Food Chemistry 205 (2016) 1–8

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Rapid determination of organochlorine pesticides in fish using selective pressurized liquid extraction and gas chromatography–mass spectrometry

Minkyu Choi *, In-Seok Lee, Rae-Hong Jung

Marine Environment Research Division, National Institute of Fisheries Science (NIFS), Busan 46083, Republic of Korea

ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form 24 February 2016
Accepted 27 February 2016
Available online 3 March 2016

Keywords:
Organochlorine pesticides
Persistent organic pollutants (POPs)
In-cell cleanup
Seafood
Selective pressurized liquid extraction (SPLE)

ABSTRACT

A rapid automated extraction and cleanup method using selective pressurized liquid extraction was developed and validated for 14 organochlorine pesticides in fish. The lipid-removal efficiencies achieved by adding alumina, Florisil, acid-treated silica gel, and silica gel to the extraction cell were determined and optimized. In the optimized method, fish (2–3 g) was placed above alumina (30 g) in the extraction cell, then the sample was extracted using a 7:3 mixture of hexane and dichloromethane. The method was validated using certified reference materials (NIST SRM 1946 and 1974c), spiked fish, and four lipid-rich fish samples. The mean low- and high-concentration spike recoveries were 91% and 93% with RSD < 20%, respectively. Measured concentrations of target OCPs showed good agreement with the certified concentrations in certified reference materials. It suggests the good accuracy and precision of the SPLE method. The proposed method met the most important requirements of an extraction and cleanup procedure, including having a short preparation (cleanup and concentration) time and minimal sample contamination and being able to be automated.

1. Introduction

Organochlorine pesticides (OCPs) were used extensively to control pests and improve agricultural yields between 1940 and 1980, and many are classed as persistent organic pollutants (POPs). The use of a number of OCPs, including 1,1,1-trichloro-2,2-bis(4-chloro phenyl)ethane (DDT), has been banned or restricted around the world because these compounds can accumulate in biota, become biomagnified through the food chain (i.e., the concentrations in biota increase as the trophic level increases), and cause adverse health effects (including causing developmental toxicity and disrupting the endocrine system) in humans and animals (Beard, 2006). Because many OCPs are persistent and bioaccumulative, they are still widespread in the environment, particularly in biological matrices, despite their use having been banned or restricted for several decades. OCPs have been found in samples from coastal environments (Arienzo, Masuccio, & Ferrara, 2013), in seafood (Moon, Kim, Choi, & Choi, 2009), in marine mammals (Robinson, Jia, Trumble, & Usenko, 2015), in birds (Hong et al., 2014), and in humans (Moon et al., 2012). Humans are still predominantly exposed to OCPs, such as DDT and its related compounds, through consuming contaminated seafood (Moon et al., 2009; Zhou et al., 2012). The analysis of OCPs in environmental samples is an essential part of monitoring and managing the risks posed by OCPs in the environment, providing the information required to develop and enforce regulations and to perform ecotoxicological risk assessments. Methods for determining OCP concentrations in seafood such as fish have therefore become routine.

Biological samples are complex, so many steps are usually involved in preparing biological samples for OCP analysis. The most critical steps of a procedure for analyzing OCPs in terms of achieving good recoveries are the extraction (Soxhlet, saponification, homogenization, pressurized liquid extraction [PLE]) and the purification and/or fractionation of the extract (acid treatment, alkali digestion, gel permeation chromatography [GPC], solid-phase extraction [SPE], normal-phase high-performance liquid chromatography [n-HPLC], column chromatography). Acid treatment and/or alkali digestion are commonly used to remove undesirable co-extracts (such as lipids and pigments) when sample extracts are prepared for the analysis of POPs such as polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (Poster et al., 2003; Tsutsumi, Amakura, Sasaki, Toyoda, & Maitani, 2003). Although many OCPs are classed as POPs, some OCPs (including dieldrin, endrin, endosulfan, and heptachlor epoxide) are sensitive and are degraded when an

* Corresponding author.
E-mail address: mkchoi3@korea.kr (M. Choi).

http://dx.doi.org/10.1016/j.foodchem.2016.02.156
0308-8146/

extract is subjected to an acid treatment or alkali digestions (Chung & Chen, 2011). Methods that do not involve an acid treatment or alkali digestion step generally have multiple steps and are generally more time-, solvent-, and labor-intensive than methods that do involve a digestion step. This has led to less monitoring data being available for OCPs than for other POPs. A simple, rapid, and cost-effective automated method for analyzing OCPs is therefore required to allow more OCP monitoring data to be acquired.

Using a selective pressurized liquid extraction (SPLE) method, in which target compounds would be extracted and potential interferences removed by adsorbents in the extraction cell, would allow fewer sample preparation steps to be used than in methods that have previously been used. Samples can be prepared more cost-effectively (in terms of the time, amounts of solvents, laboratory space, labor, and training required) by SPLE than by previously available methods (Subedi et al., 2015). SPLE has been successfully used in analyses of polybrominated diphenyl ethers, PCBs, and polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediment (Aguilar, Williams, Brooks, & Usenko, 2014; Do, Lundstedt, & Haglund, 2013), sewage sludge (Chuang, Van Emon, & Schrock, 2009), fish tissue and fish oil (Wardrop, Morrison, Hughes, & Clarke, 2015), marine mammal tissue (Robinson, Trumble, Subedi, Sanders, & Usenko, 2013), and feedstuffs (Pena-Abaurrea, Ramos, Gonzalez, & Ramos, 2013). However, SPLE has been used very little in methods for analyzing OCPs in seafood (Shen et al., 2011).

The objective of this study was to develop a simple, rapid, and reliable SPLE method for the analysis of 14 OCPs (three hexachlorocyclohexane (HCH) isomers, hexachlorobenzene (HCB), four constituents of chlordane, and six DDT and related compounds) in fish tissues for use as an alternative to currently available methods used for certification such as extraction (Soxhlet, homogenization, PLE) and cleanup (GPC, SPE, n-HPLC, column chromatography) (Poster et al., 2003; Otake, Aoyagi, Yarita, & Numata, 2010). The aim was to develop an automated SPLE method combining PLE with the cleanup procedures required to produce an extract ready for instrumental analysis. We validated the method using spiked fish tissue samples and a certified reference material (CRM), then we used the method to analyze samples of the tissues of four species of fish with high lipid contents. The fish species that were analyzed were mackerel (*Scomber japonicus*), hairtail (*Trichiurus lepturus*), eel (*Conger myriaster*), and yellow croaker (*Pseudosciaena manchurica*). The efficiencies at which lipids were removed when different adsorbents (alumina, Florisil, acid-treated silica gel, and silica gel) were added to the extraction cell were determined. The amount of lipid removed per gram of adsorbent and the fat-to-adsorbent (FA) ratio is reported here for each adsorbent that was tested.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were analytical or HPLC grade. Hexane and dichloromethane (DCM) were purchased from Merck Millipore (Darmstadt, Germany). Washed sea sand, diatomaceous earth (DE) and moisture-absorbing polymer (MAP) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Silica gel containing 44% (by weight) concentrated sulfuric acid (later called acid–silica) was obtained from GL Science (Tokyo, Japan). Alumina (0.063–0.200 mm; Merck Millipore), silica gel (70–230 mesh; Merck Millipore), and Florisil (60–100 mesh; J.T. Baker, Pittsburgh, PA, USA) were activated at 600 °C for 16 h, 450 °C for 2 h, and 120 °C for 16 h, respectively, and then allowed to cool in a desiccator. The sea sand and DE were baked at 450 °C for 4 h, then allowed to cool in a desiccator.

A stock standard solution containing 14 OCPs (*cis*-chlordane, *trans*-chlordane, HCB, α-HCH, β-HCH, γ-HCH, *cis*-nonachlor, *trans*-nonachlor, *o*,*p*′-dichlorodiphenyldichloroethane (DDD), *p*,*p*′-DDD, *o*,*p*′-dichlorodiphenyldichloroethylene (DDE), *p*,*p*′-DDE, *o*,*p*′-DDT, and *p*,*p*′-DDT) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Five isotopically labeled OCPs (HCB-$^{13}C_6$, α-HCH-d_6, γ-HCH-d_6, *p*,*p*′-DDE-d_8, and *p*,*p*′-DDT-d_8), for use as surrogate standards, were obtained from Dr. Ehrenstorfer, and 4-terphenyl-d_{14}, for use as an internal standard, was obtained from Supelco (Bellefonte, PA, USA).

2.2. Fish tissue samples

Fish tissue samples were collected from the Busan cooperative fish market, which is the largest fish market in South Korea. Immediately after the samples had been collected they were placed in a cooler box containing ice and transported to the laboratory. The skin was removed from each fish sample, then the muscle tissues were homogenized using an ultra-disperser. Each sample was then freeze-dried at below −70 °C in a vacuum freeze-dryer (FSDSM12L; Samwon Freezing Engineering, Busan, Korea). The freeze-dried samples were kept at −4 °C until they were extracted.

The accuracy of the method was determined by analyzing CRM of a fish tissue (SRM 1946) and a mussel tissue (SRM 1974c), for which the contents of eleven and nine OCPs were certified, respectively. This CRM was provided by the US National Institute for Standards and Technology (NIST; Gaithersburg, MD, USA).

2.3. Gas chromatography–mass spectrometry conditions

The sample extracts were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies; Santa Clara, CA, USA) coupled to an Agilent 5973N mass spectrometer (Agilent Technologies). The instrumental analysis procedure has been described in detail elsewhere (Moon et al., 2009). Separation was achieved using a DB5-MS capillary column (30 m long, 0.25 mm inner diameter, 0.25 μm film thickness; J & W Scientific, Palo Alto, CA, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature program started at 150 °C, which was held for 2 min, increased at 30 °C/min to 170 °C, increased at 4 °C/min to 200 °C, which was held for 5.5 min, then increased at 30 °C/min to 320 °C, which was held for 10 min. The injector temperature was 250 °C. The mass spectrometer was operated in selective ion monitoring mode, and the molecular ions of the OCPs (shown in Table S1 in the Supplementary material) were monitored.

2.4. PLE and cleanup procedures

The samples were extracted using a Dionex ASE 350 system (Thermo Fishere Scientific). A number of operating parameters (the solvent, pressure, temperature, extraction time, number of cycles, and flush time) can potentially influence the efficiency at which analytes are extracted from a sample in a PLE system. Optimized extraction parameters for a range of analytes and sample types have previously been published (e.g., Choi, Kim, Lee, & Choi, 2014; Chung & Chen, 2011; Subedi et al., 2015). We used a mixture of hexane and DCM in our method because mixtures of hexane and DCM containing between 10% and 30% DCM have previously been found to efficiently extract multiple classes of POPs from fish tissue samples (Ghosh, Hageman, & Björklund, 2011; Losada, Santos, Covaci, & Galceran, 2010; Shen et al., 2011). We selected a 7:3 (v/v) mixture of hexane and DCM for use in our method because it is a more polar mixture than the other mixtures of hexane and DCM that were used in the studies mentioned above. The samples were extracted using extraction conditions recommended in US Environmental Protection Agency method 3545A