prepare systematic management plans.

Conclusions

In this study, the pretreatment method that applied the QuEChERS method was evaluated and used in combination with online SPE LC–MS/MS to simultaneously analyze 27 residual pharmaceuticals in the solid samples from municipal WWTPs. The results can be summarized as follows:

1) The highest recovery rate was obtained when Na_2SO_4 was used as an absorbent during pretreatment without the addition of Na acetate and with the injection of Na_2EDTA during the extraction process.
2) The recoveries of solid samples ranged from 61.3 to 137.2%, and the relative standard deviation was 6.8% on average, indicating that the 27 target residual pharmaceuticals can be analyzed in an accurate and reproducible manner through simultaneous analysis.
3) When the suspended solids in the solid samples from two municipal WWTPs were analyzed using the proposed method, 19 compounds were detected in plant A and 13 compounds in plant B. Their concentration ranges were 1–830 ng g^{-1} and 5–675 ng g^{-1}, respectively.
4) Seventeen compounds were detected in the activated sludge of plant A and 21 compounds in the activated sludge of plant B, and their concentration ranges were <LOQ–2353 ng g^{-1} and <LOQ–852 ng g^{-1}, respectively. Nineteen compounds were detected in the waste sludge of plant A and 21 compounds in the waste sludge of plant B, and their concentration ranges were <LOQ–1929 ng g^{-1} and <LOQ–1096 ng g^{-1}, respectively.
5) It was possible to quantitatively detect residual pharmaceuticals adsorbed on solid samples, such as suspended solids and sludge, using the analytical method established in this study. The results of this study can be utilized in other studies to identify the concentrations of residual pharmaceuticals in municipal WWTPs more accurately and to evaluate the behavior and removal characteristics of each process.

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Data availability The data generated and/or analyzed during the current study are not publicly available for legal/ethical reasons but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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