Analytica Chimica Acta 810 (2014) 61–70

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Screening for pharmaceutical transformation products formed in river sediment by combining ultrahigh performance liquid chromatography/high resolution mass spectrometry with a rapid data-processing method

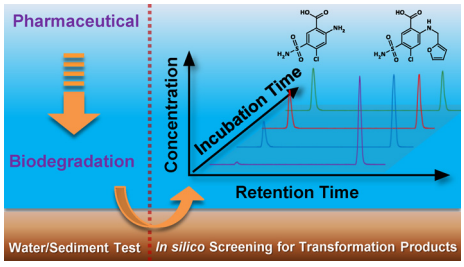
Zhe Li [a], Michael P. Maier [b,1], Michael Radke [a,*]

[a] Department of Applied Environmental Science, Stockholm University, 10691 Stockholm, Sweden
[b] Department of Hydrology, BayCEER, University of Bayreuth, 95440 Bayreuth, Germany

HIGHLIGHTS

- Knowledge gaps exist on transformation products of organic micropollutants.
- We present a screening method for transformation products in water/sediment tests.
- State-of-the-art UHPLC/HRMS is combined with a three-step in silico data treatment.
- The method is reliable, has a satisfactory efficiency, and low data processing costs.
- 16 dominant transformation products stemming from 9 pharmaceuticals are identified.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 August 2013
Received in revised form
29 November 2013
Accepted 5 December 2013
Available online 14 December 2013

Keywords:
Pharmaceuticals
Transformation products
Water/sediment test
Ultrahigh performance liquid chromatography/high resolution mass spectrometry
In silico data processing

ABSTRACT

While the occurrence of pharmaceuticals in the aquatic environment has been extensively investigated, their environmental fate is less thoroughly explored. Scarce information on their transformation pathways and transformation products (TPs) limits conventional target analytical approaches. In this study, samples from water/sediment tests were analyzed by ultrahigh performance liquid chromatography interfaced with quadrupole time-of-flight mass spectrometry (UHPLC/QToF-MS). A data processing method based on peak detection, time-trend filtration and structure assignment was established to provide an efficient way for identifying the key TPs in terms of persistence; all software used for the individual steps of this method is freely available. The accurate mass and meaningful time-trends were major contributors in facilitating the isolation of plausible TP peaks. In total, 16 TPs from 9 parent pharmaceuticals were identified. Eleven out of the 16 TPs were confirmed by corresponding reference standards; no standards were available for the remaining TPs. For additional 6 potential TPs, a molecular formula was suggested but no additional structural information could be generated. Among the TPs identified in the water/sediment tests, carbamazepine-10,11-epoxide (parent: carbamazepine), saluamine (parent: furosemide), chlorothiazide and 4-amino-6-chloro-1,3-benzenedisulfonamide (parent of both: hydrochlorothiazide), and 1-naphthol (parent: propranolol) accumulated over the entire incubation period of 35 days.

* Corresponding author at: Department of Applied Environmental Science (ITM), Stockholm University, 10691 Stockholm, Sweden. Tel.: +46 8 6747136.
E-mail address: michael.radke@itm.su.se (M. Radke).
[1] Present address: Institute of Groundwater Ecology, Helmholtz Zentrum München, 85764 Neuherberg, Germany.

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.12.012

1. Introduction

Pharmaceuticals are environmental pollutants of growing concern due to high consumption, ubiquitous presence in the environment and potential adverse effects [1–5]. A large number of investigations have documented their occurrence in rivers, lakes, and groundwater, where they can undergo abiotic and/or biotic transformation processes [3,6]. This may ultimately lead to the mineralization of these contaminants. Alternatively, transformation products (TPs) can be formed that are not readily degraded in the environment. While transformation is frequently associated with the detoxification of contaminants, products can also be formed that are as toxic or even more toxic than their parent compounds [5,7–9]. Despite their potential importance, the formation and fate of TPs from pharmaceuticals has only been scarcely investigated. This lack of knowledge hampers a thorough assessment of their environmental risk.

At present, fragmented information about pharmaceutical transformation pathways and the lack of reference standards for TPs limit conventional targeted analytical approaches. Modern high resolution mass spectrometry (HRMS) based on time-of-flight (ToF) or Orbitrap technology provides accurate mass data at satisfactory sensitivity, which opens up for comprehensive non-target screening [10,11]. Such an approach is comprised of three elements: (1) automated chromatogram deconvolution and peak detection; (2) molecular formula assignment to each specific peak; and (3) proposing a molecular structure for each assigned formula [12]. For the structure assignment, an empirically derived database can be established for currently known TPs [13]. Additionally, a database containing predicted TPs can be created using tools such as the University of Minnesota Pathway Prediction System (UM-PPS) [14].

This study aimed at the identification of microbial TPs from 9 pharmaceuticals in a water/sediment test, with a special focus on TPs that are not easily degradable and thus accumulate during the incubation period. We used a test system with artificial river water and sediment collected from two rivers, analyzed samples by rapid liquid chromatography–high resolution mass spectrometry (LC/HRMS), and applied an integrated in silico screening method to post-process the LC/HRMS data.

2. Experimental methods

2.1. Chemicals and reagents

All pharmaceuticals (purity >98%) were purchased from Sigma–Aldrich (Steinheim, Germany) and Toronto Research Chemicals Inc. (North York, Canada) (see Supplementary Material for chemical details). The isotope-substituted internal standards bezafibrate-D_4, diclofenac-D_4, furosemide-D_5, hydrochlorothiazide-^{13}C-D_2, metoprolol-D_7, naproxen-D_3 and propranolol-D_7 were purchased from Toronto Research Chemicals Inc. (North York, Canada). Carbamazepine-D_{10} and ibuprofen-D_3 were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). LC/MS-grade water (H_2O), acetonitrile (ACN), methanol (MeOH) and acetic acid (HAc) were purchased from VWR (Darmstadt, Germany).

2.2. Water/sediment incubation experiment

Three batches of sediments were collected from two rivers in Germany, i.e. Roter Main upstream and downstream of the city of Bayreuth, and Gründlach downstream of the city of Heroldsberg. Artificial river water was prepared in the laboratory (see Supplementary Material for details). The composition of the water was adapted to the aquatic chemistry of the river Roter Main. All incubation experiments were conducted in 1 L glass bottles which were filled with approximately 200 g of wet sediment and 600 mL of artificial river water. The system was equilibrated for one week before the supernatant was replaced with artificial river water that was spiked with a mixture of pharmaceuticals as described below. The bottles were continuously aerated with pressurized air and kept in the dark at a temperature of 15 °C. A daily dose of 200 μg of D-glucose was added to each bottle to avoid a carbon limitation of the system.

A mixture of the 9 pharmaceuticals was spiked at two concentration levels: 500 μg L^{-1} for Roter Main upstream sediment and 200 μg L^{-1} for Roter Main downstream and Gründlach sediment. These initial concentrations were 3–4 orders of magnitude higher than typical levels in the environment to facilitate the detection of TPs without any enrichment steps that would discriminate certain compounds, especially potential TPs. Three types of control experiments were conducted: (1) blank control (artificial river water and sediment, no addition of pharmaceuticals); (2) sterile control; (3) water-only control. Sterile control incubations contained the same amount of pharmaceuticals, but the sediment was sterilized by autoclaving and sodium azide (NaN_3) was added to the solution (final concentration: 1 g L^{-1}) to maintain sterile conditions. The water-only control contained only artificial river water and pharmaceuticals, but no sediment. Details on the experimental design and conditions are available as Supplementary Material (Tab. S2).

Water samples from all incubations were withdrawn periodically over a period of 35 days. All samples were filtered with syringe filters (0.45 μm, PTFE; Chromacol, Partille, Sweden); 400 μL were then transferred to a glass vial and stored at −18 °C. Prior to analysis, the samples were thawed and 100 μL of a solution containing the isotope-substituted standards (final concentration: 100 μg L^{-1}) and HAc (final concentration: 10 mmol L^{-1}) in ACN were added.

2.3. Analytical method

Samples were analyzed with ultrahigh performance liquid chromatography (UHPLC) coupled to a quadrupole time of flight (QToF) mass spectrometer (Acquity QToF Premier system; Waters, Manchester, UK) using electrospray ionization (ESI). Separation was achieved by an HSS T3 column (100 mm × 2.1 mm, particle diameter 1.8 μm; Waters) and a binary gradient. The mobile phase consisted of (A) H_2O:ACN (95:5, v:v) and (B) ACN:H_2O (95:5, v:v), respectively, both containing 10 mM of HAc. The UHPLC was programmed to deliver a linear gradient from 100% A to 95% B in 5 min, followed by 5 min of 95% B, at a constant flow rate of 0.3 mL min^{-1}. The QToF-MS was operated in full scan mode at a mass resolution of approximately 8000. Each sample was analyzed in positive and negative ionization mode in two separate runs. To obtain additional structural information, two acquisition functions were concurrently acquired in MS^E mode, at low (2 eV) and high collision energy (20 eV). A methanolic sulfadimethoxine solution (100 ng mL^{-1}) was introduced every 5 s through the LockSpray probe at a flow rate of 20 μL min^{-1} to compensate for mass drifts.

2.4. Transformation product identification

A three-step screening strategy was applied to identify TPs formed in the incubation systems: (1) peak detection; (2) time-trend filtration; and (3) structure assignment and confirmation. This approach is detailed below and schematically illustrated in a flowchart in the Supplementary Material (Fig. S1).

2.4.1. Step I: peak detection

The open-source software MZmine (version 2.3) was applied to automatically deconvolute the full scan data and to generate peak lists. The acquired data was constrained to a mass to charge ratio (m/z) range of 100–423 Da based on observations by Helbling et al.