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Simultaneous detection and quantification of select nitromusks, antimicrobial agent, and antihistamine in fish of grocery stores by gas chromatography–mass spectrometry

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HIGHLIGHTS

- Nitromusks, antimicrobial and antihistamine are found first time in edible fish.
- Fish from grocery stores were homogenized, extracted, concentrated and analyzed.
- GC-SIM-MS confirmed the presence of target compounds in 8 different fish extracts.
- HHCB, AHTN, TCS and DPH were consistently observed in grocery store fish fillets.
- Detected compounds are ~1 to 3 orders of magnitude lower than environmental fish.

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ABSTRACT

Continually detected biologically persistent nitromusks; galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK), antimicrobial triclosan (TCS), and antihistamine diphenhydramine (DPH) were examined for the first time in edible fillets originating from eight fish species grown in salt- and fresh-water. The sampled fish collected from local grocery stores were homogenized, extracted, pre-concentrated and analyzed by gas chromatography–mass spectrometry (GC–MS) using selected ion monitoring (SIM). The presence of the target compounds in fish extracts was confirmed based on similar mass spectral features and retention behavior with standards. Internal standard based calibration plots were used for quantification. The HHCB, AHTN, TCS and DPH were consistently observed with concentration of 0.163–0.892, 0.068–0.904, 0.189–1.182, and 0.942–7.472 ng g^{-1}, respectively. These values are at least 1–3 orders of magnitude lower than those obtained in environmental fish specimens. The MK was not detected in any fish.

1. Introduction

Nitromusks, antimicrobial agent triclosan, and antihistamine diphenhydramine are known as pharmaceuticals and personal care products (PPCPs) and are regularly used in human and animal applications. PPCPs and their metabolites are typically introduced into the environment from wastewater treatment plants and are non-regulated water contaminants (Ternes et al., 2004; Daughton, 2004). Their continuous release into aquatic systems has reached detectable and potentially harmful concentration levels (Kolpin et al., 2002; Snyder et al., 2003). As such, ubiquitous occurrence and fate of PPCPs and their metabolites as pollutants in the environment and aquatic organisms have driven many scientists to research this emerging concern (Kelly et al., 2007; Brodin et al., 2013).

Synthetic nitromusks, a group of personal care products (PCPs), such as HHCB, AHTN, and MK are frequently used as fragrance ingredients in formulation of perfumery and toiletry products. The estimated annual production of HHCB and AHTN alone was reported to be about 1 million pounds (Peck, 2006). Commercial and domestic use and discharge of these compounds into municipal sewage have contributed to their occurrence in aquatic environment and organisms. Recent studies have indicated that many of them are environmentally persistent, bioactive, and have potential for bioaccumulation (Peck, 2006; Mackay and Barnthouse, 2010). For example, these compounds and their metabolites have been detected in aquatic and terrestrial organisms (Oost et al., 2003), air (Kallenborn et al., 1999), sewage effluent (Osemwengie and

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Steinberg, 2001), industrial sewage sludge (Berset et al., 2000), marine mammals (Nakata, 2005), effluent-dominated river water fish (Ramirez et al., 2009), Pecan Creek fish (Mottaleb et al., 2009), and German fish specimen Bank (Subedi et al., 2012). Nitromusks were also detected in human tissues (Liebl and Ehrenstofer, 1993), mussels and shrimp (Rimkus and Wolf, 1995) and human blood (Hu et al., 2010). Balmer et al. (2005) and Mottaleb et al. (2008) have demonstrated that environmental exposure to PCPs results in accumulation of parent compounds and their metabolites in tissues of aquatic organisms. More significantly, series of studies have also identified that nitromusks are not only accumulated but are subsequently metabolized to reactive intermediates that form covalent bound protein adducts in human and aquatic organisms (Riedel and Dekant, 1999; Mottaleb et al., 2012).

Another PCP, the TCS, an antimicrobial agent, has been widely used in dental care products, deodorants, disinfectants, hand soaps, footwear, skin care creams and textiles. The production of TCS has been relatively consistent in the recent years, approximately 350 tons per year, in the US (Halden and Paull, 2005). TCS and its methyl metabolites were detected in surface waters, (Hua et al., 2005; Wu et al., 2007), biosolids (Ying and Kookana, 2007; Behera et al., 2010) fish (Balmer et al., 2004; Rudel et al., 2013) and algae (Coogan et al., 2007). Still, the fate and chemistry of TCS are not fully understood. TCS is quite stable for hydrolysis; however its photolysis was identified as one of the major pathways of degradation in surface waters (Tixier et al., 2002; Aranami and Readman, 2007). Other research groups discussed that TCS in surface water may be toxic to certain algae species. Orvos et al. (2002) and Reiss et al. (2002) found no observer-effect concentration (72-h growth) at 500 ng L^{-1} for algae *Scenedesmus subspicatus* while Wilson (2003) reported that TCS may cause significant increase in Synedra algae and a substantial reduction of the rare genus *Chlamydomonas* algae at 15 ng L^{-1} and 150 ng L^{-1}. Levy et al. (1999) demonstrated that TCS can block bacterial lipid biosynthesis inhibiting the enzyme enoyl–acyl carrier protein reductase, which leads to a possible development of bacterial resistance to TCS. Schettgen et al. (1999) studied the pH dependence of bioconcentration of TCS in zebra fish and demonstrated that the uptake as well as clearance rate decreases with increasing pH values. Recent studies have shown that TCS impaired swimming behavior and altered expression of excitation–contraction coupling proteins in fathead minnow (*Pimephales promelas*) (Fritsch et al., 2013), interfered with thyroid axis in the zebra fish (*Danio rerio*) (Pinto et al., 2013) and toxicity in the swordtail fish (Liang et al., 2013).

Pharmaceuticals including drugs and their active metabolites have been recognized as emerging environmental contaminants because of their increased occurrence in water, wastewater, soil, sediment, and biosolids (Daughton and Ternes, 1999; Bedoux et al., 2012). The DPH, an antihistamine drug, has repeatedly been spotted in many environmental compartments such as in water (Stackelberg et al., 2004), sediment (Ferrer et al., 2004) and fish (Ramirez et al., 2007; Wang and Gardinali, 2012). An earlier U.S. EPA pilot study, conducted by our group, found a higher concentration of DPH in the muscle and liver (up to 11 ng g^{-1}) in fish residing near multiple large metropolitan areas in the USA (Ramirez et al., 2009). Actual DPH muscle concentrations might be as high as 0.2–8.0 mg kg^{-1} if the percent of DPH bound to protein in fish is similar to the 86% and 99% protein binding reported in humans (Au-Yeung et al., 2006).

As PPCPs have been continually detected in water and fish, the consumption of fish and water can lead to an ingestion of the HHCB, AHTN, MK, TCS and DHP in humans and animals. The objective of this study is to monitor the concentration of the compounds in eight different species of edible fish fillets that were purchased from local grocery markets in the Midwest region of the United States. The sampled fish have been analyzed simultaneously by gas chromatography–mass spectrometry (GC–MS) method using selected ion monitoring (SIM) mode. To our knowledge, this is the first report on determination of nitromusks, TCS and DPH in edible fish fillets obtained from the local grocery stores. Therefore, these data could generate interest from scientific community and regulatory authorities for screening other PPCPs in edible fish.

2. Experimental

2.1. Solvents, chemicals and materials

Commercially available highest purity grade solvents and materials were purchased from local vendors. The reference standard materials that include tonalide (⩾98% purity), galaxolide (⩾50.0% purity), musk ketone (⩾98% purity), triclosan (⩾98% purity), and diphenhydramine (⩾98% purity), surrogate standard naphthalene d8 (⩾99% purity), and internal standard (IS) phenanthrene-d10 (⩾98% purity) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Silica gel (grade 60, 70–230 mesh, 60 Å), n-hexane (HPLC grade), and acetone (HPLC grade) were obtained from Fisher Scientific, Pittsburg, PA, USA. Distilled water was purified and deionized to 18 mega Ω with an ELGA PureLab Ultra water purification system.

2.2. Fish samples

Fresh- and salt-water growing fish were used as samples in this study. Eight different genus of edible frozen fish fillets were purchased from grocery stores located at Maryville, Missouri. The average weight of each fish genus group was approximately 0.91 kg. Details of the information of fish specimens, together with growing environment and collection condition are summarized in Table 1.

2.3. Homogenization and preservation of fish samples

Homogenization and compositing of fish tissues followed standard US Environmental Protection Agency (EPA) protocols (U.S. EPA, 2006). After purchasing, the fish fillets were transported to the laboratory within an hour and then individually wrapped with aluminum foil and placed in food-grade polyethylene bags at −80 °C. In the laboratory, the muscle tissue of each group of fish fillets (see Table 1) was homogenized using a Tissuemiser (Fisher Scientific Power Gen 125) set to rotate at 30 000 rpm. After homogenization, homogenate of each group of fish fillets was combined uniformly following U.S. EPA protocols (U.S. EPA, 2006) for a representative sample specimen and then placed in heavy-duty aluminum foil with appropriate sample identification mark. Following, all tissue specimens were stored at −80 °C prior to extraction.

2.4. Extraction of target analytes from fish homogenates

Detailed descriptions of the target compounds extraction from fish samples and analytical procedures were described in our

Table 1
Information of different fish specimens used.

Fish name	Genus	Growing environment	
Tilapia	*Tilapia*	Fresh water	Farm
Catfish	*Oreochromis*	Fresh water	Farm
Swai	*Pangasius*	Fresh water	Farm
Flounder	*Paralichthys*	Salt water	Wild
Salmon	*Salmo*	Salt water	Wild
Whiting	*Merlangius*	Salt water	Wild
Pollock	*Pollachius*	Salt water	Wild
Yellow fin Tuna	*Thunnus*	Salt water	Wild