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Determination of 24 personal care products in fish bile using hybrid solvent precipitation and dispersive solid phase extraction cleanup with ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry

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ABSTRACT

Personal care products (PCPs) are ubiquitous in aquatic environments owing to the continuous discharge of domestic wastewater from highly urbanized regions. These PCPs can be adsorbed by fish and thereafter usually enter the bile of the fish through biliary excretion. In this study, a sensitive method based on a combination of hybrid solvent precipitation and dispersive solid phase extraction (d-SPE) purification was developed to simultaneously extract and detect 24 PCPs, namely, 16 biocides, 4 synthetic musks, and 4 benzotriazoles, from fish bile. Hybrid precipitation on solid phase extraction (SPE) tubes was applied to remove phospholipids and proteins, and a d-SPE procedure was used for further purification. The extraction solvents for the hybrid precipitation/SPE tubes and d-SPE materials were optimized. The method performance for bile samples both with and without enzyme hydrolysis using β-glucuronidase/arylsulfatase were validated. The 24 PCPs in fish bile were spiked with standard concentrations of 10 ng/mL, 20 ng/mL, 100 ng/mL, and 200 ng/mL to evaluate recoveries, which ranged from 70 to 120% for 16, 16, 22, and 21 analytes with hydrolysis, respectively, and 70–120% for 14, 15, 23, and 23 analytes without hydrolysis, respectively. The quantification limits for target PCPs were in the range 0.26–7.38 ng/mL [excluding musk xylene (MX) and musk ketone (MK)] and 0.20–9.48 ng/mL (excluding MX and MK) for bile samples with and without enzyme hydrolysis, respectively. After enzyme hydrolysis, 12 PCPs were detected in bile from fish collected from the Yangtze River, with a maximum detected concentration of 460 ng/mL, for triclosan (TCS). The hydrolysis reaction indicated that high percentages of glucuronide and sulfate metabolites for some PCPs, i.e. four parabens and TCS, existed in the bile.

1. Introduction

Currently, personal care products (PCPs) are indispensable to the daily life of most people. The active ingredients in PCPs, such as biocides, synthetic musks, and benzotriazoles, have elicited concerns from both scientific researchers and the public because of their potential adverse effects on animals and humans [1–3]. After use, PCP compounds are released directly to aquatic environments or discharged to municipal wastewater treatment plants (WWTPs) [4]. Owing to incomplete removal in WWTPs [1,4], PCPs have been frequently detected in effluents [5,6], surface water [2,7], and sediments [7]. Consequently, aquatic organisms inhabiting these environments may bioaccumulate PCPs. In previous studies, ecotoxicity seldom has been evaluated via the internal exposure of pollutants in aquatic organisms, compared to external environmental exposure [8]. However, internal exposure in the biological compartments of aquatic organisms is vital for assessing toxicity mechanisms for certain contaminants [9,10].

In fish, biliary excretion after liver metabolism is an important removal mechanism for many environmental contaminants

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[11,12]. Therefore, fish bile is often used as an indicator of internal exposure and uptake of xenobiotic substances in biota [13], especially for hydrophilic contaminants, which are readily excreted via the bile compartment [11]. In addition, hydrophobic compounds also can be biotransformed to more water-soluble forms, by either hydroxylation or conjugation in the liver, and then excreted via bile [11,13]. Glucuronidation and sulfation are the main conjugation processes and result in contaminants existing in bile in conjugated form [14]. Enzyme-assisted hydrolysis can release conjugated contaminants and facilitate the determination of most parent compounds in fish bile [15,16]. Several studies have reported the concentrations of PCPs in fish muscle and liver tissues in their free forms [17–19], whereas the concentrations of PCPs in bile are seldom reported, either in free or conjugated form. Because PCPs in fish bile may be present in either free or conjugated form, comparisons of concentrations of these PCP forms in bile is meaningful for characterizing the uptake and metabolism mechanisms for PCPs in biota [11,15].

Bile comprises complex biological matrices, in which biological compounds, including phospholipids, proteins, cholates, pigments (bilirubin and biliverdin), and cholesterol, are present [20,21]. The existence of phospholipids and some other substances in biofluids have been reported to suppress the response of analytes in the electrospray ionization (ESI) mode of liquid chromatography-mass spectrometry [22,23]. Hence, an effective sample pretreatment method is necessary to remove matrix interferences and achieve sensitive and rigorous detection of PCPs in bile. Conventional solid phase extraction (SPE) has been a widely used approach for purifying matrices and concentrating analytes in fish bile and is performed by loading aqueous samples onto solid sorbents in an SPE cartridge and then eluting the analytes with appropriate organic solvents. For example, estrogenic compounds and some pharmaceuticals were extracted from bile via the SPE method using Oasis HLB cartridges [24,25] and Strata X-AW cartridges [26]. Some PCPs, such as triclosan (TCS), tonalide (AHTN), and galaxolide (HHCB), were also extracted from fish bile with HLB or Plexa cartridges [27,28].

Analysts continually strive for faster analyses, shorter run times, lower limits of detection, and fewer matrix effects by adequately removing interference from samples. To extract pollutants from fish bile, the above SPE approaches usually require long pretreatment times and display low removal efficiencies because of biological matrix interferences [29,30]. Usually, matrix components in biological samples are quite different from those in water samples. Hence, some special biological matrix removal SPE cartridges have been used to purify biofluids, which can selectively retain biological matrix interferences, such as phospholipids, proteins, and pigments, but allow analytes to pass [31]. These analytes can be collected directly without extra elution processes. This simple and efficient method of hybrid precipitation/SPE plates has been used to remove phospholipids and proteins from plasma samples [32,33]. The method has also been applied to cleaning other types of biological fluid samples for pharmaceuticals, through combination with extra purification processes such as dispersive solid phase extraction (d-SPE) [34].

The objective of this study was to develop a pretreatment, i.e., extraction and purification method by combining hybrid precipitation/SPE tubes and d-SPE kits, for extraction and analysis of 24 PCPs in fish bile samples. The target PCP compounds comprised 16 biocides, 4 synthetic musks, and 4 benzotriazoles. Various parameters, i.e., extraction solvent, elution ratio, and d-SPE materials, were tested to obtain optimal matrix removal efficiency for bile samples. Then, PCP concentrations were analyzed in the extracts using ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC–MS). In addition, to compare the conjugated and free PCP forms, the bile was treated with or without enzyme hydrolysis using β-glucuronidase/aryl-sulfatase. The method was validated for precision, repeatability, recoveries, method qualitative limits, method quantification limits, and matrix effects. The developed method was applied to investigate the concentrations of these 24 PCPs in bile from fish collected from the Yangtze River, China.

2. Experimental

2.1. Standards, reagents, and materials

The basic and supplier information for all 24 analytical standards (purity ≥ 95%) is listed in Table S1 (Supplementary materials). The abbreviations of the target compounds are displayed in Table 1. The selection of internal standards is based on the use of corresponding isotope-labeled analogs or labeled standards with similar retention time. The 12 internal standards (purity ≥ 95%) were supplied by several vendors. Imazalil-D_5, thiabendazole-D_6, fluconazole-D_4, AHTN-D_3, and musk xylene-D_{15} were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Ketoconazole-D_8, miconazole-D_5, and methylparaben-D_4 were obtained from Campro Scientific (Berlin, Germany). Clotrimazole-D_5 was purchased from Toronto Research Chemicals (North York, Canada). Propylparaben-D_4 was obtained from CDN Isotopes (Pointe-Claire, Canada). $^{13}C_{12}$-TCS and triclocarban-D_7 (TCC-D_7) were supplied by Cambridge Isotope Laboratories (Andover, USA). The positive control standard of triclosan O-β-D-glucuronide sodium salt was purchased from Toronto Research Chemicals (North York, Canada), and the hydrolysis enzyme β-glucuronidase/aryl-sulfatase, isolated from *Helix pomatia*, was supplied by CNW Technologies (Dusseldorf, Germany). The tricaine methanesulfonate was obtained from CNW Technologies (Dusseldorf, Germany).

HPLC-grade solvents of acetonitrile and methanol were supplied by Merck (Darmstadt, Germany) and the dichloromethane and dimethyl sulfoxide were purchased from CNW Technologies (Dusseldorf, Germany). Reagents of formic acid, ammonium acetate, acetic acid (AA), and sodium acetate (CH_3COONa) were purchased from CNW Technologies (Dusseldorf, Germany). All materials for phospholipid and other matrices removal were commercially available. The Captiva ND^{Lipid} Protein and Phospholipid Removal tubes and d-SPE materials, kit b: anhydrous magnesium sulfate/PSA/GCB and kit c: anhydrous magnesium sulfate/PSA/C18, were obtained from Agilent Technology (Palo Alto, USA). The d-SPE materials, kit a: Z-Sep/C18, was supplied by Sigma Aldrich (Santa Clara, USA). Ultrapure water was prepared with a Milli-Q water purification system (Millipore, United Kingdom).

Stock solutions (0.1 g/L or 1 g/L) for individual standards and internal standards were prepared in methanol. Individual or mixed working solutions were prepared via proper dilution of each stock solution with methanol to various final concentrations. Stock and working solutions for all standards and internal standards were stored at −20 °C. A 0.2 M acetate buffer (pH 5) was prepared with a mixture of 0.2 M AA and 0.2 M CH_3COONa ($V_{AA}/V_{CH3COONa}$ = 3/7), prepared in ultrapure water. The hydrolysis enzyme β-glucuronidase/aryl-sulfatase (100,000 units/mL) was stored in its original solution form at 4 °C, and enzyme work solution (10,000 units/mL) was diluted with 0.2 M acetate buffer (pH 5) before use.

2.2. Study area and sample collection

Bile samples were obtained from fish in the Yangtze River, China, which is the largest river in China in terms of mean annual water discharge, 900 km^3/y [35]. Sampling campaigns were carried out in July, the wet season, and in November, the dry season, of 2013.

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