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Multi-target determination of organic ultraviolet absorbents in organism tissues by ultrasonic assisted extraction and ultra-high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A sensitive and reliable method was developed for multi-target determination of 13 most widely used organic ultraviolet (UV) absorbents (including UV filters and UV stabilizers) in aquatic organism tissues. The organic UV absorbents were extracted using ultrasonic-assisted extraction, purified via gel permeation chromatography coupled with silica gel column chromatography, and determined by ultra-high performance liquid chromatography–tandem mass spectrometry. Recoveries of the UV absorbents from organism tissues mostly ranged from 70% to 120% from fish filet with satisfactory reproducibility. Method quantification limits were 0.003–1.0 ng g^{-1} dry weight (dw) except for 2-ethylhexyl 4-methoxycinnamate. This method has been applied to analysis of the UV absorbents in wild and farmed aquatic organisms collected from the Pearl River Estuary, South China. 2-Hydroxy-4-methoxybenzophenone and UV-P were frequently detected in both wild and farmed marine organisms at low ng g^{-1} dw. 3-(4-Methylbenzylidene)camphor and most of the benzotriazole UV stabilizers were also frequently detected in maricultured fish. Octocrylene and 2-ethylhexyl 4-methoxycinnamate were not detected in any sample. This work lays basis for in-depth study about bioaccumulation and biomagnification of the UV absorbents in marine environment.

1. Introduction

Organic ultraviolet (UV) absorbents, including UV filters and UV stabilizers are widely used in commodities such as cosmetics, sunscreens, and lotions to reduce harmful effects on skin and hair caused by solar radiation [1–3]. In addition, UV stabilizers are also applied in a variety of industrial products such as plastics, textile, building materials, and automobile components to prevent yellowing and degradation of the products due to their capability to effectively absorb UV-A and UV-B radiations [4]. Some of the organic UV absorbents are categorized as high production volume chemicals with great productions worldwide [1]. As a result, they have been widely found in human urine, water, dust, sediment, and organism tissues [5–21]. Some UV filters were quantified up to 3992 ng g^{-1} dry weight (dw) in mussels collected from beaches of south Portugal [19]. Presence of organic UV absorbents in the environment has drawn increasing concerns due to their ecological potential, such as endocrine disrupting properties and acute toxicity [22–25]. Some organic UV absorbents were suspected to be responsible for coral bleaching by promoting viral infections [26]. Exposure to elevated benzophenone-3 levels was speculated to be associated with endometriosis of women [27]. However, compared to legacy contaminants, such as traditional persistent organic pollutants, environmental occurrence, behavior, and ecological impacts of the organic UV absorbents are far from well-studied.

Reliable and feasible analytical methods are prerequisite for comprehend research of occurrence, fate, and consequently ecological impacts of organic UV absorbents in the environment. So far, there have been some reports about analyzing UV filters or UV stabilizers in various environmental matrices [4,9,14,19,28–50]. The UV absorbents, including various UV filters or UV stabilizers were usually extracted from solid environmental matrices such as sediment, sewage sludge, and organism tissues using ultrasonic-assisted extraction [10], mechanical shaking [3,39], accelerated solvent extraction [9,14,36], soxhlet extraction [4], and microwave-assisted extraction [12,13,41]. The compounds were determined by gas chromatography–mass spectrometry (GC–MS) [39], GC–MS/MS [13,19], high performance

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liquid chromatography–tandem mass spectrometry [3], and ultra-performance liquid chromatography–tandem mass spectrometry [9,36]. However, few efforts have been made to simultaneously analyze different groups of UV filters and stabilizers in environmental samples. It is known that UV absorbents can enter the environment indirectly via wastewater and directly via washoff through recreational activities as swimming and leaching from polymers [13]. It is therefore very likely that various UV absorbents accumulate concurrently in the environment, especially in coastal waters where are usually served for agricultural (e.g., fishing and mariculture), industrial (e.g., shipping and offloading) as well as recreational purposes. In addition, most of the available research focused on water and sediment [30,31,33–41,45–47] whereas methods for determination of the UV filters and stabilizers in organism tissues are still limited [4,34,43]. However, many organic UV absorbents have potential for bioaccumulation and biomagnification through trophic web given their strong lipophilicity [14]. Thus, it is valuable to develop a sensitive method for simultaneously measuring commonly used organic UV filters and stabilizers in organism tissues in order to better illustrate their occurrence and consequently ecological potentials in the environment.

In China, more than 20 organic UV absorbents, including cinnamate, dibenzoylmethane derivatives, benzophenone derivatives, salicylate, camphor derivatives, cyanoacrylate, benzoate, benzotriazole derivatives, are being allowed to be added in cosmetics with contents of 4–10%, with the top three being 2-ethylhexyl 4-methoxycinnamate, avobenzone, and benzophenone-3 [44,51,52]). In addition, benzotriazole derivatives (i.e. tinuvins) are one group of UV stabilizers with the highest production and most widespread use in plastics, especially in automobile surface coatings [10]. Consumption of UV stabilizer has reached 7000 t in 2010 in China [52]. Available researches have revealed the presence of some UV filters and UV benzotriazole stabilizers in wastewater [53], sewage sludge [54], sediment [39], and human blood and urine in China [55,56].

This work aimed to develop a feasible and sensitive method for multi-target determination of trace amount of most commonly commodity and industrially applied organic UV absorbents in aquatic organism tissues. The targets included six UV filters and seven benzotriazole UV stabilizers belonging to several groups as shown in Table 1. In addition to their widespread use and high production, the investigated UV absorbents are moderately to highly hydrophobic indicated by their log K_{OW} values (Table 1), implying their moderate to high potential for bioaccumulation. The UV absorbents were extracted by ultrasonic-assisted extraction, purified by gel permeation chromatography (GPC) coupled with silica gel column fractionation, and finally determined by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS). The method was validated and applied to detect the UV absorbents in wild and farmed aquatic organisms collected from the Pearl River Delta and Estuary, South China. This work laid basis for comprehensive research about bioaccumulation/biomagnification of the UV absorbents in the environment. To the best of our knowledge, this is the first work about bioaccumulation of the UV absorbents in aquatic organisms in China.

2. Experimental

2.1. Chemicals and reagents

The UV absorbent standards and deuterated standard benzophenone-d_{10} (BP-d_{10}) were purchased from Sigma–Aldrich (St. Louis, MO, USA) with purity of 97% or higher. Deuterated standard (±)3-(4-methylbenzylidene)camphor-d_4 (4-MBC-d_4) (98%) was purchased from C/D/N isotopes (Pointe-Claire, Quebec, Canada). The structures and physicochemical properties of the UV absorbents are summarized in Table 1. HPLC methanol, acetonitrile, dichloromethane, hexane, formic acid, and ammonium acetate were purchased from Merck (Darmstadt, Germany). Ultra-pure water was generated by a Milli-Q ultra-pure water system (Millipore, Billerica, MA, USA). Analytical grade ethyl acetate and cyclohexane were bought from Fuyu Chemical (Tianjing, China) and were redistilled before use.

Stock standard solutions were prepared in methanol individually at 10 mg L^{-1}. A working standard solution containing all the analytes was subsequently prepared in methanol. All the standard solutions were stored in amber glass vials and kept at −20 °C.

2.2. Samples and preparation

2.2.1. Samples

Red snappers (about 2800 g) were collected from a mariculture farm located in the Pearl River Estuary, South China. Several wild aquatic organisms (e.g., hairtail, squid, goby, pomfret, and squilla) were collected from the Pearl River Estuary using trawling in April 2013. The organism samples were stored at −20 °C until treatment.

2.2.2. Extraction

Fishes, hairtails, and squids were skinned while squillas were deshelled before being dissected carefully with a stainless steel scalpel. Only carcasses of the organisms were used in this work whereas the heads and internal organs were put away. For the big red snappers, the filet and belly were collected separately, while for the wild species, the whole body was used due to their small sizes.

Samples were freeze-dried, ground, and homogenized. About 4 g of each sample (2 g for the belly) was weighed into a 50-mL polytetrafluoroethylene centrifugal tube (Kimble, Vineland, NJ, USA) and spiked with internal standards BP-d_{10} and 4-MBC-d_4 at 100 ng g^{-1} dw (dw). The sample was then added with 20 mL of methanol and shaken on a vortex mixer (XW-80A Mixer, Shanghai, China) for 2 min prior to being subjected to ultrasonic-assisted extraction on a YJ-5200D ultrasonic water bath (40 kHz, 300 W) for 15 min. The sample was then centrifuged at 4000 rpm for 10 min at 4 °C (Avanti^{TM}30 centrifuge, Beckman, CA, USA). The clear supernatant was collected in a glass tube. The above extraction procedure was repeated three times and the extracts were combined (about 60 mL in total). One tenth of the extract was split for lipid content measurement and the rest was concentrated on a Syncore® Polyvap R-12 evaporator (Buchi, Flawil, Switzerland) to just dryness.

2.2.3. Cleanup and fractionation

The extract was re-dissolved in 1 mL of ethyl acetate/cyclohexane (50/50, v/v) prior to being subjected to a glass GPC column (1 cm × 40 cm) packed with Biobeads S-X3 (200–400 mesh, Bio-Rad Laboratories, Hercules, CA, USA) for lipids removal. The analytes were eluted with ethyl acetate/cyclohexane (50/50, v/v). The first 15 mL of eluate was discarded and the following 16 mL were collected. The collected eluate was concentrated and the solvent was exchanged to hexane prior to further purification with silica gel column (0.7 cm × 15 cm) fractionation. The UV absorbents were eluted with 15 mL of dichlomethane/ethyl acetate (50/50, v/v) from the silica gel column. The sample was brought to dryness under a gentle stream of nitrogen and then reconstituted in 1 mL of methanol prior to UHPLC–MS/MS analysis.

The lipid content was determined by gravimetric analysis. One-tenth of the ultrasonication extract was transferred into a pre-weighed glass vial and was evaporated to complete dryness. The content of lipids was calculated by the difference of the weight of the vial before and after addition of the extract.