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Selective pressurized liquid extraction technique for halogenated organic pollutants in marine mammal blubber: A lipid-rich matrix

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ABSTRACT

Analytical methods for unique and rare samples, such as marine mammal tissue, strive to reduce opportunities for analyte loss and contamination. Historically, analytical methodologies for marine mammal tissues required an extraction followed by multiple cleanup and concentration steps. These steps increase the opportunity for analyte loss and sample contamination. Selective pressurized liquid extractions (SPLE; an analytical technique that combines PLE with in-cell adsorbent cleanup) have the potential to reduce and/or eliminate the number of steps. A SPLE method was developed for the simultaneous extraction of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) from bowhead whale blubber. This SPLE utilized acidic silica with a fat-to-fat retainer ratio of 0.02 as well as eliminated post-extraction cleanup steps, such as size-exclusion chromatography step. In addition, neutral silica was placed beneath the acidic silica as an acid buffer, thereby preventing acid from contaminating the extraction system. Analysis was performed using gas chromatography/mass spectrometry in electron capture negative ionization mode. PBDE, PCB and OCP triplicate recoveries averaged 84 ± 1%, 83 ± 3%, and 76 ± 11%, respectively. Overall, measurements of NIST Whale Blubber SRM 1945 were within ± 30% of certified values. PBDEs were measured for the first time in bowhead whale blubber; average concentrations ranged from 0.2 to 1.4 ng g^{-1} wet weight (ww). Average OCPs and PCBs concentrations ranged from 0.4 to 37 ng g^{-1} ww and 0.1 to 3.0 ng g^{-1} ww, respectively, which were within one order of magnitude lower than those previously reported in bowhead whale blubber.

1. Introduction

Persistent organic pollutants (POPs), such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs), undergo long-range atmospheric transport and bioaccumulate in lipid-rich biological matrices, such as marine mammal blubber [1–4]. POP trends and profiles from marine mammal matrices have provided valuable information regarding contaminant behavior and environmental fate [1,5–7]. POP trends and profiles are generated from marine mammal samples are often unique and irreplaceable due to the difficulty of obtaining marine mammal samples.

Historically, sample preparation for POP extraction from lipid-rich matrices (i.e. >30% lipid) utilized multiple steps: soxhlet or pressurized liquid extraction (PLE) followed by traditional column cleanup, acid digestion, and gel permeation chromatography (GPC) [1,8,9] (Fig. 1 and Fig. S1). During each preparation step (i.e. traditional packed column cleanup, GPC cleanup, and nitrogen concentration step) there are possibilities for analyte loss and contamination. Extraction methods for POPs should seek to reduce the overall number of steps.

One solution to reduce the number of sample preparation steps for POPs analysis is selective pressurized liquid extraction (SPLE), a technique that combines pressurized liquid extraction with cleanup adsorbent/s [10–13]. By reducing the number of sample preparation steps, SPLE has the potential to reduce possibilities for analyte loss and sample contamination. One of the recent analytical challenges for extracting POPs from lipid-rich matrices is the optimization of SPLE methods to eliminate all post-extraction cleanup steps. Trumble et al. extracted PBDEs, PCBs, and OCPs from Weddell seal (*Leptonychotes weddelli*) blubber using SPLE incorporating neutral silica and required GPC as post-extraction cleanup (Fig. 1B) [3]. Other adsorbents have been incorporated into SPLE and eliminated additional cleanup steps for lipid-rich matrices.

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For example, SPLE incorporating Florisil™ eliminated additional cleanup steps, for the extraction of: PCBs from dried spoonbill eggs (42% lipid) [14], PBDEs from whale blubber standard reference material (SRM) 1945 (72%) [15], and PBDEs, PCBs, and OCPs from a unique lipid-rich matrix, whale earwax [10]. Similarly, SPLE incorporating acidic silica eliminated additional cleanup steps for the extraction of: PCBs from pure lard (fat) [16] and PCBs and PBDEs from sheep liver (31% lipid) [17].

The objective of this study was to develop an SPLE analytical method that was capable of measuring PBDEs, PCBs, and OCPs in bowhead whale blubber and required no additional post-extraction cleanup steps. In literature, blubber masses ranging from 0.03 to 5 g have been utilized for POP analysis [3,18–20]. This method will be designed to extract POPs from 1.5 g blubber (or ~1 g of lipid) within a single PLE extraction cell. This study expands on previous lipid-rich SPLE techniques by using acidic silica [16] for the simultaneous extraction of PBDEs, PCBs, and OCPs from a lipid-rich matrix and elimination of additional cleanup steps as compared to previous marine mammal blubber methods [3,9]. This method was validated using National Institute of Standards and Technology standard reference material (NIST SRM) blubber 1945 and compared to previously published bowhead whale (*Balaena mysticetus*) blubber OCP and PCB measurements [1]. PBDE measurements in bowhead whale blubber were also reported for the first time.

2. Materials and methods

Chemicals were purchased commercially at reagent grade or better and stored in accordance with the manufacturer's recommendations. The majority of chemical specifications have been described previously [10]. Sulfuric acid of 95–98% purity was purchased from EMD (Darmstadt, Germany). Acidic silica was prepared in accordance with Bjorklund et al. [16] and Muller et al. [21] (2:3 w/w sulfuric acid to neutral silica ratio; 40% sulfuric acid silica gel) by adding 200 g of sulfuric acid to 300 g of previously baked neutral silica (baked at 200 °C for 12 h and allowed to cool). The acidified silica was mixed overnight on an Orbital Shaking Incubator (VWR, Model: 1575R, Radnor, PA, USA) [16,21]. The analyte list consisted of 30 analytes of interest and 7 isotopically-labeled quantification standards including PBDEs, PCBs, and OCPs (Supplementary Content). Bowhead whale blubber sample was collected in 1999–2001 from Barrow and Kahtovik, AK ($n = 6$; two from each year). Samples were stored at −80 °C until analysis.

2.1. Selective pressurized liquid extraction

For this study, POPs were extracted from blubber homogenates using SPLE, which combined PLE with acidic silica cleanup techniques into a single automated technique. SPLE was performed using an accelerated solvent extractor (ASE; ASE 350, Thermo Scientific Dionex™, Salt Lake City, UT, USA) with 100 mL extraction cells. First, an aliquot of ~1.5 g wet weight (ww) bowhead whale blubber (>1 g lipid; blubber samples were gravimetrically determined to have an average of 70% lipid, ranging from 64 to 83% lipid) was homogenized with anhydrous sodium sulfate to remove water. Sodium sulfate was previously baked at 500 °C for 12 h and allowed to cool. Blubber homogenates were placed on top of adsorbents in the following order from top to bottom: acidic silica (55 g) and baked neutral silica (5 g; Fig. 1C). To correct for analyte loss during sample preparation, blubber homogenates were fortified with isotopically-labeled surrogate standards and were allowed to come to equilibrium for 1 h prior to extraction. Briefly, ASE parameters were 100 °C, 1500 psi, 2 cycles (5 min each), and 100% rinse volume with n-hexane as the extraction solvent. Blubber extracts were concentrated to ~0.3 mL using a Turbo Vap II from Caliper (Hopkinton, MA), then transferred to a gas chromatography (GC) vial and spiked with isotopically-labeled internal standards prior to analysis using gas chromatography/mass spectrometry (GC/MS; analysis parameters described previously [10]).

2.2. Adsorbent and adsorbent mass (fat-to-fat retainer ratio) selection

Adsorbents were selected based on their densities as well as their ability to retain/destroy interferences but not analytes of interest (based on analyte recoveries). SPLE studies have shown that Florisil™ and treated adsorbents (i.e. acidic silica and basic and acidic alumina) can successfully retain potential interferences present in lipid-rich matrices [12] and reduce chromatogram baselines [10]. In the literature, use of alumina has resulted in high variability for PBDEs (relative standard deviation (RSD) >30% [15] and select organosulfide pesticides (RSD >56%) [10]. Initially, adsorbent mass was selected based on an optimized fat-to-fat retainer (FFR) ratio of 1:40 or 0.025 for complete fat removal from lard extracts by Bjorklund et al. [16]. Florisil™ and acidic silica were examined at a 1:40 FFR ratio. FFR ratios using acidic silica gel were optimized to 1:50 or 0.02 in order to incorporate larger masses of acidic silica (fat-retaining adsorbent) to accommodate larger masses of lipid in a 100 mL extraction cell.

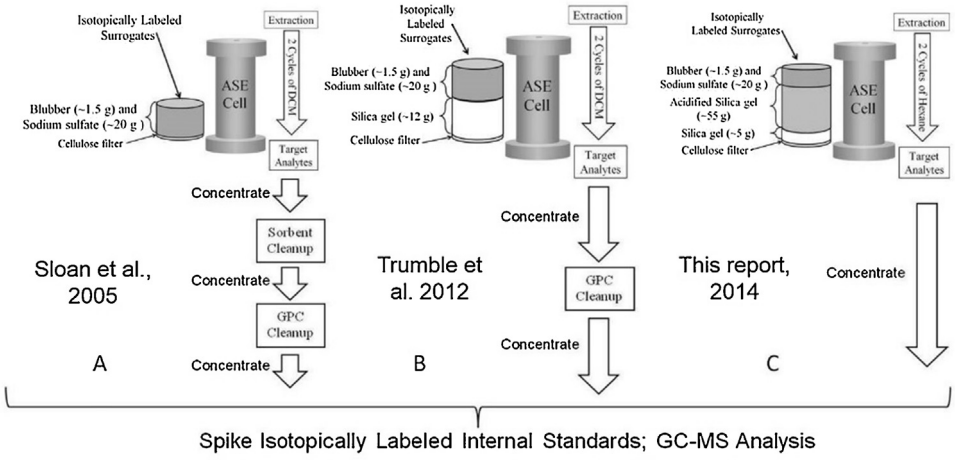

Fig. 1. Progression of extraction methods for blubber samples from PLE to SPLE based on (A) Sloan et al., 2005 [9], (B) Trumble et al., 2012 [3], and (C) this report, 2014.