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Analytical Methods

Optimization of matrix solid-phase dispersion for the rapid determination of salicylate and benzophenone-type UV absorbing substances in marketed fish

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ABSTRACT

A simple and effective method for the rapid determination of five salicylate and benzophenone-type UV absorbing substances in marketed fish is described. The method involves the use of matrix solid-phase dispersion (MSPD) prior to their determination by on-line silylation gas chromatography tandem mass spectrometry (GC–MS/MS). The parameters that affect the extraction efficiency were optimized using a Box–Behnken design method. The optimal extraction conditions involved dispersing 0.5 g of freeze-dried powdered fish with 1.0 g of Florisil using a mortar and pestle. This blend was then transferred to a solid-phase extraction (SPE) cartridge containing 1.0 g of octadecyl bonded silica (C18), as the clean-up co-sorbent. The target analytes were then eluted with 7 mL of acetonitrile. The extract was derivatized on-line in the GC injection-port by reaction with a trimethylsilylating (TMS) reagent. The TMS-derivatives were then identified and quantitated by GC–MS/MS. The limits of quantitation (LOQs) were less than 0.1 ng/g.

1. Introduction

Organic UV absorbing substances are widely used in sunscreens, cosmetics and skin care products to protect our skin from UV radiation. They are also used as UV stabilizers or sun-blocking agents in plastic food packaging materials to prevent polymers or foods from undergoing photodegradation. However, based on *in vitro* and *in vivo* bioassay studies, they have been reported to have both estrogenic and anti-androgenic properties in a variety of organisms (Ma, Cotton, Lichtensteiger, & Schlumpf, 2003; Schlumpf et al., 2001; Suzuki et al., 2005). Moreover, some of these UV absorbing substances are relatively lipophilic and therefore have the potential to bioaccumulate in aquatic biota, and even enter and accumulate in the food-chain (Bachelot et al., 2012; Balmer, Buser, Muller, & Poiger, 2005; Fent, Zenker, & Rapp, 2010; Gago-Ferrero, Díaz-Cruz, & Barceló, 2012; Zenker, Schmutz, & Fent, 2008). Although the concentrations of these residues in aquatic biota are generally assumed to be too low to pose an acute risk, the potential adverse effects from long-term exposure on the intake of aquatic foodstuffs, as in the case of exposure to other persistent organic contaminants, promoted us to develop a simple and reliable method to determine their presence in commercially marketed fish. Fig. 1 displays the structures, names and the log octanol–water partition coefficients (log*Kow*) (Negreira, Rodríguez, Ramil, Rubí, & Cela, 2009) of five commonly occurring salicylate and benzophenone-type UV absorbing substances that were employed in the method development and validation of this study.

Many attempts have been made to quantitatively determine various organic UV absorbing substances in aquatic biota. These techniques have been comprehensively reviewed by Gago-Ferrero et al. (2012). Among them, Soxhlet extraction, pressurized liquid extraction (PLE) or microwave-assisted extraction (MAE), followed by additional time and solvent-consuming clean-up steps, based on gel permeation and/or column purification, constitute the most commonly used procedures for the extraction of organic UV absorbing substances in aquatic biota samples (Bachelot et al., 2012; Balmer et al., 2005; Fent et al., 2010; Gago-Ferrero et al., 2012; Meinerling & Daniels, 2006; Mottaleb et al., 2009; Zenker et al., 2008). These approaches generate large amounts of organic wastes and are quite time consuming. Compared with the above approaches, in the case of matrix solid-phase dispersion (MSPD), first reported by Barker, Long, and Short (1989), extraction and clean-up are integrated in a single step, thus making the procedure simple, low-cost, and convenient. This method has been used successfully for the extraction of organic UV absorbing substances in indoor dust and sediment samples (Carpinteiro, Abuín, Ramil, Rodríguez, & Cela, 2012; Negreira, Rodríguez, Rubí, & Cela, 2009). MSPD has also been successfully applied to the determination of various

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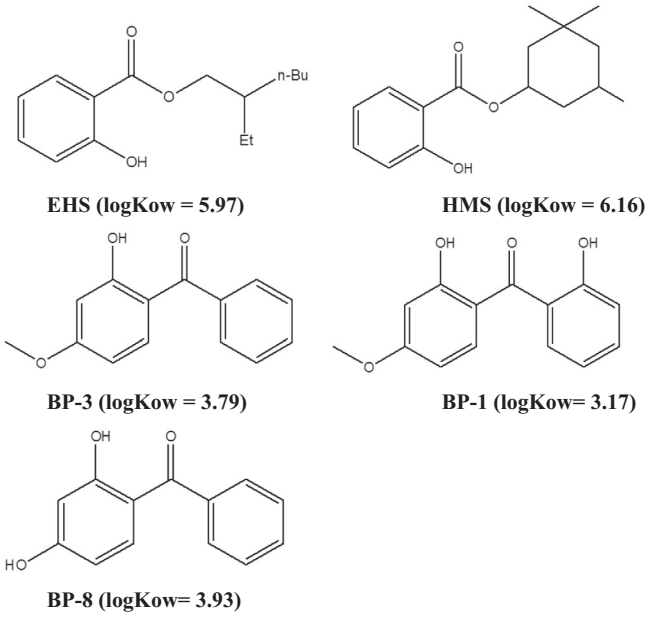

Fig. 1. Structures, names and log*Kow* values for the five salicylate and benzophenone-type UV absorbing substances employed in the development and validation of the method.

micropollutants in aquatic biota, animal tissue and foodstuff samples, and these applications have been reviewed extensively by Capriotti et al. (2010). Moreover, gas chromatography coupled with mass spectrometry (GC–MS) or tandem mass spectrometry (GC–MS/MS) is a frequently used technique for detecting lipophilic organic UV absorbing substances due to its superior separation, high distinguishing power and availability (Bachelot et al., 2012; Balmer et al., 2005; Carpinteiro et al., 2012; Fent et al., 2010; Gago-Ferrero et al., 2012; Mottaleb et al., 2009; Negreira, Rodríguez, Ramil, et al., 2009; Negreira, Rodríguez, Rubí, et al., 2009; Zenker et al., 2008). To improve the GC chromatographic separation, derivatization is typically used to increase the volatility of hydroxylated analytes and to improve sensitivity. However, off-line TMS-derivatization is laborious and time-consuming. In a previous study, we reported on the development of a direct injection-port silylation method for the determination of benzophenone-type UV absorbing substances in aqueous samples; this approach reduces solvent waste, simplifies the derivatization procedure, and avoids the need for hazardous reagents (Ho & Ding, 2012). Furthermore, the large-volume (10 μL) sample injection is attractive for improving detection sensitivity, and preventing the discrimination inside the syringe needle and injector liner that occurs when a small sample volume is injected.

Although MSPD is a commonly used sample pretreatment method, further development and validation is needed, depending on the nature of the specific target analyte, the matrix and the concentration of analyte. In line with attempts to study the spread of lipophilic organic UV absorbing substances in our foodstuffs, we report herein on the development of a simple MSPD method coupled with on-line silylation GC–MS/MS, for the first time, to determine the levels of five salicylate and benzophenone-type UV absorbing substances in samples obtained from marketed fish. The parameters affecting MSPD (i.e., the types and amounts of dispersant, clean-up co-sorbent, and elution solvent) were systematically investigated and the conditions optimized. Accuracy and precision were evaluated, and the suitability of the method for the determination the trace levels of the target analytes in marketed fish samples was demonstrated.

2. Experimental

2.1. Chemicals and reagents

All chemicals and solvents were purchased in high purity grade from Sigma–Aldrich (St. Louis, MO, USA), Mallinckrodt Baker (Phillipsburg, NJ, USA) and Merck (Darmstadt, Germany), and were used without further purification. Standards: ethylhexyl salicylate (EHS), 3,3,5-trimethylcyclohexyl salicylate (HMS), 2-hydroxy -4-methoxybenzophenone (BP-3), 2,4-dihydroxy-benzophenone (BP-1) and 2,2′-dihydroxy-4-methoxybenzophenone (BP-8), (purities of all compounds > 99%) and [$^2H_{10}$]-benzophenone (purity >99%, used as an internal standard) were purchased from Sigma–Aldrich. Phenyl-$^{13}C_6$-BP-3 (>99%, used as a surrogate) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was purchased from Sigma–Aldrich. Stock solutions of each analyte (1.0 mg/mL) were prepared in ethanol. Mixtures of the analytes for preparing working standards and for sample fortification were prepared in ethanol. All stock solutions and mixtures were stored in the dark at 4 °C. Deionized water was further purified using a Millipore water purification device (Billerica, MA, USA).

2.2. Sample collection

Two freshly killed fish (a striped bass and a tilapia) were purchased from a local fish market, and two imported frozen fish fillets (cod and salmon) were purchased from a supermarket in Chung-Li city, Taiwan. The fish samples were stored in a thermo insulator box, and then transported to the laboratory. At the laboratory, the fish samples were washed several times with Milli-Q water produced by a Millipore Elix® 10 RO system and a Millipore Synergy® UV system (Millipore SAS, Molsheim, France). The skin of the cod and salmon, as well as the scales of the striped bass and tilapia were removed from the fish, and the muscle tissue was cut into small pieces, and then homogenised in a commercial blender. The homogenate was then freeze-dried for 24 h, and ground into powder.

To eliminate contamination, all glassware was soaked in a solution of 5% (w/w) sodium hydroxide in ethanol for at least 12 h, and then cleaned and subsequently rinsed with deionized water, ethanol and acetone before drying, followed by an overnight heating at 250 °C. After performing this procedure, none of the target compounds were detected by GC–MS/MS analysis as described below. In addition, the use of sunscreen by laboratory personnel was restricted.

2.3. Matrix solid-phase dispersion procedure

The procedure used for MSPD has been described in previous studies (Canosa, Rodríguez, Rubí, Ramil, & Cela, 2008; Negreira, Rodríguez, Rubí, et al., 2009), and was performed with minor modifications. Briefly, a portion of powdered fish sample (0.5 g) was mixed with 0.5 g of anhydrous sodium sulfate, and dispersed with 1.0 g of Florisil in a mortar with a pestle. This blend was carefully transferred to a polypropylene SPE cartridge containing 1.0 g of octadecyl bonded silica (C18), as the clean-up co-sorbent packing at the bottom. A frit was placed over the dispersed sample and slight compression was applied. The target analytes were then eluted by gravity flow with 7 mL of acetonitrile (optimized, see Section 3.1), and at the end of the elution, a slight vacuum was applied. The eluent was collected and then evaporated to dryness under a gentle stream of nitrogen. The residue was re-dissolved in a solution (100 μL) of dichloromethane containing 0.1 g/mL of