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Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC/tandem MS and GC/MS

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Abstract

Analytical methods have been developed that allow for the determination of antiphlogistics, lipid regulators, the antiepileptic carbamazepine, cytostatic agents, the psychiatric drug diazepam and iodinated contrast media (ICM) as well as two major polycyclic musk fragrances HHCB (galaxolide) and AHTN (tonalide) in activated and digested sludge. The procedures consist of ultrasonic solvent extraction (USE) using methanol/acetone or pressurized liquid extraction (PLE) using 100% methanol. Clean-up was performed with C_{18ec} material and silica gel followed by LC tandem MS (electrospray or atmospheric pressure chemical ionization) detection for pharmaceuticals and iodinated contrast media as well as GC/MS in the SIM mode for musk fragrances. Absolute recoveries from spiked activated sludge in general ranged from 88 ± 4 to 119 ± 20% for ICM and were 78 ± 15 and 87 ± 10% for the AHTN and HHCB, respectively. For the pharmaceuticals, absolute recoveries in activated sludge ranged between 43 and 78%. Subsequently, compensation of losses was carried out by using surrogate standards (acidic pharmaceuticals: fenoprop, neutral pharmaceuticals: dihydro-carbamazepine, musk fragrances: AHTN-D_3). With one exception the recoveries were also adequate in digested sludge ranging from 43% to 120%.

Keywords: Antiepileptics; Cytostatic agents; Psychiatric drugs; Antiphlogistics; Lipid regulators; Contrast media; Musk fragrances; Sewage; Sewage sludge; GC/MS; SPE; PLE; LC/MS/MS

1. Introduction

In human medicine of many countries, pharmaceuticals and iodinated contrast media (ICM) are consumed in the ton-range per year. It can be expected that worldwide consumption of pharmaceuticals will increase due to a developing health care system and a higher life expectancy in industrial countries.

Musk fragrances are used in high quantities in cosmetic products. In 1996, about 5600 t of polycyclic musk fragrances (PMF) were used worldwide [1]; the production of HHCB alone was estimated to be 1000 t. In recent years, nitro musk fragrances have successively been replaced by PMF in some countries (e.g. Germany).

Human pharmaceuticals, iodinated contrast media (ICM) and ingredients of cosmetic products such as PMF are introduced into sewage to a high extent by households. Recently, their occurrence in municipal sewage treatment plants (STPs) and the receiving water has been reported in Europe, Brazil and North America by several authors [2–15]. Since pharmaceuticals, ICM and PMF are not totally removed during sewage treatment [9,10,14,16–20] they are discharged in appreciable quantities into receiving waters through STP effluents.

Concentrations of pharmaceuticals present in sewage sludge are needed to perform flow studies and total mass balances in STPs. Although many pharmaceuticals are relatively polar, a specific sorption might occur as found for

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fluoroquinolone antibiotics [14]. To the best of our knowledge, no analytical methods have been reported in literature for the analysis of the selected pharmaceuticals (see Table 1) in sewage sludge.

Most PMF are more hydrophobic than pharmaceuticals. Due to their elevated lipophilicity ($\log K_{OW}$ (AHTN) = 5.90−6.35) [1,18,21,22] PMF are, therefore, sorbed onto sludge and suspended matter. In literature, analytical methods are reported for analyzing PMF in sewage sludge using Soxhlet or pressurized liquid extraction (PLE) with dichloromethane, silica gel, alumina columns and gel permeation chromatography (GPC) clean-up and GC/MS [16,23,24]. Concentration levels for AHTN and HHCB in municipal sludge have been reported to be in the mg/kg range [23–25].

The objective of this paper is to present reliable analytical methods developed for the detection of antiphlogistics, lipid regulators, the antiepileptic carbamazepine, cytostatic agents, the tranquilizer diazepam, ICM, and two major polycyclic musk fragrances HHCB and AHTN in activated and digested sludge (see Table 1). The individual compounds were selected according to current data available on their occurrence in municipal sewage [2–15] (Table 1a and b; list of selected pharmaceuticals, iodinated contrast media and musk fragrances).

2. Experimental section

A scheme of the analytical methods is illustrated in Fig. 1a and b.

2.1. Sampling of Swiss sewage treatment plants

Anaerobically digested sludges ($n = 2$) and two activate sludges ($n = 2$) were collected from the mechanical–biological STPs in Kloten–Opfikon and Altenrhein near Zurich, Switzerland. The samples were collected in glass bottles, filtered through a glass fibre filter (GF 8, diameter 90 mm Schleicher & Schuell, Dassel, Germany) to obtain the solid fraction and were frozen at −20 °C. Afterwards, the sewage sludge samples were freeze-dried, ground in a mortar, mixed thoroughly and stored in amber bottles until analysis. The STP of Kloten–Opfikon serves a residential population of 55,000 population equivalents. The total solid sludge retention time is 10–12 days. The plant consists of primary clarification, denitrification, nitrification and sand filtration. The primary sludge from mechanical treatment and the excess sludge from biological treatment and filtration are mixed and fed into a mesophilic anaerobic digester. The conventional STP of Altenrhein treats the mixed sewage of 120,000 population equivalents. The plant is equipped with mechanical treatment, secondary treatment consisting of a denitrification and nitrification and tertiary sand filtration. For additional details see Joss et al. [26].

2.2. Sampling of a German sewage treatment plant

The solid fraction of activated sludge was obtained by filtration through glass fibre filters (GF8 Schleicher & Schuell, Dassel, Germany) of the slurry of activated sludge samples taken from the nitrification tank of the Wiesbaden STP. The samples were collected in glass bottles, homogenized, filtered and used immediately. Digested sludge was collected from the respective digester. The Wiesbaden STP (population equivalent: 350,000) consists of a preliminary clarification, a denitrification-nitrification cascade with an internal recirculation of sludge, and a phosphate removal by addition of $Fe(II)Cl_2$ into the final clarification. The total solid sludge retention time is 11–13 days. Primary and secondary sludge is fed into a mesophilic anaerobic digester. Further details can be found in Andersen et al. [27].

3. Extraction and clean-up

3.1. PLE of musk fragrances

For extraction, an automated ASE 200 from DIONEX (Sunnyvale, CA) was used. An aliquot of freeze-dried sludge (0.2 g) was placed into 11 mL stainless steel extraction cells from Dionex and was thoroughly mixed with ∼10 g of quartz sand. The extraction solvent was methanol. The selected operating conditions included: extraction temperature, 100 °C; extraction pressure, 100 bar; pre-heating period, 5 min; static extraction period 5 min; number of extraction cycles, 2; solvent flush, 100% of cell volume; nitrogen purge 30 s. The final extraction volume (20 mL) was quantitatively transferred (rinsed with ∼80 mL of de-ionized water in 2–3 portions) to a 1000 mL volumetric flask and filled with 900 mL de-ionized water. The samples were shaken and spiked with 500 ng of the surrogate standard Tonalid-D_3 (AHTN-D_3 from Dr. Ehrenstorfer, Augsburg, Germany) (50 μL from a 10 ng/μL solution). Because PLE extractions were done at elevated temperature, potential thermal degradation of the PMF was checked by spiking and recovery experiments on purified sand. The spiked sand was extracted twice for 5 min at 100 °C. Procedural blanks (quartz sand) were extracted for each extraction series to control the laboratory contamination. Multiple sequential extractions of the same sludge sample were conducted to assure for quantitative extraction.

3.2. Ultrasonic solvent extraction (USE)

An aliquot (PMF: 0.2 g, others: 0.5 g) of freeze-dried sludge was extracted successively with 4 and 2 mL methanol and then two times (musk fragrance: three times) with 2 mL acetone. In a subsequent extraction step with 2 ml acetone none of the analytes could be detected anymore. In each extraction step, the sample slurry was ultrasonicated for 5 min. The sludge was centrifuged at 19,000 rad/min for 5 min and the supernatants combined. Surrogate standards (musks: