Talanta 155 (2016) 1–7

Contents lists available at ScienceDirect

Talanta

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

Development of a multi-residue analysis of diclofenac and some transformation products in bivalves using QuEChERS extraction and liquid chromatography-tandem mass spectrometry. Application to samples from mesocosm studies

Gaëlle Daniele [a,*], Maëva Fieu [a], Sandrine Joachim [b], Alice James-Casas [b], Sandrine Andres [b], Patrick Baudoin [b], Marc Bonnard [c], Isabelle Bonnard [c], Alain Geffard [c], Emmanuelle Vulliet [a]

[a] Université de Lyon, Institut des Sciences Analytiques, UMR5280, CNRS, Université Claude Bernard Lyon1, ENS-Lyon, 5 rue de la Doua, 69100 Villeurbanne, France
[b] INERIS, Laboratoire d'écotoxicologie in vitro et in vivo, Parc Technologique Alata, BP n°2, 60550 Verneuil-en-Halatte, France
[c] UMR-I 02 INERIS-URCA-ULH SEBIO, Campus du Moulin de la Housse, BP 1039, 51687 Reims cedex 2, France

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form
4 April 2016
Accepted 5 April 2016
Available online 8 April 2016

Keywords:
Pharmaceuticals
QuEChERS
LC-MS/MS
Mesocosm
Metabolite
Photoproduct

ABSTRACT

Pharmaceuticals are ubiquitously present in the aquatic environment, mainly due to insufficient removal in wastewater treatment plants. Although these compounds are often found at trace levels in waters, long-term exposure can have negative impacts on biotic communities due to their inherent biological activity. The non-steroidal anti-inflammatory drug diclofenac (DCF) is one of the most frequently detected human pharmaceuticals in water and has recently been included in the "watch" list of the European Union. However little data are available on the detection of this substance and its transformation products in aquatic organisms. In this context, an analytical methodology has been developed to quantify traces of DCF along with its biotic and abiotic transformation products in a wild species of bivalve, the zebra mussel *Dreissena polymorpha*. A modified QuEChERS extraction was implemented on a small quantity of soft bivalve tissue (100 mg). This was followed by liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS) with electrospray ionization in positive mode (ESI+). Whole analytical method was validated on spiked real samples, with regard to linearity (from 1 to 50 or 100 ng/g depending on the target compounds, $R^2 > 0.99$), intra-day precision (relative standard deviation (RSD) < 18%), inter-day precision (RSD < 25%), (recoveries 78–117%), and limits of detection and of quantification (both inferior or equal to 1 ng/g). The optimized method was successfully applied to organisms collected from mesocosm experiments. Bioconcentration factors comprised between 4 and 13 were observed for DCF in the zebra mussels. To the best of our knowledge, the product 2-indolone was for the first time detected in bivalves, with levels up to 6 ng/g.

1. Introduction

Pharmaceuticals are widely used for human and veterinary medicine purposes. They can enter aquatic systems via different ways. Effluents of wastewater treatment plant (WWTP) could be identified as the main one due to incomplete removal of many drugs [1,2]. Indeed WWTP designed to eliminate natural organic matter and microorganisms are not effective to remove most of the pharmaceuticals that are generally of lower molecular weight and polar. Moreover, run-off from agricultural soils, industrial and domestic deposits contribute to the environmental contamination by pharmaceuticals. As a consequence, pharmaceuticals have been increasingly detected in field samples throughout the world with concentrations ranging from a few ng/L to some μg/L [3–5]. One of the main concerns related to the presence of pharmaceuticals in the environment is their potential for unintentional effects on nontarget species as they are inherently biologically active compounds.

Once released in the environment in their native form or as metabolites, pharmaceuticals can undergo abiotic and biotic processes that induce a great variety of transformation products. While the occurrence of pharmaceuticals in the aquatic environment has been reported in many studies, information on the

* Corresponding author.
E-mail address: gaelle.daniele@isa-lyon.fr (G. Daniele).

http://dx.doi.org/10.1016/j.talanta.2016.04.016
0039-9140/

corresponding metabolites and transformation products is less documented [6–8]. Nevertheless the relevance of studying metabolites and transformation products is a significant issue as they could exhibit an environmental impact as large as or larger than the parent compound [8–11].

In order to improve the quality of rivers, lakes and coastal waters in the European Union, in January 2012 the European Commission suggested adding fifteen chemical substances that represent a significant risk to or via the aquatic environment, including three pharmaceuticals. Among them, the common non-steroidal anti-inflammatory drug diclofenac (DCF) was identified for priority investigation because of risk perception [12]. DCF presents analgesic, antipyretic and anti-inflammatory properties. It has been detected in almost all water surveys probably due to its high consumption rate and polarity, with concentrations up to 380 ng/L, 1200 ng/L, and 7100 ng/L for groundwater, surface water and WWTP effluents, respectively [1,13–15].

The studies of drug residues in aquatic invertebrates in natural ecosystems have been less frequently reported than in aquatic vertebrates. However bivalves are excellent sentinel species for monitoring contamination of environmental waters [16]. Indeed they filter large volumes of water during breathing and feeding and they are sensitive to environmental stressors. Bivalves contain lots of lipids and proteins that can affect the analysis of target compounds, especially as the analytes are present at low concentrations.

Suitable extraction and clean-up have to be developed to limit the presence of matrix compounds during the analysis. Various techniques are implemented by different authors for the extraction of pharmaceuticals in bivalves: Pressurized Liquid extraction (PLE) [16,17], microwave assisted extraction [18,19], extraction through sonication [20] or QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction [21]. The advantages of the QuEChERS method are a low solvent consumption together with short extraction and clean-up times. Most current analytical methods for the separation and detection of pharmaceuticals are based on gas chromatography-mass spectrometry (MS) or to a larger extent on coupling liquid (LC) chromatography with ultraviolet (UV), diode array (DAD), fluorescence (FL) detectors or mass spectrometry (MS) and tandem mass spectrometry (MS/MS). In the investigation of metabolites or degradation products, high resolution mass spectrometry (HRMS) has been successfully employed [21].

This paper describes the development and validation of a simple, rapid, robust and sensitive analytical methodology based on modified-QuEChERS extraction followed by an accurate analysis using LC-MS/MS for the detection and quantification of DCF and nine of its biotic or abiotic transformation products in bivalves (*Dreissena polymorpha)*. Considering the large diversity of transformation products and metabolites, we have selected in this study chemically stable and commercially available substances, both metabolites and transformation products (S1). The chosen metabolites were those observed in plants and organisms, as well as in waters. The transformation products are mainly photoproducts as photodegradation is the main degradation pathway of DCF [9,15,22,23].

To the best of our knowledge, there is not currently available any analytical multi-residue method for bivalves that includes a pharmaceutical parent compound and its metabolites and transformation products [21].

2. Materials and methods

2.1. Chemicals and standards

Diclofenac-sodium (purity $\geq$98.5%) and 4′-hydroxy-diclofenac standards (purity $\geq$98%) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Standards of 2-indolone (purity $\geq$97%), DCF-lactam (purity $\geq$98%) and DCF-alcohol (purity $\geq$98%) standards were purchased from TLC Pharma (Vaughan, Ontario, Canada). The standard of CPAB was obtained from Synchem OHG (Felsberg, Germany). Standards of 5-OH-DCF (purity $\geq$98%), DCF carboxylic acid (purity $\geq$97%), DCF-aldehyde (purity $\geq$98%), and DCF-glucuronide (purity $\geq$95%) were acquired from Toronto Research Chemicals (Toronto, Canada). Isotopically labelled standards diclofenac-d_4 (purity $\geq$92%), used as internal standard was purchased from CDN Isotopes (distributed by Cluzeau, Sainte Foy La Grande, France), and phenacetin-ethoxy-1-^{13}C (98 atom % ^{13}C, at 1 mg/L in ACN), used as injection standard, was obtained from Isotec (Ohio, USA).

Individual stock solutions of each compound were prepared in methanol (MeOH) at concentrations of 200–1000 mg/L and stored at -20 °C (3 months). Working solutions were prepared by the appropriate mixture and dilution of the stock solutions in MeOH.

Ultra-pure quality water was obtained using a MilliQ® gradient A10 water purification system equipped with an EDS-PAK cartridge and a 0.2 μm Millipak® 40 filter from Merck-Millipore (St. Quentin en Yvelines, France). Acetonitrile (99.97% purity), MeOH (purity $>$99.98%), formic acid (purity 99%) and heptane (purity $\geq$90%) were purchased from Biosolve Chimie (Dieuze, France). Dimethylsulfoxide (DMSO) (purity $\geq$99.7%) was obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). Acetic acid (purity $\geq$99%) was acquired from Fluka.

The citrate and acetate buffer packets were supplied from Agilent Technologies (Massy, France). The citrate buffer contained 1 g of sodium citrate, 4 g of $MgSO_4$, 1 g of NaCl and 0.5 g of disodium citrate sesquihydrate (pH 5–5.5) whereas the acetate one was composed of 1.5 g sodium acetate and 6 g of $MgSO_4$ (pH=4.8). Two different materials were evaluated for dispersive solid-phase extraction (dSPE): primary secondary amine (PSA) and PSA/C_{18}. Both were purchased from Macherey Nagel (Düren – Germany).

2.2. Collection and maintenance of Dreissena polymorpha

Zebra mussels (*Dreissena polymorpha*) between 20 and 25 mm long were collected from the Lake of Der (eastern France). The organisms were maintained in a laboratory for 15 days in 20 L tanks with constant aeration. The tanks were filled with Cristaline Aurèle drinking water at a constant temperature of 10 ± 1 °C (Conductivity, 427 ± 46 μS/cm). Water parameters were measured photometrically (colorimetric method, spectrophotometer DR/2000 Hach), corresponding to nitrates, 10 ± 3.5 mg/L; nitrites $<$LD and ammonium $<$LD, with respective detection limits: nitrates, 0.3 mg/L; nitrite, 0.005 mg/L and ammonium 0.08 mg/L. During this 15-days period, mussels were fed ad libitum twice a week with a mixture of two species of microalgae, *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Two days before the experiment in mesocosms, organisms were distributed into 2 mm-mesh polyethylene experimental cages in laboratory (45 mussels per cage, some organisms were intended for biological response in other studies; 24 cages: 2 cages by mesocosm) to ensure their attachment in the cage.

2.3. Sampling and sample pre-treatment

The experiment was performed using twelve lotic mesocosms of 20 m long and 1 m wide, located in the North of France (INERIS,