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Anticipating the fate and impact of organic environmental contaminants: A new approach applied to the pharmaceutical furosemide

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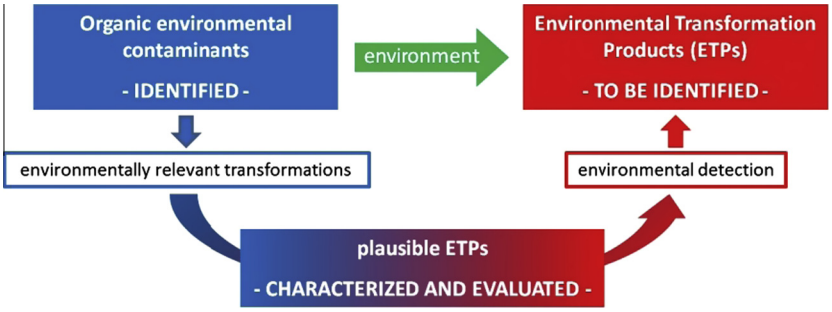
HIGHLIGHTS

- A multidisciplinary method able to anticipate the fate of environmental contaminants is presented.
- Electro-Fenton and bioconversion were used to obtain transformation products.
- A plausible environmental transformation product of the pharmaceutical furosemide revealed to be toxic.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of trace levels of organic contaminants in the environment is currently an environmental concern. When these contaminants are subjected to environmental transformations, environmental transformation products (ETPs) are obtained, whose structures often remain unknown. The absence of information concerning these new compounds makes them unavailable and consequently makes their environmental detection as well as their (eco)toxicological study impossible. This report describes a multidisciplinary approach that seeks to both anticipate the fate and evaluate the impact of organic environmental contaminants.

Our approach consists of three steps. First, isolated and fully characterized transformation products (TPs) of the parent molecule are obtained. In the second step, the parent molecule is subjected to environmentally relevant transformations to identify plausible ETPs. The detection of previously characterized TPs allows the concomitant identification of plausible ETPs. The third step is devoted to the toxicological evaluation of the identified plausible ETPs.

Such an approach has recently been applied to furosemide and has allowed the identification of its main TPs. This report now seeks to identify and evaluate toxicologically plausible ETPs of this drug, which is also known as an environmental contaminant.

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1. Introduction

Emerging pollutants are a class of compounds that result from human activity, are detected in the environment and whose impact on ecosystems remains unknown (Levi, 2009). These pollutants consist of compounds that were originally developed as pharmaceuticals and personal care products (Daughton, 2002; Khetan and Collins, 2007; Fatta-Kassinos et al., 2011a), bactericides or industrial additives (Jurado et al., 2012; Rodil et al., 2012). Regarding the pharmaceuticals, the progress made in trace analysis during the last decade (Buchberger, 2011) has allowed their detection and quantification in the effluents of sewage treatment plants (STPs), rivers, ground water, and sometimes even in drinking water (Heberer, 2002; Kuemmerer, 2004). The presence of pharmaceuticals in the environment mainly occurs after their excretion and results from an incomplete degradation in STPs (Deblonde et al., 2011). Despite their low environmental concentrations the continuous release of these substances makes them pseudo-persistent, thus presenting a risk of toxicity for non-target organisms (Daughton and Ternes, 1999).

Assessing the impact of the presence of drugs (i.e., molecules designed to exert a biological effect) on aquatic biota requires an ecotoxicity or a chronic toxicity study to be associated with a specific chemical structure (or alternatively, to a well-defined mixture of molecules). For this purpose, molecules detected in the environment have been tested in biological models (Henschel et al., 1997). However, the primary limitation of such studies is that they do not consider the environmental transformation products (ETPs), i.e., compounds stemming from the parent molecule and likely to appear in the environment consecutively to (a) biotic transformations. Attempts to identify ETPs from the analysis of complex environmental matrices, such as soils, sediments or biota, have been demonstrated to be prone to failure (Ibanez et al., 2004; Richardson, 2010). As a consequence, even powerful techniques like LC/MS in full-scan detection mode cannot achieve the detection limits required to characterize these unknown compounds that are present at trace levels. In the absence of characterization, the majority of ETPs remain ill-defined, thus ruling out the evaluation of their (eco)toxicities, which is problematic because the toxicity of ETPs may exceed that of their parent molecules (Dirany et al., 2011; Fatta-Kassinos et al., 2011b; Dirany et al., 2012).

In this report, we describe a new approach that enables both the identification of plausible ETPs and their toxicological evaluation. Because the main difficulty in studying the fate of drugs in the environment lies in the lack of authentic samples of their ETPs, we propose a new stepped approach. The first step consists of isolating the transformation products (TPs) of a drug that is known to be an environmental contaminant. This step, which requires the tools of organic synthesis, focuses first on providing fully characterized compounds assumed to be real (at least similar to) ETPs, and second developing methods for their detection in complex mixtures. In the second step, the contaminant is submitted to environmentally relevant transformations, i.e., processes that are representative of the transformations undergone by contaminants in the environment. By highlighting previously characterized TPs, this step allows the identification of plausible ETPs. In the third step, a toxicological study is initiated on each of the TPs that are identified as plausible ETPs.

In a recent work, we described the behavior of furosemide **1** (Fig. 1) under oxidative conditions (Laurence et al., 2011). This drug, which has been widely used as a diuretic since the nineteen sixties, is one of the forty compounds having the highest risk with a predicted environmental concentration greater than 100 ng L^{-1} (Besse and Garric, 2008). Poorly metabolized and eliminated either unmodified or as glucuronide conjugate (Lee et al., 1997) (which releases furosemide after hydrolysis), this drug has been detected at concentration ranges between 61 and 200 ng L^{-1} in European rivers (Castiglioni et al., 2006; Khalaf et al., 2009). During the course of our preliminary study, three TPs of furosemide were identified: furfural **2**, saluamine **3** (both resulting from the oxidation of the amino group) and a zwitterionic pyridinium **4** (resulting from the oxidation of the furan ring). These three TPs, which are obtained by anodic oxidation, have been isolated and fully characterized, thereby allowing their detection by LC–MS in complex mixtures.

This study seeks to confirm whether these three TPs, which were obtained without any environmental relevance, are indeed plausible ETPs. For this purpose, environmentally relevant degradation pathways were applied to furosemide. To be representative of the transformations likely to occur in the environment, two protocols were selected. The first protocol is abiotic and uses an electrochemical advanced oxidation process (electro-Fenton); the second protocol is biotic and requires microorganisms. The TPs identified as plausible ETPs were then subjected to a preliminary toxicological study.

2. Material and methods

2.1. Chemicals

Furosemide (>99.0%) was purchased from TCI Europe N.V. Furfural (99%) and 1-methyl-4-phenylpyridinium iodide were purchased from Sigma–Aldrich. Saluamine **3** and pyridinium **4** employed for the toxicological evaluation and required as standards for the analysis of complex mixtures were prepared as previously described (Laurence et al., 2011). Dulbecco's Minimal Essential Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, trypsin-EDTA solution and phosphate buffered saline (PBS) were purchased from GibcoBRL (France). The caspase fluorogenic substrate N-Acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethylcoumarin (Ac-DEVD-AFC) was purchased from Calbiochem (France). Sodium chloride (NaCl), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), triton X-100, dithiothreitol (DTT), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (Germany).

2.2. Analytical procedure

ESI–MS/MS analyses were conducted using a Q-TOF Premier instrument equipped with a Z-spray electrospray source (Waters, Saint Quentin-en-Yvelines, France) operating in the positive mode. For fragmentation studies, solutions were introduced into the electrospray ionization source using a syringe pump at an infusion rate of 10 μL min^{-1}. To investigate the degradation products, the sample was analyzed using a 2690 liquid chromatography module

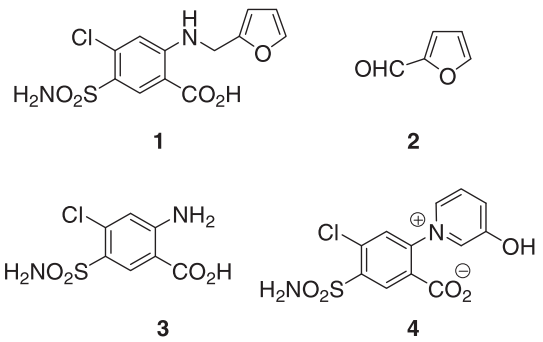

Fig. 1. Furosemide **1** and its TPs.