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Phosphodiesterase type V inhibitors: Occurrence and fate in wastewater and sewage sludge

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ABSTRACT

The contamination of wastewater and sewage sludge has been examined for three phosphodiesterase type V inhibitors sildenafil, vardenafil and tadalafil, active agents of Viagra®, Levitra® and Cialis®, respectively. Sensitive quantification methods based on solid-phase extraction (SPE) and pressurized liquid extraction (PLE) followed by high performance liquid chromatography – tandem mass spectrometry (HPLC–MS/MS) have been developed to analyse these compounds in wastewater and sewage sludge.

Effluent water of nine sewage treatment plants (STPs) has been analysed to assess the impact of the phosphodiesterase type V inhibitors on the environment. One municipal STP (Tarragona, Spain) has been thoroughly studied over the year 2008 (i) with respect to the distribution of these compounds among influent and sewage sludge and (ii) the elimination efficiency.

The developed methods allowed quantification at trace concentrations. Sildenafil was present in all investigated samples at the low ng/L and ng/g range, respectively. Tadalafil was not detected or below the limit of detection (LOQ) in effluent water taken in Spain but in sewage sludge (12 ng/g – < LOQ). Vardenafil was present only in one sludge sample and between 5 ng/g and < LOQ in effluent water. The overall removal efficiency of the STP in Tarragona (Spain) is 68%, 69% and 80% for sildenafil, tadalafil and vardenafil, respectively. This study shows for the first time the determination of these compounds in wastewater and sewage sludge.

1. Introduction

Sewage water contains various pollutants, including a broad spectrum of pharmaceutically active compounds that can be discharged into the environment via the sewage treatment plant (STP) effluent water (Daughton and Ternes, 1999). Besides, reports about stimulating drugs like cocaine and amphetamines, e.g. 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) in natural waters (Castiglioni et al., 2006; Boleda et al., 2007; Huerta-Fontela et al., 2007) have attracted attention to the scientific community. The consumed amount of stimulating drugs is usually higher than legally prescribed quantities. Another class of drugs which are often illegally acquired are the active agents of Viagra®, Levitra® and Cialis®

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namely sildenafil citrate, vardenafil and tadalafil, respectively. They are used in therapies for erectile dysfunction acting as a selective inhibitor of phosphodiesterase type V which cleaves cyclic guanosine monophosphate (cGMP) (Berzas et al., 2002; Daraghmeh et al., 2001). Viagra®, or sildenafil citrate, was devised to treat pulmonary hypertension, or high blood pressure in arteries of the lungs. The drug works by suppressing the enzyme that controls blood flow, allowing the vessels to relax and widen. The same mechanism facilitates blood flow into the penis of impotent men. In the case of athletes, increased cardiac output and more efficient transport of oxygenated blood to the muscles can enhance endurance. For that reason, sildenafil has been recommended to be listed as a doping substance (Longman, 2008).

Until now, no information can be found in literature concerning the concentration of phosphodiestarase type V inhibitors in environmental samples. However, these compounds have been determined in dietary supplements for male sexual potency (Zhu et al., 2005), in pharmaceutical samples (Berzas et al., 2002; Abad-Elbary et al., 2004) and in biological samples such as plasma (Cooper et al., 1997; Eerkes et al., 2002; Al-Ghazawi et al., 2007; Kim et al., 2003). Sildenafil is readily absorbed and metabolized in the liver to desmethylsildenafil by cytochrome P450 3A4 (Eerkes et al., 2002). Several authors (Cooper et al., 1997; Eerkes et al., 2002; Al-Ghazawi et al., 2007; Kim et al., 2003) provide information about this principal metabolite, which is present at 30–40% of the parent drug concentration in plasma after single oral dosing (Cooper et al., 1997).

For the determination of sildenafil in biological tissues and in dietary human supplements, gas chromatography (GC) has been applied (Berzas et al., 2002), but the polarity of sildenafil, vardenafil and tadalafil, favours high performance liquid chromatography (HPLC) (Zhu et al., 2005; Cooper et al., 1997; Eerkes et al., 2002) as separation technique. For detection, tandem mass spectrometry is most appropriate since it exhibits both, high sensitivity and selectivity (Weinmann et al., 2001).

Since the concentrations of the target analytes in environmental samples are expected to be in the low ng/L range, an efficient sample preparation to extract the analytes from the respective matrix is required. For aqueous samples solid-phase extraction (SPE) is widely used and has already been applied to extract sildenafil from rat serum (Guermouche and Bensalah, 2006). For solid samples pressurized liquid extraction (PLE) has successfully used to determine pharmaceutical compounds in sewage sludge (Nieto et al., 2008, 2009; Chu and Metcalfe, 2007).

The aim of this work was to develop a method to determine the phosphodiesterase type V inhibitors sildenafil, tadalafil and vardenafil in influent and effluent wastewater as well as in sewage sludge. For sample preparation SPE and PLE have been optimized and applied prior analytical detection with HPLC – tandem mass spectrometry. The analytical method was applied to determine these compounds in grab effluent wastewater samples from different STPs in Germany and Spain ($n = 9$). The STP in Tarragona (Spain) has been studied thoroughly. Influent and effluent wastewater as well as sewage sludge have been analysed in order to assess the elimination efficiency on the one hand, and the contribution of STPs to the contamination of the aquatic environment with these phosphodiesterase type V inhibitors on the other hand.

2. Experimental

2.1. Reagent and standards

All solvents were HPLC-grade. Hexane, acetonitrile, acetic acid and aluminum oxide were obtained from Merck/VWR-International (Darmstadt, Germany), methanol was from Merck/VWR-International and SDS (Peypin, France), respectively. Acetone and dichloromethane were from SDS (Peypin, France), nitrogen was purchased from Carburos Metálicos (Tarragona, Spain).

SPE cartridges (OASIS-HLB 60 mg) were from Waters (Eschborn, Germany). Fluazifop-butyl served as an internal standard (IS) and was supplied by Sigma Aldrich (Gillingham, UK). Sildenafil, vardenafil and tadalafil were pharmaceutically grade in tablet form with concentrations of 50 mg of sildenafil and 10 mg of tadalafil and vardenafil.

Stock solutions were prepared by dissolving each compound in methanol at a concentration of 1 g/L and stored at −20 °C. Working solutions were prepared weekly by diluting this solution with acetone. Ultra pure water was obtained with a Milli-Q water purification system (18.2 MΩ cm) (Millipore, Bedford, MA, USA).

2.2. Sampling

In order to check the approximate concentration of sildenafil in the effluent water, grab samples ($n = 8$) have been investigated from the STP of Beuerbach (Germany). Further, nine municipal STPs were sampled. They were divided in two groups: (i) major cities (Frankfurt/Main, Darmstadt, Giessen and Wiesbaden in Germany, and Tarragona and Reus in Spain) and (ii) spas in Germany (Bad Orb, Bad Homburg von der Höhe and Bad Nauheim).

The STP of Tarragona has been thoroughly investigated. Twenty-four hours composite influent and effluent samples as well as sludge samples were taken every two month over the year 2008 and have been analysed for the target compounds. This STP receives mostly urban wastewaters and some industrial discharges (population equivalent approx. 140,000; $BOD_5 = 400$ mg/L). Aqueous samples were adjusted to pH 2 and stored at −25 °C before sample preparation. The average flow (calculated for 2008) was 529,000 m^3 per month for influent water and 516,000 m^3 per month for effluent water. The annual production of sewage sludge (dry weight, d.w.) in 2008 was 1915 t.

2.3. Sample preparation

For *SPE*, two different materials were tested to extract the target analytes from water, Waters OASIS-HLB and Waters OASIS-MCX. The extracted volume was 200 mL for effluent water and 100 mL for influent water. Elution of the analytes from OASIS-HLB was done with 1.5 mL acetone:ethylacetate (1:1, v:v); this step was repeated twice. When OASIS-MCX was used, the elution was carried out with 3 × 1.5 mL acetone.

The final preconcentration step based on OASIS-HLB. The sorbent was sequentially conditioned with 2 mL hexane, 6 mL