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Analytical Methods

Environmentally friendly procedure based on VA-MSPD for the determination of booster biocides in fish tissue

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Diuron (PubChem CID: 3120)
Diuron-d6 (PubChem CID: 16212191)
Ethanol (PubChem CID: 702)
Acetonitrile (PubChem CID: 6342)
Acetone (PubChem CID: 180)
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ABSTRACT

Booster biocides have been widely applied to ships and other submerged structures. These compounds can be released into the marine environment as the result of vessel hull leaching and may remain in different environmental compartments. This study aimed at introducing an environmentally friendly procedure for the extraction of irgarol and diuron from fish samples by vortex-assisted matrix solid phase dispersion (VA-MSPD) with detection by liquid chromatography tandem mass spectrometry. Different types of solid supports and solvents were evaluated. The best results were found when 0.5 g mussel shell, 0.5 g sodium sulfate and 5 mL ethanol were used. Analytical recoveries ranged from 81 to 110%, with RSD below 10%, whereas the matrix effect was between −17 and 1% (for all samples under study). LOQ values of irgarol and diuron were 5 and 50 ng g^{-1}, respectively. The method under investigation proved to be a promising alternative to controlling contamination of fish by booster biocides, with low consumption of biodegradable reagents.

1. Introduction

Around 90% of the world trade has been conducted by the international shipping industry (ICS, 2016). Ships and other structures submerged in water can be affected by marine biofouling, which can be defined as the undesirable accumulation of microorganisms, plants and animals on artificial surfaces immersed in seawater (Yebra, Kiil, & Dam-Johansen, 2004). Aggregation of organisms leads to some problems, i.e., the ship hull becomes a rough surface that requires the engine to work more and consume more fuel (Mukherjee, Rao, & Ramesh, 2009; Yebra et al., 2004). Moreover, biofouling causes oxidation on the surface and increases the number of dry dock operations needed to remove biofouling (Yebra et al., 2004). Antifouling paints, whose active principles are booster biocides, have been developed and widely applied to avoid biofouling on ships. (Yebra et al., 2004).

The term booster biocide has been associated with a class of compounds that complements the biocidal action of copper, which was replaced as an antifouling because it is ineffective against some widespread algal species (Yebra et al., 2004). These compounds may be released into the marine environment as the result of vessel hull leaching and may remain in different environmental compartments because of their stability (Thomas, Blake, & Waldock, 2000; Yebra et al., 2004). Booster biocides have been detected in marine water (Hall, Killen, Anderson, Balcomb, & Gardinali, 2009; Sakkas, Konstantinou, Lambropoulou, & Albanis, 2002), sediment (Batista-Andrade et al., 2016; Hamwijk et al., 2005) and biota samples (Alejandro, Torres-Padron, Sosa-Ferrera, & Santana-Rodriguez, 2014; Kaonga, Takeda, & Sakugawa, 2015).

The most common booster biocides are irgarol and diuron, which have been detected in the water column (Dominguez, Caldas, Primel, & Fillmann, 2014; Hall et al., 2009; Harino, Mori, Yamaguchi, Shibata, & Senda, 2005; Sánchez-Rodríguez, Sosa-Ferrera, Santana-del Pino, & Santana-Rodríguez, 2011), sediment (Comber, Gardner, & Boxall, 2002; Gatidou, Thomaidis, & Zhou, 2007; Harino et al., 2005) and fish (Alejandro et al., 2014; Kaonga et al., 2015). Both compounds are herbicides that act in the photosystem by inhibiting

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electron transfer. As a result, they have been considered dangerous to certain species, such as algae and phytoplankton (Fai, Grant, & Reid, 2007; Mochida & Fujii, 2009). Some studies have shown that both may be harmful to rainbow trout larvae (Okamura, Watanabe, Aoyama, & Hasobe, 2002). Others have reported that the effects of diuron, irgarol and their metabolites on fish include morphological, biochemical and physiological alterations (Kaonga et al., 2015; Key et al., 2009; Mhadhbi & Beiras, 2012). Therefore, the development of reliable analytical procedures for the extraction and determination of booster biocides and other contaminants in fish samples is needed (Chen et al., 2009; Gan et al., 2016). Besides, very little is known about these compounds, mainly regarding biological samples, such as fish, a key species in the study of the biomagnification/bioaccumulation process, once it is in a high trophic level in the food chain (Alejandro et al., 2014; Duarte et al., 2013). Moreover, the development of efficient and sensitive methodologies to determine these compounds in organisms and in seafood is also needed because they are potentially harmful to human health.

Several extraction techniques have been reported in the literature for the extraction of biocides from a diversity of samples. However, only two methods were reported for the extraction of irgarol and diuron from fish samples: microwave assisted extraction (MAE) (Alejandro et al., 2014) and mechanical shaking (Kaonga et al., 2015). These methods need costly apparatus (e.g., microwave), high volume of toxic solvents, such as hexane, and a clean-up step, besides exposing the analyst to chemicals (Alejandro et al., 2014; Kaonga et al., 2015). Modern methods that use low amounts of organic solvents, need little time and can be easily developed, while keeping acceptable accuracy and extraction efficiency, are preferred (Chen, Guo, Wang, & Qiu, 2008).

Therefore, matrix solid phase dispersion (MSPD) (Barker, 2007), which consists in blending a proper solid support and a sample with the aid of a mortar and pestle (Capriotti et al., 2015), seems to be a good choice. MSPD is simple and accepts the use of renewable materials and green solvents, besides enabling the extraction and the clean up to be performed in a single step. Besides, the use of a vortex (VA-MSPD) makes it faster and mitigates human exposure to chemical compounds. Several studies have successfully applied VA-MSPD and have had acceptable results when different biological matrices were used (Barker, 2007; Escarrone et al., 2014; Hertzog, Soares, Caldas, & Primel, 2015; Rombaldi et al., 2015). Although VA-MSPD is a potential method to extract organic contaminants, no reports of the use of VA-MSPD for the extraction of booster biocides from fish have been found.

Recent trends in MSPD have shown the possibility of using alternative solid supports instead of traditional ones (e.g., C18 and C8 silica bonded phase, silica and florisil) (Capriotti et al., 2015). Cheaper and greener solid supports, such as mussel shell (Rombaldi et al., 2015), chitin, chitosan and diatomaceous earth (Hertzog et al., 2015), have been used without affecting the performance of the method.

Despite recent advances in the use of alternative solid supports and biodegradable compounds, little attention has been focused on the use of bio-solvents and other solvents that are less dangerous to the environment. The most common ones are methanol (Alejandro et al., 2014), acetonitrile (Harino et al., 2005) and acetone (Tsang, Lei, & Lam, 2009).

Therefore, the aim of this study was to develop and validate an analytical method which employed VA-MSPD with determination by liquid chromatography tandem mass spectrometry (LC-MS/MS) to the extraction of irgarol and diuron from fish tissue samples. The use of alternative solid supports and different solvents, such as a bio-solvent, was investigated.

2. Materials and methods

2.1. Chemicals

High-purity analytical standards (≥98%) of irgarol 1051, diuron and deuterated diuron (diuron-d6) were purchased from Sigma-Aldrich (Germany). High-performance liquid chromatography (HPLC) grade acetonitrile (MeCN), ethanol (EtOH), methanol (MeOH), acetone, hexane and ethyl acetate were bought from J. T. Baker (USA). Tetrahydrofuran (THF) was purchased from Sigma-Aldrich (Germany). Aluminum oxide, chitosan, florisil and silica were bought from Sigma-Aldrich (Germany). Endcapped C18, diatomaceous earth and sulfate sodium were purchased from Agilent Technologies (USA), Proquímios (Brazil) and Synth (Brazil), respectively, whereas mussel shell was obtained in the laboratory, having been previously characterized (Rombaldi et al., 2015). Ultrapure water was purified by the Direct-Q UV3® (resistivity 18.2 MΩ cm, Millipore, USA) water purification system (Millipore, USA). Acetic acid (98–100%) was purchased from Merck (Germany).

2.2. Standard solution preparation

Stock solutions of individual compounds were prepared in MeOH at 1000 mg L^{-1} and stored in a freezer. A mix of irgarol, diuron and diuron-d6 (2000 μg L^{-1}) was used as the working standard solution.

2.3. Sampling and sample preparation

Samples of *Micropogonias furnieri*, *Mugil liza* and *Cynoscion guatucupa* were bought as fish fillets (~500 g each) from fishermen in Rio Grande, a city located in Rio Grande do Sul state, Brazil. Samples were homogenized by mechanical blending in a stainless steel mixer and then completely ground. Afterwards, they were wrapped in aluminum foil and placed in a polyethylene bag in a freezer (≤ −20 °C). *Micropogonias furnieri* was used throughout the method development.

2.4. Sample extraction

Initial extraction conditions were based on a previously developed and validated VA-MSPD procedure (Hertzog et al., 2015). To reach the purpose of this study, some variables were evaluated so that the highest recoveries for irgarol and diuron in fish muscle could be achieved. Parameters evaluated by this study were the types of solid support and extraction solvent, the influence of salt and the volume of extraction solvent. Thus, preliminary experiments for the optimization of the VA-MSPD procedure were carried out with 0.5 g fish muscle (*Micropogonias furnieri*) spiked with 125 μL standard solution of irgarol and diuron (2000 μg L^{-1}), 0.5 g C18 and 0.5 g Na_2SO_4. The extraction step was carried out with 5 mL methanol by a vortex for one minute.

In order to carry out the extraction, 0.5 g sample (muscle tissue), 0.5 g mussel shell (solid support) and 0.5 g Na_2SO_4 were blended by a mortar and pestle for 5 min. Then, the homogeneous mixture was transferred to a PTFE tube (15 mL full capacity) and 5 mL EtOH was added to it. The tube was shaken by a vortex for 1 min and centrifuged for 5 min at 8000 rpm. Finally, 1 mL extract was collected for the chromatographic analysis.

2.5. Instrumentation

Liquid Chromatography was performed by a Waters Alliance 2695 Separations Module fitted to an autosampler, a membrane degasser and a quaternary pump. LC separation was carried out by an XTerra analytical column C18 3.5 μm (3.0 × 50 mm i.d.) (Waters, USA). Ten μL was injected by an autosampler. The mobile phase consisted of acetonitrile:water (52:48 v/v); both were acidified by 0.1% formic acid at 0.4 mL min^{-1} flow rate, as described by a previous study (Dominguez