

Assessment of sulfamethoxazole UV-C/H₂O₂ oxidation: Elucidation and stability of transformation products

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Abstract

Antibiotics are now well-acknowledged contaminants of emerging concern. Advanced Oxidation Processes (AOPs) have exhibited enhanced removal capacity of antibiotic microcontaminants from urban wastewater treatment plants (UWTP) matrices, and have been proved to be powerful treatment processes for the removal of organic persistent and biologically-recalcitrant compounds (Michael. *et al.*, 2013). At the same time, the available scientific literature has extensively dealt with the optimization of the evaluated technologies for the removal and optimization of individual compounds, and at a lesser extent with the identification and structural elucidation of their transformation products (TPs). Sulfamethoxazole (SMX) is one of the most widely prescribed sulfonamide drugs (Hu *et al.*, 2007). It has been frequently detected in surface and drinking waters as well as in effluents of UWTPs (Hu *et al.*, 2007; García-Galán *et al.*, 2008; Bahnmüller *et al.*, 2014). The literature reports degradation and transformation of SMX by different treatment processes including, among others, phototransformation under simulated sunlight (Trovó *et al.*, 2009a; Gmurek *et al.*, 2015), solar photo-Fenton (Trovó *et al.*, 2009b), low and medium pressure UV and UV/H₂O₂ treatment (Lekkerkerker-Teunissen *et al.*, 2012), ozonation (Gómez-Ramos *et al.*, 2011) and sulfate radical anion oxidation (Ahmed *et al.*, 2012). Within this context, the aim of this study was the investigation of the fate and transformation of SMX during UV-C/H₂O₂ oxidation. The experiments were run in a photochemical apparatus, batch type bench-scale cylindrical reaction vessel with a capacity of 600 mL and a 9 W low-pressure mercury lamp. Two different initial concentrations of SMX were tested; an environmentally relevant concentration of 100 µg L⁻¹ and a sufficiently high concentration of 2 mg L⁻¹. The low enough concentration of 100 µg L⁻¹ was used to simulate real environmental conditions. Prior to TPs analysis, the treated samples were extracted by means of solid-phase extraction (SPE) in order to preconcentrate the candidate TPs and acquire their MS/MS spectra. The significantly higher concentration of 2 mg L⁻¹ (compared to the concentration of SMX in wastewater effluent samples) was

applied during the UV-C/H₂O₂ oxidation experiments in order to enable slower degradation kinetics and facilitate the identification of TPs. For each initial concentration of the parent compound, the treatment process was optimized with respect to the initial oxidant dose. Under the optimum oxidant dose, the time interval samples were also analyzed for the structural elucidation of TPs of SMX. The identification of the formed TPs was based on an integrated Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS)-based workflow, using both suspect and non-target screening approaches. Analysis was performed by both reversed phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) in order to investigate their complementarity for the detection of more polar compounds. To the authors' knowledge, this is the first work dealing also with an environmentally relevant initial spiked concentration of SMX in order to assess the formation, persistence and stability of its TPs. The two-step post-acquisition data processing approach which was employed to detect and identify the candidate TPs of SMX was based on the study of Beretsou *et al.* (2016) but it was adjusted for the objectives of our research. As a first step, a suspect database of plausible TPs was compiled by using two different *in silico* prediction tools: (1) the Eawag-Biocatalysis/Biodegradation Database Pathway Prediction System (Eawag-BBD/PPS) (<http://eawag-bbd.ethz.ch/predict/>), an artificial intelligence system, which predicts microbial metabolic reactions based on biotransformation rules set in the Eawag-BBD and scientific literature. Eawag PPS was used with the "relative reasoning mode" switched off, and (2) the MetabolitePredict software (Metabolite Tools 2.0, Bruker Daltonics, Bremen, Germany), a rule-based expert system, which predicts metabolites from Phase I, II and Cytochrome P450 reactions. The prediction results from both programs included the molecular formula as well as the structures of the generated TPs from two subsequent reactions in the metabolic pathway. However, since oxidative reactions are predominant in UV-C/H₂O₂ oxidation, TPs resulting either from the oxidation of the parent compound or through the breakdown of the parent compound's structure are expected to be formed and thus,

only those TPs were included in the suspect database. Already known and reported TPs from the literature were also added to the suspect database (Trovó *et al.*, 2009a; Trovó *et al.*, 2009b; Gómez-Ramos *et al.*, 2011; Ahmed *et al.*, 2012; Lekkerkerker-Teunissen *et al.*, 2012; Gmurek *et al.*, 2015). All samples taken at different sampling times (time interval samples) were screened in full scan, in both chromatographic systems and in both ionization modes, for the detection of suspect TPs from the database. The criteria used for the reduction of features in both chromatographic modes included a threshold in peak area, a threshold in intensity counts, a threshold in mass accuracy of ± 5 ppm on the monoisotopic peaks and the existence of a good isotopic pattern fit. Additional criteria for the identification of the suspects were the existence of a meaningful time trend during the batch experiments, their absence (or presence at very low levels) in the zero-time samples, the blank and the control samples. As a second step, samples were also screened for additional TPs not present in the suspect database, following a non-target approach. Background subtraction and peak picking were carried out using Metabolite Detect (Metabolite Tools 2.0, Bruker Daltonics, Bremen, Germany) in order to find TPs present in the treated samples, but absent in the control samples, and that showed a meaningful time trend. Chromatograms of the treated samples were compared with those of the control samples. Structural elucidation of both suspect and non-target TPs was based on the use of characteristic fragmentation (i.e. fragmentation pattern) during data-dependent MS/MS fragmentation events. The level of confidence for the identification of the detected compounds was determined according to Schymanski *et al.* (2014). Parallel to the removal of SMX (initial spiked concentration 2 mg L^{-1}) during the UV-C/H₂O₂ oxidation, six TPs were formed and identified, in total, through the use of the suspect and non-target screening approaches. All TPs were detected in the ESI(+) mode, while analysis performed in ESI(-) mode did not reveal any additional TPs. All TPs were detected in both RPLC and HILIC systems. The TPs of SMX upon UV-C/H₂O₂ oxidation were mainly formed, either by the cleavage of the parent compound structure or by its hydroxylation and they were tentatively identified according to their fragmentation pattern in ultrapure water and secondary treated wastewater samples. A transformation pathway of SMX during UV-C/H₂O₂ oxidation was proposed exploring the effect of the initial antibiotic concentration on the stability of TPs. The choice of the initial analyte concentration may not only impact the kinetics of removal but also the transformation pathways.

Keywords: sulfamethoxazole; transformation products; UV-C/H₂O₂ oxidation; liquid chromatography/quadrupole-time-of-flight mass spectrometry

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