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Research article

Application of fluorescence polarization immunoassay for determination of carbamazepine in wastewater

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ABSTRACT

Carbamazepine is an antiepileptic drug that can be used as a marker for the cleaning efficiency of wastewater treatment plants. Here, we present the optimization of a fast and easy on-site measurement system based on fluorescence polarization immunoassay and the successful application to wastewater. A new monoclonal highly specific anti-carbamazepine antibody was applied. The automated assay procedure takes 16 min and does not require sample preparation besides filtration. The recovery rates for carbamazepine in wastewater samples were between 60.8 and 104% with good intra- and inter-assay coefficients of variations (less than 15 and 10%, respectively). This automated assay enables for the on-site measurement of carbamazepine in wastewater treatment plants.

1. Introduction

A large variety of pharmaceuticals enter wastewater treatment plants (WWTPs) where many of them are not efficiently removed. Due to the disposal of treated wastewater into surface water, a high amount of various pharmacologically active compounds are found in surface waters, that may influence the ecosystem and the natural organization (Osorio et al., 2016). Through irrigation with treated wastewater, pharmaceuticals can also be found in vegetables (Miller et al., 2016; Wu et al., 2014). Therefore additional sewage treatment stages are under development. Several approaches like ozonation, hydrodynamic-acoustic cavitation, heterogeneous Fenton-like reactions, production of singlet oxygen and other reactive oxygen species, enhanced biodegradation, pulsed corona discharge and activated carbon filtration have shown high efficiency for reducing the load of micropollutants (Andreozzi et al., 2002; Banaschik et al., 2015; Bräutigam et al., 2012; Eggen et al., 2014; Fernández et al., 2016; Nadejde et al., 2015; Rosal et al., 2008). A method for verification and monitoring of the cleaning efficiency directly in the WWTPs would be desirable for an effective control of those additional purification steps. Monitoring of all pharmaceuticals would obviously not be possible due to their large number. Therefore, a suitable indicator should be considered.

Carbamazepine (CBZ) has often been reported as a marker for wastewater input into water bodies (Bahlmann et al., 2012; Clara et al., 2004; Dickenson et al., 2011; Gasser et al., 2011; Kahle et al., 2009). This antiepileptic drug is excreted by humans to about 14% in non-metabolized form and enters in this way or through incorrect disposal of pills and tablets via the toilet the water cycle (Bahlmann et al., 2014). Once CBZ has arrived in surface water, it can negatively influence the health status of aquatic organisms (Almeida et al., 2014, 2015; Ferrari et al., 2003; Freitas et al., 2016, 2015a, 2015b; Tsiaka et al., 2013). During common wastewater treatment, mostly less than 30% of CBZ is degraded due to its low biodegradability (Andreozzi et al., 2002; Joss et al., 2005; Ternes, 1998; Zhang et al., 2008). On the contrary, even higher CBZ concentrations were found in effluent than in influent samples of WWTPs due to degradation of CBZ metabolites (Bahlmann et al., 2014; Zhang et al., 2008). CBZ is usually found in any wastewater sample, what illustrates the ubiquitous occurrence of this substance (Bade et al., 2015; Bahlmann et al., 2014; Rossmann et al., 2014). A degradation rate of CBZ of almost 100% through

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additional, especially oxidative, purification steps has been proven in several studies (Andreozzi et al., 2002; Bräutigam et al., 2012; Eggen et al., 2014; Nadejde et al., 2015). Therefore, CBZ can be used as a marker for an effective elimination of micropollutants from wastewater.

For prompt monitoring of this marker, a system is required that enables for on-site measurements. One approach is the immunoanalytical determination of CBZ. Several studies for CBZ determination in water samples using antibodies for detection have been reported. Heterogeneous enzyme immunoassays have been used, which are very sensitive but do not offer the possibility of an on-site measurement due to several long incubation and washing steps (Bahlmann et al., 2009, 2012; Calisto et al., 2011; Grandke et al., 2013a; Wade et al., 2015). Fluorescence polarization immunoassay (FPIA), as a homogeneous assay, does not require these steps and therefore could prove capability for on-site monitoring. The assay is based on the change of fluorescence polarization of a fluorophore-labeled analyte when it is bound to an analyte-specific antibody. This labeled analyte, the so-called tracer, competes with the analyte from the samples for the antibody binding sites. The principle of this assay has been described in detail many times (Jameson and Ross, 2010; Maragos, 2009; Smith and Eremin, 2008). For the determination of CBZ, FPIAs have been previously developed for the application to serum and to surface water (Lin et al., 2012; Oberleitner et al., 2015).

Recently, a new monoclonal anti-CBZ antibody was produced and characterized (Oberleitner et al., 2016). This antibody showed low cross-reactivity against other pharmaceuticals like cetirizine, loratadine or opipramol. In previous studies, using another, commercially available antibody, cetirizine led to high overestimation of CBZ in water samples (Bahlmann et al., 2009, 2011; Grandke et al., 2013a). Due to the low cross-reactivity of the new antibody towards relevant environmental pollutants, this antibody offers the opportunity for a more accurate CBZ determination in environmental samples. The applicability of this antibody to wastewater samples using FPIA was to be verified in this study. To the best of our knowledge, this is the first time that a FPIA is used for CBZ determination in wastewater. Actually only one FPIA for wastewater analysis has been reported until now using a preconcentration by solid-phase extraction (Sanchez-Martinez et al., 2007). The difficulty with the application of FPIA to this matrix lies in the complexity of wastewater, which contains a lot of different ingredients like salts, proteins and pharmaceuticals in a wide concentration range. Thus, one of the prerequisites for on-site measurements, the avoidance of washing steps, is at the same time the main problem that needs to be solved for the application of FPIA to this complex matrix.

2. Material and methods

2.1. Reagents

All solvents and chemicals were purchased from Sigma-Aldrich, Merck KGaA, Serva, AppliChem GmbH and J.T. Baker. The tracer CBZ-triglycine-5-(aminoacetamido)fluorescein (CBZ-AAF) was previously synthesized (Oberleitner et al., 2015). Calibrators, dilutions and the following buffers were prepared in ultrapure water (Synthesis A10 Milli-Q® water purification system, Millipore): sample buffer (250 mmol/L glycine, 50 mmol/L sodium chloride, 0.5% disodium ethylenediaminetetraacetate dihydrate (EDTA), 35 mmol/L sodium hydroxide, pH 8.5), phosphate buffered saline (PBS, 10 mmol/L sodium dihydrogenphosphate, 70 mmol/L disodium hydrogenphosphate, 145 mmol/L sodium chloride, pH 7.6), tracer stabilization buffer (70% PBS, 20% glycerol, 10% methanol), antibody stabilization buffer (80% PBS, 20% glycerol, 0.2% sodium azide, 0.1% bovine serum albumin, 0.05% Tween20). CBZ calibrators for calibration and spiking were prepared gravimetrically in ultrapure water from a 1.15 g/kg methanolic stock solution.

2.2. FPIA in cuvettes

For FPIA measurements, aokin spectrometer FP 470 (aokin AG, Berlin, Germany) was used. The optical filter system in this instrument is designed for fluorescein tracer and is able to measure parallel and perpendicular intensities simultaneously and time-resolved. For instrument control and sample evaluation, aokin software mycontrol (ver. 4.1.12) was used. The spectrometer was connected to aokin liquid handling workstation (LHW) for automated assay performance.

For the CBZ FPIA, all steps were performed automatically. Here, the optimized protocol is described. First, 1.7 mL sample buffer were pipetted into the round-bottom cuvette, which contained a magnetic stir bar. The buffer background was measured for 5 s. Next, 100 μL CBZ calibrator or sample were added. The pipetting tube was rinsed with 100 μL sample buffer so in total a volume of 200 μL was added during this step. After measuring the sample background (SBG, 5 s), 100 μL tracer dilution, 1:20,000 in tracer stabilization buffer, were added, followed by 100 μL sample buffer. Subsequently, the fluorescence intensities of the free tracer were measured (5 s). Then 100 μL antibody BAM-mab 01 (CBZ) dilution in antibody stabilization buffer (1.5 μg/mL) were added and flushed again with 100 μL sample buffer. The total volume in the cuvette after this step was then 2.3 mL. The measurement time, after addition of antibody, was set to 600 s. In total, the assay procedure took 16 min, including automated rinsing of the cuvette.

Sixteen CBZ calibrators in the range of 0.01–40,000 μg/L were measured in triplicate for setting up the sigmoidal calibration curve and a precision profile determined as the relative error of concentration according to Ekins (1981). The measurement range was defined as the range with relative errors of concentrations less than 30% as described previously (Grandke et al., 2013a, 2013b; Oberleitner et al., 2014). For the calibration and evaluation of sample concentrations with the software mycontrol, point-to-point interpolation is applied. For this, seven CBZ calibrators (2.5–180 μg/L) were measured in triplicate. Additionally, a low CBZ calibrator (0.01 μg/L) was taken into consideration to have a reference point for CBZ concentrations that are below the calibration range. All samples were measured in triplicate. The concentrations were determined over a time range from 400 to 550 s after the addition of antibody. Single measurements were repeated when the signals were too noisy (*e.g.* due to air bubbles in the cuvette). Approximately 10% of the sample measurements were repeated.

The degrees of polarization for calibration curves were calculated by using SBG-corrected fluorescence intensities and subtraction of the degree of polarization value of the free tracer. The G factor was fixed to 1.10. For evaluation of degrees of polarization of the free tracer, SBG-corrected fluorescence intensities were used. For samples, the degree of polarization was determined without any correction of fluorescence intensities and the G factor was set to 1. Total fluorescence intensities are given as the sum of parallel and double perpendicular intensity. For calculation of these values for the free tracer, again SBG-corrected intensities were used.

2.3. Sample preparation

The samples were obtained from four Berlin WWTPs, one influent and effluent sample from each. The samples were filtered through folded filters and then through glass fiber syringe filters (1–2 μm, neoLab, Heidelberg, Germany). The samples were stored at 4 °C.