Journal of Chromatography A, 1216 (2009) 5949–5954

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry

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ARTICLE INFO

Article history:
Received 6 March 2009
Received in revised form 8 June 2009
Accepted 10 June 2009
Available online 13 June 2009

Keywords:
Sewage sludge
Fluoroquinolones
Tetracyclines
Sulfonamides
PLE
SPE
HPLC–ESI-MS

ABSTRACT

A new scheme for the quantitative determination of traces of fluoroquinolones (FQs), tetracyclines (TCs) and sulfonamides (SAs) in sewage sludge was developed. The compounds were simultaneously extracted from sewage sludge by pressurized liquid extraction (PLE). A novel and effective method for PLE was developed. Solid-phase extraction was used for cleaning up the extracts. Identification and quantification of the compounds was done using high-performance liquid chromatography with electrospray ionization mass spectrometry in selected reaction monitoring mode. The best recovery of FQs and TCs was obtained by using hydrophilic–lipophilic balance cartridges, recoveries ranged 59% for norfloxacin to 82% for ofloxacin and 95% for doxycycline; for SAs strong cation-exchange cartridges were more efficient, recoveries were 96% for sulfamethoxazole and 43% for sulfadimethoxine. Limit of quantification ranged from 0.1 ng/g for SAs to 160 ng/g for tetracycline. Method precision for TCs was 5.06% and 1.12%, and for SAs 0.43% and 2.01%. FQs precision ranged from 0.77% to 1.89%.

1. Introduction

The amount of sewage sludge increases rapidly all over the world [1]. Although by its nature a hazardous waste, sewage sludge may be used as a fertilizer, if after relevant treatment it is safe for soil, surface and ground water, plants, people and animals [2]. Before utilization the sludge should be analyzed for heavy metals, *E. coli* and helmints' eggs. If the contents of named pollutants do not exceed the trigger values, the sewage sludge is considered to be safe as an ingredient of a fertilizer [3]. Recent research has shown that in addition to these pollutants treated sewage sludge always contains traces of several pharmaceuticals, including antibiotics [4–6]. Although their concentrations are much lower than the levels of traditionally known organic pollutants the potential long-term effects of these compounds to humans, plants and animals cannot be ignored. No trigger values exist for drug residues in sewage sludge neither in Estonia [3] nor in the European Union [7]. The most closely related act is the EU directive EMEA/CVMP/055 establishing trigger values for drug residues in manure [8]. The content of drug residues should not exceed 100 μg/kg in manure and 10 μg/kg in the soil fertilized with manure.

The sewage sludge from Estonian wastewater treatment plants has never been analyzed for pharmaceuticals and as a rule the relevant levels are unknown. The selection of drugs was made by considering their stability in soil [4,9] and/or their potential accumulation into plants [10–13]. It has been shown that tetracyclines (TCs) and fluoroquinolones (FQs) can bind strongly to solid particles and this phenomenon might be an additional reason for their slow degradation [14–17]. Sulfamethoxazole is relatively stable in sewage sludge [18] and sulfonamides (SAs) are found to be present there [5]. Therefore, these antibiotics may contaminate agricultural fields, disturb natural balance and accumulate in crops and vegetables [11–13]. Even very small amounts of antibiotics in everyday food may generate the strains of resistant bacteria in human and animal bodies, provoke allergy and affect the liver [13].

Analytical tools have been developed for different media, e.g. feed products [19–22], environmental water samples [23–26], soil [4,13,15,27–29] and manure [15,30], while only few methods

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doi:10.1016/j.chroma.2009.06.029

have been designed for extraction of antibacterial agents from sewage sludge [4,31]. The existing methods [15] cannot be used for simultaneous extraction of FQs, SAs and TCs from sewage sludge.

Several extraction techniques have been applied for the determination of antibiotics from solid phase, such as ultrasonic-assisted extraction (USE) [6,32,33], microwave-assisted extraction (MAE) [34,35], pressurized liquid extraction (PLE) and accelerated solvent extraction (ASE) [4,15]. For extracts clean-up liquid–liquid extraction (LLE) [30,36] and solid-phase extraction (SPE) [4,21,23,25] were used. ASE or PLE have clear advantages over other methods such as higher precision, smaller amounts of extraction solvent and reduced sample preparation time [37]. Sludge extraction is usually followed by pre-concentration and clean-up of the PLE extracts using SPE with different cartridges [4,28,31].

Most of the relevant analytical methods reported are based on liquid chromatography–mass spectrometry (LC–MS) [38,39], LC–tandem MS (LC–MS/MS) [19,40] and HPLC-UV [24,35]. Optimum conditions with regard to extraction solvent and number of extraction cycles were established. Identification and quantification of the pharmaceuticals were carried out by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) using electrospray ionization (ESI).

The aim of this work was to develop a specific extraction method for the quantification of FQs, SAs and TCs in sewage sludge using the same extraction process.

2. Experimental

2.1. Chemicals and materials

Antibiotics were purchased from Riedel-de-Haën (Seelze, Germany)—three fluoroquinolones: ciprofloxacin (CIP, purity 99.8%), norfloxacin (NOR, purity 99.9%) and ofloxacin (OFL, purity 99.3%); two tetracyclines: tetracycline hydrochloride (TCL, purity 97.3%) and doxycycline hyclate (DOX, purity 99.5%); two sulfonamides: sulfadimethoxine (SDM, purity 99.4%) and sulfamethoxazole (SMX, purity 99.9%). Strong cation-exchange (SCX) cartridges (Strata SCX (55 μm, 70 Å) 500 mg/6 mL) were supplied by Phenomenex (Torrance, CA, USA); Hydrophilic–lipophilic balanced (HLB) cartridges (Oasis HLB (60 μm), 500 mg/6 mL) by Waters (Milford, MA, USA). Acetonitrile and methanol were obtained from J.T. Baker (Deventer, The Netherlands), phosphoric acid from Lachema (Brno, Czech Republic), citric acid monohydrate from Fisher Scientific (Pittsburgh, PA, USA), formic acid from Riedel-de-Haën, ammonium acetate from Fluka (Buchs, Germany). All solvents were of reagent grade or higher quality.

2.2. Sample collection and storage

The samples were taken from anaerobically digested sludge (before mixing with peat) in Tallinn and from untreated sludge (before composting with tree bark) in Tartu. Approximately 200 g of sludge (content of dry matter was 28% in Tallinn and 25% in Tartu) was placed into a 500 mL glass jar and mixed thoroughly. The jar was covered hermetically with a lid. Before analyzing the samples were stored in refrigerator at temperature +4 °C in the dark. The samples were analyzed as soon as possible, typically within a week. Alternatively they were stored in polypropylene vials frozen at temperature −80 °C.

2.3. Pressurized liquid extraction (PLE)

PLE was performed using an in-house designed system schematically depicted in Fig. 1. The extractor was designed using ultra high vacuum components. For surviving high pressure the stainless steel chamber cylinder wall thickness was 10 mm and for sealing flanges copper gaskets were used. Volume of the pressure chamber was 55 mL. Standard HPLC valves and stainless steel tubing were used.

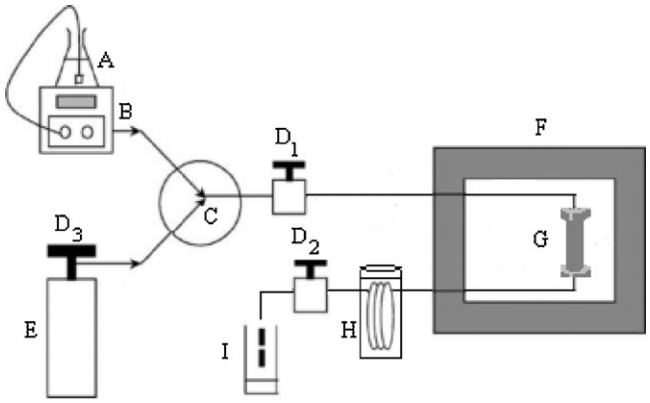

Fig. 1. Pressurized liquid extraction (PLE) system: A, extraction solvent; B, HPLC pump; C, three-way switching valve; D1 and D2, static valves; D3, the valve of argon gas; E, argon tank; F, oven; G, extraction cell; H, cooling coil; I, extract collection vial.

9 ± 1 g (wet weight, ww) of sewage sludge sample was mixed 1:1 with sand, and 9 ± 1 g of sludge/sand blend was packed into cellulose filter and placed into the extraction cell mounted in an oven. Extraction was performed with 0.35% phosphoric acid and acetonitrile mixture (1:1, v/v) adjusted to pH 2.50 with 0.01 M citric acid monohydrate. For one extraction cycle approximately 30 mL of solvent was pumped into the extraction cell with static valve D1 open. The system was pressurized with argon using valve D3; subsequently the cell was heated. The operating conditions were as follows: temperature in the range 100–110 °C with a 30 min heat-up time, pressure in the range 100–110 atm (10,130–11,143 kPa), static extraction 10 min, 5 cycles and solvent flush volume 60%. The extracted analytes were purged from the sample cell using pressurized argon for 40 s. The solvent used for flushing of the extraction cell was collected with static valve D2 open after the first cycle of extraction. Subsequent cycles of extraction were carried out using the same operating conditions. The extract cooling was accomplished by stainless steel tubing in cold water. The total volume of the extract collected was in the range of 150–160 mL.

2.4. Solid-phase extraction

The extracts collected by PLE were cleaned up by SPE. Antibiotics as CIP, NOR, OFL, TCL, DOX, SDM and SMX were extracted using SCX and HLB cartridges. Two different cartridges were tested with the aim of securing the best possible recoveries. For SPE procedure the vacuum manifold, supplied by Agilent Technologies, was used. For extraction with SCX cartridges the cartridges were preconditioned with 6 mL of methanol and 6 mL of buffer solution (1 mM ammonium acetate and 0.1% formic acid, pH 2.8). A portion (80 mL) of sludge PLE extract was diluted to 500 mL with H_2O (pH adjusted to 2.0) and then percolated through the cartridge at a flow rate ~1.5 mL/min using the vacuum manifold. After extraction, the compounds were eluted from cartridges using 20 mL of 20% ammonia water solution in 40% methanol. For extraction with HLB cartridges the cartridges were preconditioned with 20 mL of methanol and 10 mL of Milli-Q water. Dilution of PLE extract was preformed as for SCX cartridges. Flow rate of sample loading was ~6 mL/min. After extraction, the compounds were eluted from cartridges using 12 mL of methanol. The SPE extracts were concentrated on polypropylene vials in N_2 stream. Polypropylene vials were used to avoid sorption to glass walls and samples were not evaporated to complete dry-