Science of the Total Environment 536 (2015) 261–267

Contents lists available at ScienceDirect

Science of the Total Environment

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

Determination of endocrine disrupting compounds and their metabolites in fish bile

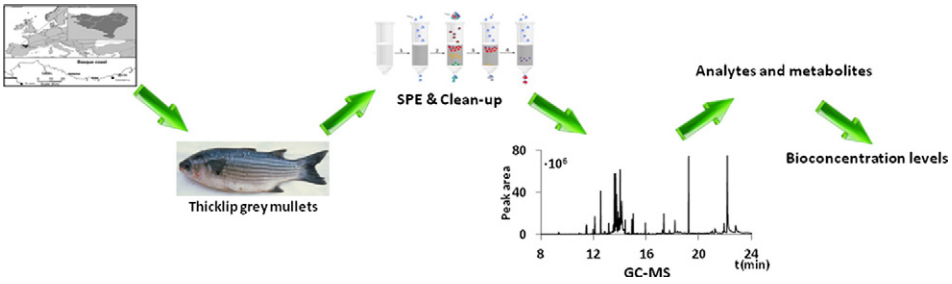
Oihana Ros [a], Jon Kepa Izaguirre [a], Maitane Olivares [a], Cristina Bizarro [b], Maren Ortiz-Zarragoitia [b], Miren Pilar Cajaraville [b], Nestor Etxebarria [a], Ailette Prieto [a], Asier Vallejo [a,*]

[a] Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), P.O. Box 644, 48080 Bilbao, Basque Country, Spain
[b] Research Centre for Experimental Marine Biology and Biotechnology (PIE), University of the Basque Country (UPV/EHU), Areatza z/g, E-48620 Plentzia, Basque Country, Spain

HIGHLIGHTS

- A simple method for the analysis of EDCs and their metabolites
- Accumulation of emerging pollutants in fish bile of the Basque coast was demonstrated
- The use of fish bile as a biomarker for discriminating environmental impacted areas

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form 14 July 2015
Accepted 14 July 2015
Available online 25 July 2015

Editor: Adrian Covaci

Keywords:
Endocrine disrupting compounds
Fish bile
Chelon labrosus
Solid phase extraction
Gas chromatography–mass spectrometry
Bioconcentration factor

ABSTRACT

This work describes a new methodology for the simultaneous determination of a large variety of emerging and persistent organic compounds and some of their metabolites in fish bile samples. The target compounds were musk fragrances, alkyl phenols, hormones, pesticides, phthalate esters and bisphenol-A, all of them with a known endocrine disrupting effect. To achieve the determination these three steps were optimized: i) an enzymatic hydrolysis of the metabolites to render the unconjugated compounds; ii) the solid phase extraction of the target analytes (Plexa cartridges 200-mg); and, iii) a clean-up of the extracts (Florisil cartridges 1-g). The samples were analyzed by gas-chromatography–mass spectrometry (GC–MS), though the polar fraction required a previous derivatization with O-bis (trimethylsilyl) trifluoroacetamide. Good apparent recoveries (63–122%), repeatability (<20%) and limits of detection (LODs) ranging between 0.04 and 459 ng/mL were obtained. This method was applied to the analysis of the target analytes in bile samples of thicklip grey mullets (*Chelon labrosus*) from five different populations of the Basque Coast (South East Bay of Biscay) during the period of May–June 2012. The target analytes were found at concentrations ranging from <LOD to 19,226 ng/mL and the most polluted site was Gernika. Bioconcentration factors (log BCF) were estimated (range of 1.69–5.55) and BBP and NP mix showed the highest values. A deep discussion has been carried out in order to explain the different concentration levels of the different sampling sites.

1. Introduction

The presence of emerging contaminants (ECs) and persistent organic pollutants (POPs) is becoming one of the main concerns of the 21st century. On the one hand, ECs are those chemical compounds whose

* Corresponding author.
E-mail address: asier.vallejo@ehu.eus (A. Vallejo).

http://dx.doi.org/10.1016/j.scitotenv.2015.07.074
0048-9697/

presence and environmental concern have been raised recently. On the other hand, POPs are often referred as legacy contaminants which remain intact for long periods of time. They are widely distributed throughout the environment, accumulate in the fatty tissues of living organisms and are toxic to both humans and wildlife (Besse et al., 2012; Sauvé and Desrosiers, 2014).

It has been pointed that wastewater treatment plants (WWTPs) act as a secondary source of many of these contaminants since they are not efficiently removed and therefore they are continuously discharged to water bodies such as rivers, estuaries or sea (Bueno et al., 2012; Loos et al., 2013). Among these contaminants, endocrine disrupting compounds (EDCs) are becoming of increasing concern due to their possible adverse health effects in organisms and communities as a result of changes in the hormone homeostasis (Bergman et al., 2013). Compounds including alkyl phenols (APs), estrogens, bisphenol-A (BPA), some pesticides, phthalate esters (PEs) or synthetic musks are considered EDCs (Martínez-Gómez et al., 2013; Bergman et al., 2013) and they have been included in different legislations such as Water Framework Directive (WFD, 2013/39/EU), REACH (Registration, Evaluation and Authorization of Chemical Substances EC1907/2006) or Stockholm convention (www.pops.int).

As shown in previous works, these compounds could be released to the environment at low or very low concentrations (ng/L level) (Bizkarguenaga et al., 2012; Blanco-Zubiaguirre et al., 2014), however, this sublethal but continuous discharge causes a chronic exposure scenario that may cause different alterations in fish population such as feminization, intersex, decreased fertility and fecundity and developmental abnormalities (Ferreira-Leach and Hill, 2001; Jobling et al., 2004; Madsen et al., 2004; Martínez-Gómez et al., 2013).

Many of these ECs, usually the most lipophilic ones, are metabolized in the liver and excreted through the bile (Ferreira-Leach and Hill, 2001; Jonsson et al., 2008; Martínez-Gómez et al., 2013). According to Pedersen and Hill (Pedersen and Hill, 2002), APs are accumulated in different organs as follows: bile > liver > gonads > blood ~ gill ~ kidney > muscle. Although, fish bile and liver seems to be proper target tissues for the analysis of EDCs, the high content of lipids found in the liver increases the complexity of the analysis (Chen et al., 2009) and thus, fish bile has become an alternative target to the liver.

Since the amount and variety of compounds that are analyzed is growing up, the analytical strategies are evolving from the target approach to the non-target one as long as the instrumental capabilities can accommodate them. This leads to the so-called multi-residual or multiscreening methods, which allow the simultaneous determination of many organic pollutants in a single run (Herrera-López et al., 2014). For instance, the simultaneous analysis of different organic pollutant families has been reported for water samples (Bizkarguenaga et al., 2012; Masía et al., 2013; Prieto et al., 2010), sediments (Lazartigues et al., 2011; Cristale and Lacorte, 2013) or biota (Kim et al., 2011; Lazartigues et al., 2011; Munaretto et al., 2013). To the contrary, in the analysis of fish bile, only a single family or two families of contaminants have been simultaneously analyzed, as the case of polycyclic aromatic hydrocarbons (Johnson-Restrepo et al., 2008), hormones (Fenlon et al., 2010), musk fragrances (Fernandes et al., 2013), BPA (Fenlon et al., 2010) or pharmaceuticals (Togunde et al., 2012). To our knowledge, only Yang et al. (2014) have analyzed 2 APs, BPA and 2 hormones, becoming the method presented in this work the only multiresidue method for organic compounds in fish bile.

Besides, due to the low concentration of the EDCs present in fish bile samples (Gibson et al., 2005; Fenlon et al., 2010; Vallejo et al., 2010; da Silva et al., 2013) and the complexity of the matrix, it is a necessary preconcentration step such as liquid–liquid extraction (LLE) (Fernandes et al., 2013; Martínez-Gómez et al., 2013), solid-phase extraction (SPE) (Fenlon et al., 2010; Vallejo et al., 2010; da Silva et al., 2013) or solid-phase microextraction (SPME) (Togunde et al., 2012). In many cases, a further clean-up step is also required to get better results in terms of accuracy, repeatability and limits of detection (LODs) (Budzinski et al., 2006). In addition to this, since many of these contaminants are subjected to metabolic transformation to ease the transport along the fish organs or their elimination (Pettersson et al., 2006), the metabolic by-products are also required to obtain a real and relevant environmental data of these contaminants (Togunde et al., 2012). As consequence, not only the analytes must be studied but also their metabolites. Thus, there is a necessity of developing new methods for the simultaneous determination of different families of compounds and their metabolites in fish bile.

Therefore, in the framework of a broader study aiming to the assessment of the intersex condition and molecular markers of endocrine disruption (Bizarro et al., 2014), the objective of this work was the development of a multiresidue analytical method for the determination of different target analytes including 2 synthetic musk fragrances, 3 APs, 2 hormones, BPA, 7 pesticides, the metabolites of chlorfenvinphos and chlorpyrifos and 3 PEs and their metabolites in fish bile using SPE as preconcentration and clean-up step after an enzymatic hydrolysis and a gas chromatography–mass spectrometry (GC–MS) analysis. The developed method was applied to the biomonitoring of thicklip grey mullets collected at five different estuaries and harbors along the Basque coast during the period of May–June 2012 and a deep study of the results was carried out.

2. Materials and methods

2.1. Reagents and materials

Names, abbreviations and characteristics of the chemicals compounds used in this work are summarized in Table S1. All the chemicals were >91% purity. All analytes were individually prepared at concentrations between ~1000 and 4000 ng/μL in methanol (MeOH, HPLC grade, Lab Scan, Dublin, Ireland) except for DEHP, which was directly obtained at 2000 ng/μL in MeOH, and [$^2H_{15}$]-MX, which was obtained at 100 ng/μL in cyclohexane. 10–100 ng/μL solutions were prepared weekly according to the experiments and stored in amber vials at −20 °C.

In the same way, solvents, sorbents, enzymes and reagent features are described in the supplementary material (S1 section).

2.2. Bile analysis

2.2.1. Sample collection

Adult thicklip grey mullets larger than 20 cm were captured by traditional rod (n = 12–30) during May–June 2012 in the estuaries of Ondarroa (fishing port, +43° 19′ 11.21″, −2° 25′ 24.59″) and Deba (Marina, +43° 17′ 40.38″, −2° 21′ 20.99″), Gernika (downstream a WWTP located at the Biosphere Reserve of Urdaibai, +43° 19′ 26.18″, −2° 40′ 25.61″) and in the harbors of Santurtzi (commercial port, +43° 19′ 45.54″, −3° 1′ 44.80″) and Pasaia (commercial port, +43° 19′ 19.21″, −1° 55′ 50.38″), all sites located in the Basque Coast (South East Bay of Biscay) (see Figure S1 and S2 for a detailed information about those sites and Table S2 for gender, size and weight of the fish). After fishing, they were immersed in a saturated solution of benzocaine in order to anesthetize them before being dissected (AVMA, 2013). The bile was extracted by means of a syringe and up to ~1 mL of bile fluid was collected in sterile cryogenic vials (Corning Incorporated, Mexico), and kept in liquid N_2 until laboratory arrival where they were stored at −80 °C until analysis. 13 bile samples from Gernika, 9 from Pasaia, 12 from Deba, 10 from Ondarroa and 7 from Santurtzi were analyzed.

2.2.2. Extraction and clean-up

Under optimum conditions, 100 μL of bile samples were hydrolyzed using 1.5 mL of phosphate buffer (0.1 mol/L, pH 6), 800 μL of Milli-Q water and 200 μL of corresponding enzymes (1.000 U/mL for β-glucuronidase, 2 U/mL for sulfatase and 20 U/mL for β-glucosidase) according to Gibson et al. (Gibson et al., 2005). 100 μL of surrogate ([$^2H_4$]-DEHP, [$^2H_{15}$]-MX, [$^2H_8$]-4,4′-DDT, [$^2H_4$]-NP, [$^2H_4$]-BPA and [$^2H_3$]-E2 at