Journal of Chromatography A, 1532 (2018) 68–73

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of organophosphorus flame retardants in fish by freezing-lipid precipitation, solid-phase extraction and gas chromatography-mass spectrometry

Yin-E Liu[a,b], Li-Qian Huang[a,b], Xiao-Jun Luo[a,*], Xiao-Xin Tan[a,b], Chen-chen Huang[a,b], Pablo Zapata Corella[a], Bi-Xian Mai[a]

[a] State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Resources Utilization and Protection, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, 510640, PR China
[b] University of Chinese Academy of Sciences, Beijing, 100049, PR China

ARTICLE INFO

Article history:
Received 25 October 2017
Received in revised form 1 December 2017
Accepted 1 December 2017
Available online 6 December 2017

Keywords:
Fish
Freezing-lipid precipitation
Sample preparation
Organophosphorus flame retardants

ABSTRACT

An analytical method has been developed for measuring 12 organophosphorus flame retardants (PFRs) in fish tissue samples. After the Soxhlet extraction of PFRs with dichloromethane. The experimental parameters of the clean-up were systematically optimized. Methanol was found to be a more effective solvent than acetonitrile used in freezing-lipid precipitation. Methanol (5%) in ultrapure water, was finally selected to perform solid-phase extraction (SPE, Oasis HLB cartridge), with mean lipid removal efficiency of 94% after freezing-lipid precipitation. Further purification followed by 200 mg of Z-Sep and C18 dispersant to eliminate the remaining interferences. Quantification was performed using gas chromatography-mass spectrometry in selective ion monitoring mode. The recovery, precision, and the method detection limits (MDLs) were verified by spiking experiments. All chemicals except triethyl phosphate (TEP) showed satisfactory recoveries in the range of 73–107% and 56–108% in the spiked blanks samples and spiked fish tissue samples, respectively. MDLs for PFRs in the biological samples ranged from 0.004 to 0.059 ng/g. The proposed method successfully applied to the determination of PFRs in real fish samples with recoveries of four internal standards varying from 75 to 97%. The results demonstrated that the proposed method is highly effective for analyzing PFRs in fish samples.

1. Introduction

Organophosphorus flame retardants (PFRs) have been widely used as flame retardants in commercial products such as electronic devices, and have also been applied as plasticizers or additives in lubricants [1]. There has been a huge increase in the demand and production of PFRs because they are regarded as appropriate alternatives for brominated flame retardants (BFRs). Over the last few decades, PFRs have been found to be ubiquitous in abiotic environments such as air [2], soil [3], water [4], dust [5,6] and sediment [7,8]. However, studies examining PFRs in biota are scarce and have only started emerging in the last few years [9,10].

Available data for PFRs in biota samples are limited. The major reason is the lack of an efficient and systematic pretreatment method. PFRs contain ester bonds in their chemical structures which lead to less persistence properties and different bioaccumulation capacity compared to other persistent flame retardants. The structural differences among PFRs result in a variety of chemical and physical properties, from highly lipophilic (log K_{OW} = 10.6 for Trioctyl phosphate) to highly hydrophilic (log K_{OW} = −9.8 for Tetrakis(hydroxymethyl) phosphonium sulfate) [1]. Meanwhile, the levels of PFRs in biota are influenced by degradation/transformation processes such as metabolism [11], which decreases the concentrations of these compounds in living organisms. Therefore, a highly efficient extraction method is needed due to the low concentration of PFRs in biological samples.

Several approaches have been developed to eliminate the co-extracted lipid interferences for organism samples, including pressurized liquid extraction using aqueous solution and solid-phase microextraction [12], on-line turbulent flow chromatography [13], matrix solid-phase dispersion [14], and gel permeation chromatography and silica gel cleanup[15]. These methods are either time-consuming and organic solvent-consuming or have high equipment requirements. Meanwhile, conventional cleanup

* Corresponding author.
E-mail address: luoxiaoj@gig.ac.cn (X.-J. Luo).

https://doi.org/10.1016/j.chroma.2017.12.001
0021-9673/

methods, such as basic or acidic treatment like saponification cannot be applied to PFR analysis since PFRs are prone to degradation under extremely acidic or basic conditions.

Due to their low melting points, lipid components can be easily separated from many compounds such as organophosphorus insecticides [16], chlorinated pesticides [17], and phenols [18] by freezing-lipid filtration. The cleanup step enables efficient removal of lipids extracted from biological samples without significant loss of the target compounds and no much organic solvents are consumed.

Up to date, the method of freezing-lipid filtration was not conducted on the PFR analysis in organisms. In this study, a combination of freezing-lipid precipitation and solid-phase extraction was developed for determining PFRs in biological samples containing high levels of lipids. The developed method was validated and applied to detect PFRs in fish samples from the Pearl River Delta.

2. Materials and methods

2.1. Standards and reagents

Triethyl phosphate (TEP), tri-iso-propyl phosphate (TiPP), tri-*n*-propyl phosphate (TnPP), tri-*n*-butyl phosphate (TnBP), tri(2-chloroethyl) phosphate (TCEP), tri(2-chloro-isopropyl) phosphate (TCPP), tris(2-butoxyethyl) phosphate (TBEP), tri(2-chloro,1-chloromethy-ethyl) phosphate (TDCP), tri(2-ethylhexyl) phosphate (TEHP), 2-ethylhexyl diphenyl phosphate (EHDPP), triphenyl phosphate (TPhP), and tri-cresyl phosphate (TCrP) [19] were purchased from AccuStandard (New Haven, CT, USA), as well as TnPP-D_{21}, TnBP-D_{27}, TCPP-D_{18}, TPhP-D_{15}, TCEP-D_{12}, and TDCP-D_{15}.

Oasis HLB cartridges (200 mg, 6 mL) were purchased from Waters (Milford, Massachusetts, USA). Z-Sep and C18 sorbents were purchased from Supelco (Bellefonte, PA, USA). Methanol, dichloromethane, acetonitrile, and hexane (chromatography grade) were purchased from Oceanpk (Sweden). Ethyl acetate was obtained from Honeywell (USA).

2.2. Sample collection

Fish samples, including plecostomus (*Hypostomus plecostomus*), tilapia (*Tilapia nilotica*), mud carp (*Cirrhinus molitorella*), and catfish (*Silurus asotus*), were collected in the Pearl River Delta as described in a previous study [20]. All the collected samples were freeze-dried, triturated, wrapped in aluminum foil, sealed in zip bags and stored at −20 ° until analysis.

2.3. Sample extraction and cleanup

After being spiked with surrogate standards (TnPP-D_{21}, TnBP-D_{27}, TCPP-D_{18}, TPhP-D_{15}, 100 ng each), 2 g of the lyophilized catfish sample (with a wet weight of 8.8 g) was Soxhlet-extracted with 200 mL of dichloromethane for 24 h. The extract was preconcentrated and transferred to a 10 mL centrifuge tube, where the solvent was concentrated to near dryness, under gentle nitrogen flow, and reconstituted in a polar organic solvent. Three different solvents were tested: ethyl acetate, methanol, and acetonitrile. The polar organic solvent extract was then stored in the freezer at −20 ° for 2 h to freeze the lipids. Most of them were precipitated on the bottom of the tube as a condensed mass. The supernatant was collected in a 500 mL flat bottom flask and 300 mL of ultrapure water was added. Different organic solvent volumes (5 and 10% of organic solvent/ ultrapure water) were also tested. The mixture was subsequently purified and fractionated by SPE on an Oasis HLB cartridge, which was activated separately with 4 mL each of ethyl acetate, methanol, and ultrapure water. After loading the mixture on the cartridge, the cartridge was dried for about 20 min under a gentle nitrogen stream, and was eluted with two aliquots of 4 mL of ethyl acetate. The remaining water and the residual lipids from the elution were removed with anhydrous sodium sulfate and 200 mg of Z-Sep/C18 (1:1) dispersant. After evaporation to near dryness, the liquid was re-dissolved in 200 μL of n-hexane. TCEP-D_{12} (100 ng) and TDCP-D_{15} (100 ng) were added as recovery standards, prior to instrumental analysis.

TnPP-D_{21}, TnBP-D_{27}, TCPP-D_{18}, and TPhP-D_{15} were added as internal standards. TnPP-D_{21} was used for TEP, TiPP, and TnPP quantification, whereas TnBP-D_{27} was used for TnBP and TCEP quantification, and TCPP-D_{18} was used for TCPP and TDCP quantification. Finally, TPhP-D_{15} was used for TBEP, TPhP, EHDPP, TEHP, and TCrP quantification. TCEP-D_{12} was used as a recovery standard for TnPP-D_{21} and TnBP-D_{27}, and TDCP-D_{15} was used as a recovery standard for TCPP-D_{18} and TPhP-D_{15}.

2.4. Instrumental analysis

PFR analysis were carried out with a Shimadzu 2010 gas chromatograph (GC) equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm; SGE Analytical Science) and coupled to a mass spectra detector (MSD) . It was operated in selective ion monitoring (SIM) mode, with two characteristic ions acquired for each compound [6]. The GC temperature program was set at 70 °C and held for 2 min, increased at 15 °C/min to 300 °C, and then held at 300 °C for 10 min. Sample injection (1 μL) was performed using the splitless mode with injector temperature of 290 °C. The carrier gas was Helium, at a flow rate of 1 mL/min. The temperatures of the interface, ion source, and injector were 290, 200, and 290 °C, respectively.

2.5. Determination of the lipid content for fish samples

After Soxhlet-extraction, the extract was concentrated and the volume was adjusted to 10 mL. An aliquot of the extract (1/10) was used to determine the lipid content by gravimetrical method, while the rest of the extract was used for PFRs determination by the developed method. The frozen lipid eliminated after freezing-lipid precipitation was also determined by gravimetric measurement. The average lipid contents of the plecostomus, tilapia, mud carp, and catfish were 2.91 ± 0.592, 2.01 ± 0.268, 1.83 ± 1.10,2.40 ± 0.581(%,w/w), respectively.

2.6. Quality assurance (QA) and quality control (QC)

In consideration of PFRs are widespread used and are likely to be present in various lab equipment, any plastic and rubber material was avoided to be used to minimize possible contamination of the samples during storage, sampling, extraction and transport. All the glassware were baked at 450 ° for 5 h and rinsed with acetone, dichloromethane and *n*-hexane orderly. Anhydrous sodium sulfate was heated at 450 ° for 5 h and stored in glass drying vessel. The connecting pipe and cock of the SPE device were also rinsed with three kinds of reagents orderly.

Measures quality was controlled and assured by spiking of surrogate standards into all samples and regular analysis of procedural blanks, spiked blanks, spiked matrices, and triplicate samples. PFRs-spiked fish tissue samples and blank samples were repeatedly (n = 3) analyzed during the development of the proposed method and a procedural blank for each batch of 12 samples was processed. In the procedural blank only traces of TCEP and TCPP were found. Instrumental QC included regular injection of the solvent blank and the standard solution (spiked with 500 ng/mL of PFRs). The standard solution was injected three times within a day and this solution was injected everyday to monitor the stability of instrument. The RSDs for the intra-day were in the range from 2.7% for