Microchemical Journal 143 (2018) 92–98

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Extraction of chlorothalonil, dichlofluanid, DCOIT, and TCMTB from fish tissues employing the vortex assisted matrix solid-phase dispersion

Augusto Alves Vieira, Sergiane Souza Caldas, Larine Kupski, Ronan Adler Tavella, Ednei Gilberto Primel*

Post-graduate Program in Technological and Environmental Chemistry, Escola de Química e Alimentos, Laboratório de Análise de Compostos Orgânicos e Metais (LACOM), Universidade Federal do Rio Grande, Av Itália Km 8, Rio Grande 96203 900, Rio Grande do Sul, Brazil

ARTICLE INFO

Keywords:
Antifouling compounds
VA-MSPD
Sample preparation
GC ECD
Biological samples
Fish tissues

ABSTRACT

This paper reports the development and optimization of a method based on vortex assisted matrix solid-phase extraction (VA-MSPD) and gas chromatography with electron capture detection for the evaluation of booster biocides in fish tissues. Some parameters that affect the method performance were evaluated and suitable recoveries were obtained with 0.2 g sample, 2.0 g reused end capped C18, 0.2 g sodium sulfate and 5 mL ethyl acetate (35 mmol L^{-1} acetic acid). After validation, the method was applied to both species *Mugil liza* and *Cynoscion guatucupa* and to different tissues of *Micropogonias furnieri*. Analytical recoveries ranged from 60 to 126% whereas relative standard deviation was below 18%. The limit of quantification value of chlorothalonil was 125 ng g^{-1} while the one of dichlofluanid, DCOIT and TCMTB was 625 ng g^{-1}. The use of VA-MSPD for this proposal has shown to be a promising alternative since it is fast, simple and low cost. Besides, this is the first study that aims at extracting DCOIT and TCMTB from fish samples.

1. Introduction

Biofouling on ship hulls causes economic and environmental concerns, since they increase fuel consumption, induce corrosion processes and introduce foreign species [1,2]. In order to solve this problem, the shipbuilding industry makes use of some active substances in paints [3].

Since 2008, when the International Maritime Organization (IMO) banned organotin (OT) compounds due to its serious toxic effects on secondary aquatic organisms, mainly mussels (an effect known as *imposex*), the shipbuilding industry has adopted the use of around 18 compounds (booster biocides) as additives in copper-based antifouling paints to prevent biofouling effects [1,3,4].

Booster biocides were chosen due to their low stability in the environment and low toxicity, by comparison with OT compounds [5]. Although some booster biocides are rapidly degraded in the environment, the intense traffic of vessels may increase the input of these contaminants in the marine environment [5,6]. After having been released into the environment, these compounds may either undergo the process of bioaccumulation or provoke toxic effects on non-target species, e.g., fishes [7]. As described in a work of Harino [3], the fact of these substances have been detected in different environmental compartments (water, sediment, and biota) could indicate that they have been used in large scale or in an indiscriminate way.

Some studies have stated that chlorothalonil can have deleterious effects on chordates, crustaceans, mussels and other species in the range from 6.6 to 390 μg L^{-1} [3]. Dichlofluanid can affect the larvae of some chordates in their embryonic stage at concentrations above 282 μg L^{-1} [3]. Similarly, 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) and (1,3-Benzothiazol-2-ylsulfanyl)methyl thiocyanate (TCMTB) can cause different problems in the biota, such as growth inhibition of some algae, mortality and poor embryonic development of chordates, at concentrations above 0.42 and 46 μg L^{-1}, respectively [3]. Although these compounds have been found in water [3,8], sediments [3,8,9], and biological samples [4,10], there is no legislation or regulation to control their maximum residue level in seafood.

Since fishes, which are an important source of protein, minerals, and omega-3 fatty acids, have been exposed to many ways of contamination (diet and environment), they can affect human health, considering that a well-balanced diet must include a variety of fish and shellfish [11].

Therefore, both the development of analytical methods to determine the concentration of booster biocides in different samples and the generation of data are of major importance. However, to the best of our knowledge, there is no method to determine DCOIT and TCMTB in fish

* Corresponding author.
E-mail address: dqmednei@furg.br (E.G. Primel).

https://doi.org/10.1016/j.microc.2018.07.034
Received 21 March 2018; Received in revised form 26 July 2018; Accepted 27 July 2018
Available online 29 July 2018
0026-265X/

samples. Besides, extraction of chlorothalonil and dichlofluanid from fish samples is difficult to perform and acceptable levels of recovery (50–120%) are seldom reached [12–14].

Fast analytical methods which demand low amounts of non-toxic organic solvents while keeping acceptable accuracy and robustness are required nowadays [15]. The matrix solid-phase dispersion (MSPD) technique, described by Barker [16] allows simultaneous extraction and clean-up of analytes and appears as a good alternative to extract contaminants at low concentrations in complex matrices, such as biota [17]. Besides, when using the vortex assisted MSPD (VA-MSPD) where the manifold was replaced by a vortex, the technique demands less time and reduces exposure of the analyst to solvents [18,19].

To reach the best results, not only adequate sample preparation but also a sensitive detector is needed. Gas chromatography with electron capture detection (GC-ECD) has been applied to the determination of booster biocides [20,21], mainly when they are thermally stable, volatile and have electrophilic groups, such as halogens.

Therefore, taking into account there is no method to determine chlorothalonil, dichlofluanid, DCOIT and TCMTB simultaneously in fish tissue, thus, this study aimed at developing and validating an analytical method for this purpose using VA-MSPD with determination by GC-ECD.

2. Materials and methods

2.1. Chemicals

High-purity analytical standards (≥98%) of chlorothalonil, dichlofluanid, DCOIT and TCMTB were purchased from Sigma-Aldrich (Germany). High-performance liquid chromatography (HPLC) grade acetone, acetonitrile (MeCN), ethyl acetate (EtOAc), hexane and methanol (MeOH) were purchased from J. T. Baker (USA). Aluminum oxide (alumina) and chitosan were bought from Sigma-Aldrich (Germany). Endcapped C18 and sulfate sodium were purchased from Agilent Technologies (USA) and Synth (Brazil), respectively. Reused endcapped C18 ($C18_R$) were obtained by cleaning C18 from used SPE cartridges, as described by a previous study [22]. 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. Preparation of standard solutions

A stock solution of chlorothalonil was prepared in a mixture of MeOH/acetone (1:1 *v*/v) while dichlofluanid, DCOIT, and TCMTB were prepared in MeOH at 1000 mg L^{-1} and stored in a freezer (−18 °C). A mix of chlorothalonil, dichlofluanid, DCOIT and TCMTB (1000 μg L^{-1}) was used as the working standard solution.

2.3. Apparatus and chromatographic conditions

GC-ECD analyses were performed by a Shimadzu GC 2010 gas chromatograph instrument, equipped with a split/splitless injector system and a ^{63}Ni electron capture detector. Chromatographic conditions were based on a previous study [20]. Detector temperature was set at 300 °C and current of 1.5 nA was used. Booster biocides were separated at a dimethylpolysiloxane (Rtx®-1) column, 30 m × 0.25 mm × 0.25 μm. The temperature program used for the analysis ranged from 80 °C (3 min) to 290 °C (3 min) at 21 °C min^{-1}. The injector temperature was set to 250 °C in the splitless mode. In the GC-ECD system, hydrogen 4.5 FID (H_2) and nitrogen 5.0 (N_2) were used as carrier (1.5 mL min^{-1}) and make-up gas, respectively.

2.4. Fish samples

Samples of *Micropogonias furnieri*, *Mugil liza*, and *Cynoscion guatucupa* were chosen for this study since these species are demersal fishes (live and feed on or near the bottom of seas or lakes) which feed on small worms associated with the sediment, small fishes and mollusks [23]. Due to these characteristics, they can be potential bioindicators of contamination.

Samples of *Micropogonias furnieri*, *Mugil liza*, and *Cynoscion guatucupa* were bought as whole fish and fish fillets (~500 g each) from fishermen in Rio Grande, a city located in Rio Grande do Sul, Brazil. Whole *Micropogonias furnieri* was submitted to dissection in order to separate each tissue. Then, fish muscle was homogenized by mechanical blending by a stainless steel mixer until it was completely ground. Afterward, a fraction was separated for lyophilization and lyophilized samples were placed in amber flasks. Fresh fractions were wrapped in aluminum foil and placed in a polyethylene bag in a freezer (≤ −20 °C).

The characterization of muscle tissue was conducted in agreement with the Association of Official Analytical Chemists International – AOAC [24]. Humidity, lipid and protein contents were determined by methods no. 935.29, 920.85 and 920.87, respectively, whereas a factor of 6.25 was used for protein conversion.

2.5. VA-MSPD optimization

For the optimization of the VA-MSPD procedure, fish muscle (*Micropogonias furnieri*) samples spiked with 250 μL of 1000 μg L^{-1} standard of a mix of chlorothalonil, dichlofluanid, DCOIT and TCMTB (M4) were used. In order to find the best conditions, different parameters were evaluated by the one-factor-at-a-time (OFAT) method (solid support, extractor solvent, spiking interaction, amount of sample, acidification of extractor solvent and lyophilization) and by the central composite design (CCD) 2^3 (amount of solid support (X_1) and salt (X_2) and time of vortex (X_3)) (Table 1).

Table 1
Values of recovery observed in CCD 2^3 and relative errors for each analyte at the concentration of 50 μg L^{-1}.

Assay	Variables			Chlorothalonil		Dichlofluanid		DCOIT		TCMTB	
	X_1(g)	X_2(g)	X_3(min)	R (%)	Relative error (%)	R (%)	Relative error (%)	R (%)	Relative error (%)	R (%)	Relative error (%)
1	-1(0.5)	-1(0.2)	-1(1)	34	9	32	20	28	−2	50	27
2	1(2.0)	-1(0.2)	-1(1)	44	6	50	1	46	-2	51	−8
3	−1(0.5)	1(0.5)	−1(1)	38	−5	36	5	44	7	55	0
4	1(2.0)	1(0.5)	−1(1)	19	−8	40	−3	34	1	35	−4
5	−1(0.5)	−1(0.2)	1(9)	17	−11	22	−16	27	−7	30	−19
6	1(2.0)	−1(0.2)	1(9)	52	−3	52	4	46	−2	60	8
7	−1(0.5)	1(0.5)	1(9)	31	10	34	−0.4	42	2	68	18
8	1(2.0)	1(0.5)	1(9)	35	7	43	5	38	11	43	15
9	0(1.25)	0(0.35)	0(5)	36	8	36	−4	39	3	42	−9
10	0(1.25)	0(0.35)	0(5)	28	−19	34	−11	34	−11	33	−39
11	0(1.25)	0(0.35)	0(5)	31	−7	35	−7	37	−2	38	−21