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Optimization of diclofenac quantification from wastewater treatment plant sludge by ultrasonication assisted extraction

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ABSTRACT

A rapid quantification method of diclofenac from sludge samples through ultrasonication assisted extraction and solid phase extraction (SPE) was developed and used for the quantification of diclofenac concentrations in sludge samples with liquid chromatography/tandem mass spectrometry (LC–MS/MS). Although the concentration of diclofenac in sludge samples taken from different units of wastewater treatment plants in Istanbul was below the limit of quantification (LOQ; 5 ng/g), an optimized method for sludge samples along with the total mass balances in a wastewater treatment plant can be used to determine the phase with which diclofenac is mostly associated. Hence, the results will provide information on fate and transport of diclofenac, as well as on the necessity of alternative removal processes. In addition, since the optimization procedure is provided in detail, it is possible for other researchers to use this procedure as a starting point for the determination of other emerging pollutants in wastewater sludge samples.

1. Introduction

The presence of pharmaceuticals and their metabolites in surface waters, ground water, soil, or sediment is mainly due to the discharge of municipal wastewater treatment plant (WWTP) effluents. The occurrence and detected concentration of emerging contaminants in surface waters and ground water samples has prompted public interest regarding potential adverse ecological effects and potential contamination of drinking water sources [1,2]. Non-steroidal anti-inflammatory drugs are one of the most widely detected pharmaceutical groups in the environment [3–6]. Among them, diclofenac is a commonly used over-the-counter anti-inflammatory drug worldwide [7]. Even though only 15% of diclofenac administered is excreted as the unchanged form [8], diclofenac is frequently detected in wastewaters.

Studies point out that certain and suspected effects of diclofenac on different organisms occur even at low concentrations [9–14] and that it has the highest acute toxicity on aquatic organisms among anti-inflammatory drugs [15]. Moreover, diclofenac is listed in the high priority category by Global Water Research Coalition [16].

Diclofenac is not readily biodegradable [17] and hence high concentrations of diclofenac are discharged from WWTPs to the environment [18]. Diclofenac have been reported to be present in ground water and surface waters at significant levels [19–24], even though it is susceptible to photolysis in surface waters [25]. The results of studies showed that diclofenac concentration in most of the investigated WWTPs effluents exceeded the proposed Environmental Quality Standards (0.1 μg/L) [26] thus indicating that the fate of diclofenac in municipal WWTPs needs to be further evaluated in order to improve its removal efficiency in existing WWTPs. Moreover, rapid measurement of diclofenac in WWTPs can also assist in providing more effective conceptual treatment alternatives and in the determination of environmental compartment(s) where diclofenac can pose a risk to the ecosystem.

The fate of a pollutant in a WWTP cannot be determined by its quantification in the dissolved phase alone. Thus, the investigation of the fate of pollutants in WWTPs requires knowledge on the presence and speciation of these compounds in both liquid and solid phases to identify whether the removal from the liquid phase is due to biological processes or sorption to the sludge phase [27]. Mass balances based on only the dissolved phase may lead to significant errors if sorption to the sludge plays a significant role during removal of pollutants. In addition, the possibility of agricultural application of WWTP sludge requires careful assessment of micropollutants in the sludge phase [28–30].

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In the literature, there is a lack of knowledge on the occurrence and fate of pharmaceuticals in solid matrixes (e.g. sludge, sediments) [31–35] with most of the studies focusing on quantification of diclofenac in the liquid phase. The reason for the lack of studies dealing with both liquid and solid phases [18,36] can be attributed to current limitations of analytical measurement techniques for complex matrices [37,38].

The most frequently used extraction methods for sludge samples are Microwave Extraction (MWE), Accelerated Solid Extraction (ASE), Pressurized Liquid Extraction (PLE) and Ultrasonic Extraction (USE) [39]. USE is the most practical technique because there is no need for special equipment, but can be both time and solvent consuming [38,40]. However, solvent consumption and extraction duration can be decreased by optimizing factors that affect extraction recovery (e.g. extraction time, extraction solvent type, extraction solvent volume, number of extraction cycles, and amount of sludge sample). In the literature, some studies only report the extraction procedure and do not present details of the optimization procedure [34–42], while others focus on only a limited number of factors affecting the extraction procedure (e.g. solvent type, sample amount) [40,43–45]. Therefore, the aim of this study was to establish an easy-to-apply and efficient USE method for the extraction of diclofenac from WWTP sludge. The applicability of the optimized extraction method was assessed with WWTP sludge samples taken from different units of the WWTPs.

2. Materials and methods

2.1. Reagents

All of the solvents used for USE, SPE and LC–MS/MS measurements were HPLC grade (Sigma–Aldrich, USA) except for LC–MS grade water (Merck, Germany). Diclofenac sodium salt (98%; CAS 15307-79-6) and isotope labeled diclofenac-d_4 used as internal standards were purchased from Sigma–Aldrich (Steinheim, Germany) and C/D/N Isotopes (Canada), respectively. Stock solutions of diclofenac and diclofenac-d_4 were prepared in methanol (MeOH) and working solutions were prepared daily by diluting stock solutions in LC–MS grade water. All non-volumetric glassware used for preparation of solutions and recovery experiments was kept at 550 °C for 3 h after rinsing with MeOH and deionized water (DI).

2.2. Sample preparation

Sludge samples were collected from two municipal WWTPs located in Istanbul, Turkey. All sludge samples, except the ones taken from drying and centrifugation units, were centrifuged at 9000 rpm for 10 min to concentrate the sludge phase and then all samples were freeze dried (ThermoSavant, Modulyo D, USA) and stored at −20 °C. Samples were weighed before the application of USE.

Recovery studies were performed with the sludge sample taken from the drying unit of the WWTP-II. 2 mL MeOH was added to freeze dried sludge samples before spiking diclofenac and diclofenac-d_4 to have a homogenous concentration distribution. Then samples were mixed for 5 min with vortex. USE was applied to the samples which were kept overnight in a darkened fume hood to evaporate MeOH.

2.3. Ultrasonic Extraction (USE)

The performances of the ultrasonic homogenizator (Bandelin, Sonoplus HD 2200, Germany) and ultrasonic bath (Intersonik, MIN12, Turkey) were tested for the extraction of diclofenac from sludge samples. Experiments were performed in a basic ultrasonic bath with a 25–40 kHz ultrasonic frequency. The temperature during extraction was kept constant at room temperature. Since there are numerous combinations to test the factors affecting extraction efficiency, a systemic approach was used for optimization. A method was selected from literature [46] and several factors (e.g. optimum sludge amount, type and volume of extraction solvent, ultrasonication time, extraction cycle), were optimized in a stepwise manner. All sludge samples were ultrasonicated in 50 mL Teflon centrifuge tubes (Nalgene, USA). At the end of the solvent extraction, collected supernatants were evaporated to dryness at 45 °C by using a rotary evaporator (Heidolp, Laborota 4000, Germany). The test conditions for extraction are provided for each optimization step in related sections.

2.4. Solid phase extraction (SPE)

SPE was applied at the end of the USE process in order to clean-up and concentrate the samples. Several types of SPE cartridges (Oasis HLB cartridge 6 cc/200 mg; Oasis MCX Cartridges 3 cc/60 mg (Waters, Millford, MA, USA); Cleanert PEP-H cartridge 6 cc/200 mg; Cleanert PAX cartridge 6 cc/200 mg; Cleanert PCX 6 cc/200 mg (Bonna-Agela, Willmington, DE, USA)) were tested (data not shown). Oasis HLB cartridge (6 cc/200 mg) which provided the best recovery was selected for SPE analysis. An automatic SPE vacuum manifold (VacMaster, Biotage, NC, USA) was used in order to have homogeneous extraction efficiency. MeOH, MeOH:acetonitrile (ACN), MeOH:acetone (Ace), methyl tert-butyl ether (MTBE) and ethyl acetate (EtOAc) were tested as eluent by using DI spiked with diclofenac and diclofenac-d_4. Glassware and PTFE lined materials were used in order to prevent adsorption of diclofenac.

2.5. Liquid chromatography/tandem mass spectrometry (LC–MS/MS)

Diclofenac was measured by using Thermo Electron Cooperation TSQ Quantum Access triple quadruple mass spectrometer coupled with Accela Ultra Performance Liquid Chromatograph (UPLC) with a Thermo Hypersil Gold column (1.2 mm × 100 mm; 1.9 μm; Thermo Scientific, Germany) and an electrospray ionization interface. LC–MS/MS method used for diclofenac measurement was modified from the study of Aydinli and Talinli [20]. Chromatographic separation (Table S1) and MS parameters (Table S2) were optimized for diclofenac measurement. While quantification was made by using transitions of 294–214 m/z for diclofenac and 298–253.9 m/z for diclofenac-d_4, confirmations of these ions were provided with transitions of 294–249.7 m/z and 298–281.6 m/z for diclofenac and diclofenac-d_4, respectively. A chromatogram example for the optimized conditions can be found in Fig. S1.

2.6. Quality assurance and control

All samples were injected three times and relative standard deviations (RSD) were below 20%, which indicates a high precision for this method. Diclofenac-d_4 was used as an internal/surrogate standard for quantification of diclofenac. Correlation coefficient (R^2) of calibration curves used for the quantification were always greater than 0.995. Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined using signal to noise ratios of 3 and 10, respectively.

The accuracy of this method was tested by performing recovery experiments. Sludge samples were spiked with 100 or 400 ng/g diclofenac (corresponding to low and high concentration levels, respectively) and 50 ng/g diclofenac-d_4. The extraction method was optimized by determining the conditions that had the highest recovery (≥80%). For method validation, diclofenac was quantified