Science of the Total Environment 642 (2018) 954–967

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transformation products of amoxicillin and ampicillin after photolysis in aqueous matrices: Identification and kinetics

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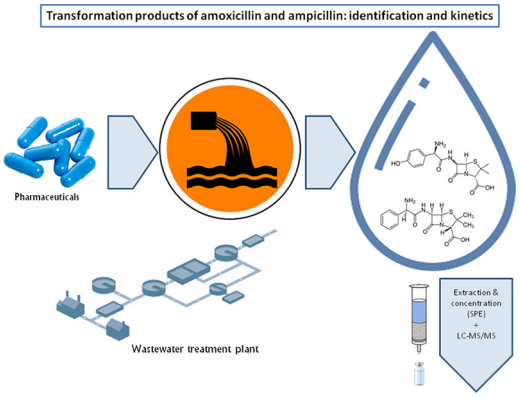
HIGHLIGHTS

- Degradation of amoxicillin/ampicillin was studied under photolysis conditions.
- A database of 65 transformation products of amoxicillin and ampicillin was build.
- Real samples of surface water and wastewater were analyzed searching all compounds.
- All data have been obtained using high-resolution mass spectrometry.
- The presence of several TPs of these beta-lactams was demonstrated in all samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 April 2018
Received in revised form 5 June 2018
Accepted 10 June 2018
Available online xxxx

Keywords:
Amoxicillin
Ampicillin
Emerging contaminants
Environmental analysis
High resolution mass spectrometry
Transformation products

ABSTRACT

Antibiotics are widely used in human medicine and veterinary production. Residues of these compounds reach the water sources through waste or direct application (e.g. aquaculture). The constant input of the parent drugs and their transformation products into the environment leads these pharmaceuticals to be considered as emerging pollutants. For some molecules, the pathway of degradation and formation in products is less known. To assess the impact of these substances in the environment and in the human health, it is necessary to elucidate the transformation products and their kinetic of degradation to evaluate the possible risks. In the present report, the characterization and the degradation kinetic of two widely used β-lactams antibiotics – amoxicillin and ampicillin – was evaluated. Surface water samples containing these antibiotics were submitted to photolysis and analyzed by liquid chromatography coupled to mass spectrometry with Orbitrap detection in order to establish the profile of degradation and the formation of transformation products. Results showed that the degradation of amoxicillin and ampicillin is almost complete and reach their maximum at 48 h in river water. Moreover, a database containing >65 transformation products of amoxicillin and ampicillin was build and real samples of industrial wastewater were analyzed to investigate the occurrence of amoxicillin, ampicillin and their transformation products.

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https://doi.org/10.1016/j.scitotenv.2018.06.122
0048-9697/

1. Introduction

In recent years, great attention has been paid to the emerging contaminants (ECs). These substances are characterized by their constant input into the environment. As a consequence and despite their - in some cases – low stability, they are considered pseudo persistent (Gavrilescu et al., 2015). Currently, ECs are defined as chemical compounds that are not regulated and whose impact on the environment and human health is poorly understood yet. Among the ECs, pharmaceuticals and personal care products are those most studied. The major concern about these compounds is their impact on the water sources. In the case of pharmaceuticals, even very low amounts can exhibit undesirable effects in non-target species. This is especially true for pharmacological classes such as antibiotics and hormones (Naidu et al., 2016).

Amoxicillin (AMX) and ampicillin (AMP) are antibiotics from the β-lactam class. These pharmaceuticals are widely used in human medicine and animal production. Even low doses of these compounds can show intense biological effects. Several authors have reported the biological activity of the antibiotics in the environment (Aga et al., 2016; Boxall et al., 2003; Majewsky et al., 2014). In some cases, not only the parent compound is biologically active, but also its transformation products (TPs), generated by both metabolism and other degradation processes (Majewsky et al., 2014).

Know the degradation and transformation pathways of the ECs is crucial for several reasons: 1) to assess the risk associated with the ECs and their TPs when they reach the environment, 2) to determine the toxicity of unknown derivatives and, 3) to study processes to promote the removal or the complete degradation of the ECs to non-hazardous compounds (Da Silva et al., 2013; Deblonde et al., 2011).

Several authors reported the presence of AMX and AMP in urban wastewater, surface water and groundwater. An average amount of 127.49 ng L^{-1} of AMX in wastewater was reported by Kim et al. (Kim et al., 2018). Amoxicillin-diketopiperazine-2′, 5′ was firstly reported in wastewater and effluent samples by Lamm et al. (Lamm et al., 2009). AMX and several transformation products were detected in surface water by Pérez-Parada et al. (Pérez-Parada et al., 2011). The concern about the presence of AMX, AMP and their degradation products led researches to develop and evaluate removal methods. Numerous techniques have been evaluated for the removal of these compounds from aqueous matrices, such as ozonation (Andreozzi et al., 2005), deionization using polythylenediimine (Martin et al., 2016), ozonation with UV radiation, homogeneous catalytic ozonation and homogeneous photocatalytic ozonation (Souza et al., 2018).

It is well known that the metabolism of AMX has two major products: amoxicilloic acid and amoxicillin piperazine-2,5-dione (DIKETO). These compounds do not display antibiotic activity, however, the amoxicilloic acid could have potential allergic properties (Reyns et al., 2008). Some minor products were obtained after acid hydrolysis (Nägele and Moritz, 2005). Pérez-Parada et al. reported a degradation study of AMX under acidic and alkaline conditions (pH 2 and 10, respectively): degradation of AMX was spontaneous and fast under both conditions (<5 min). A complete degradation of AMX in alkaline media was achieved in 24 h., whereas in acidic media, after 5 days residues of AMX were still identified (Pérez-Parada et al., 2011). For AMP, the transformation processes are very similar to those observed for AMX. The TPs identified for AMP generally show a difference of 16 amu from the equivalent product of AMX.

In terms of metabolism, usually a large extension of the β-lactams antibiotics dosage is excreted under unaltered form. In the case of milk from medicated animals, it was demonstrated that the thermal treatments applied to milk can produce TPs (Junza et al., 2014). For both AMX and AMP, the major metabolism products are the respective penilloic acids, penicilloic acids, and diketopiperazines. In the case of AMP, the formation of the two stereoisomers (3S,5R) and (3S,5S) epimers of ampicillin penilloic acid was demonstrated by Suwanrumpha et al. (Suwanrumpha and Freast, 1989).

In Brazil, there is few reports about the occurrence of AMX, AMP and their TPs in surface water and wastewater (Deschamps et al., 2012; Souza and Féris, 2016). In an industrial wastewater of a pharmaceutical company (producer of AMX), residues of AMX were found, suggesting that the effluent treatment (alkaline hydrolysis) was not efficient to complete removal/degradation of AMX and by-products (Deschamps et al., 2012). It is also important to highlight that Brazil is an important consumer market for these antibiotics: in the period 2004–2010, Brazil imported >6191 t of AMX and >561 t of AMP (De De Magalhães and Borschiver, 2012).

In order to elucidate the degradation's kinetics and pathway of AMX and AMP in natural ecosystems, river water samples were submitted to photolysis experiments under simulated sunlight. The degradation profile, as well as the tentative assignment of TPs structures, was achieved using liquid chromatography coupled to tandem mass spectrometry with Orbitrap detection. Moreover, the scientific literature reviewed was used to build a database of AMX and AMP TPs. The database developed was used to investigate the presence of AMX, AMP, and their TPs in real effluent and influent industrial wastewater samples from Brazil.

2. Experimental

2.1. Chemicals and reagents

Analytical standards of ampicillin trihydrate (AMP) and amoxicillin trihydrate (AMX) were purchased from Fluka AG (Buchs, Switzerland) with a minimum of 90% of purity.

Acetonitrile (ACN) and methanol (MeOH), all of HPLC grade, were purchased from Merck (Darmstadt, Germany) and J.T. Baker (Phillipsburg, NJ, USA), respectively. Formic acid (FA) was purchased from J.T. Baker. Water HPLC grade was supplied by Fischer Scientific.

Stock standard solutions of 1 mg mL^{-1} were prepared in HPLC grade water. The degradation profile of AMX and AMP were evaluated using solutions of 50 μg mL^{-1} of AMX or AMP and prepared with HPLC grade water and real river water. The average pH of river and HPLC water were 9.25 and 6.74, respectively. The average value of conductivity for river and HPLC water were − 125.3 and 4.6 mV, respectively.

The effect of the solvent was evaluated using solutions of 200 ng mL^{-1} of each antibiotic in HPLC water, ACN, H_2O:ACN (1:1), and H_2O:MeOH (1:1), each with or without 0.1% formic acid.

2.2. Photolysis experiments

The experiments of AMX and AMP photodegradation were carried out using simulated solar irradiation conditions, obtained through a Suntest CPS simulator (Heraeus, Hanau, Germany). The device was equipped with a xenon arc lamp and appropriate glass filters to restrict the irradiation transmission in wavelengths below 290 nm, resulting in a wavelength spectrum very similar to solar light. The lamp intensity was adjusted to an irradiance of 500 W m^{-2} corresponding to a light dose of 1800 kJ m^{-2} h^{-1}. The samples irradiated in the Suntest apparatus were contained in crimp-cap 20 mL quartz vials.

Aliquots of 50 μL were collected from the photolysis apparatus and diluted to 1.0 mL with H_2O:MeOH (95:5) and stored in the freezer in dark conditions until the moment of the analysis. Thus, the final nominal concentration of each antibiotic into the vials was 500 ng mL^{-1}. Initially, an aliquot was taken at 0, 15, 30, 60, 120 and 180 min. of the photolysis experiment; after that, samples were collected at 4, 6, 8, 10, 12, 14, 16, 20, 24, 48 and 72 h.

Samples were coded as LC (HPLC water) or RW (river water), followed by the time of sampling. Thus, for instance, AMX_RW4H means a sample of river water containing AMX and collected after 4 h of irradiation.

The samples from photolysis experiments were analyzed by UHPLC −Orbitrap-MS, using an Orbitrap Q Exactive (Thermo-Fisher, San Jose, CA) coupled to a Waters Acquity ultraperformance LC (UPLC) system