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Enhanced pressurized liquid extraction technique capable of analyzing polychlorodibenzo-*p*-dioxins, polychlorodibenzofurans, and polychlorobiphenyls in fish tissue

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ABSTRACT

A high-throughput enhanced pressurized liquid extraction technique was developed by incorporating pressurized liquid extraction and multiple cleanup techniques. USEPA methods of polychlorodibenzo-*p*-dioxins, polychlorodibenzofurans (PCDD/Fs) and dioxin-like polychlorobiphenyls (dl-PCBs) analysis in fish tissue include independent silica gel, florisil, alumina, and carbopack/celite column cleanup techniques following extraction. Under the improved method, fish composites (~10 g) were extracted and cleaned simultaneously using alumina (~10 g), florisil (~10 g), silica gel (~5 g), celite (~5 g), and carbopack (~0.5 g). Clean extracts were concentrated and then analyzed by high resolution gas chromatography coupled with electron capture negative ionization mass spectrometry. Carbopack/celite within the extraction cell provided the analytical separation of dl-PCBs from PCDD/Fs, reducing potential molecular interferences. The average recoveries ($n = 3$) of dl-PCBs in dichloromethane:hexane (1:1, v/v) extracts were $93 \pm 2.4\%$ and PCDD/Fs in toluene extracts were $85 \pm 3.0\%$. The developed method was applied to measure the PCDD/Fs and dl-PCBs in catfish from San Jacinto River Waste Pits, a Superfund site in Houston, TX. The dl-PCBs were measured at 5.0–17,000 pg g^{-1} ww. Sample preparation time and solvents were reduced as much as 95% and 65%, respectively, as compared to USEPA method 1613.

1. Introduction

Polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), and dioxin-like polychlorobiphenyls congeners (dl-PCBs) constitute a class of chlorinated compounds of great concern due to their persistent, bioaccumulative, and toxic characteristics [1–4]. These compounds enter into the environment as byproducts of municipal and industrial waste incinerators [5], chlorophenols industries [6], and pulp and paper mill effluents [7]. PCDD/Fs and dl-PCBs have been measured, employing different analytical methodologies, such as EPA 1613, EPA 1668, EPA 8290A, and European Standard Method EN1948-1/2/3, in different environmental matrices such as sediments [8], fish [9], birds [10], human serum, and adipose tissue [11].

Analytical methods used in the routine analysis of PCDD/Fs and dl-PCBs in complex biological matrices such as fish tissues usually require the removal of more polar and nonpolar interferences using the individual adsorbent column cleanup and/or gel permeation chromatography [12–14]. Analytical methods that utilize multiple individual cleanup techniques are time, cost, space, and labor intensive as well as increase potential loss of target analytes and surrogates during sample preparation. Excluding these cleanup steps typically resulted in additional instrument maintenance and/or reduced analyte response.

Significant analytical improvements have been made over the past few years by combining the necessary extraction techniques with individual cleanup techniques. Silica gel cleanup has been combined with PLE for the analysis of different classes of analytes such as polynuclear aromatic hydrocarbons in fish and mussel tissue [15], pharmaceuticals and personal care products in fish tissue [16], polybrominated biphenyl ether and PCBs in sheep liver [17], and 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) in sediments [18]. PLE combined with the silica gel cleanup technique for TCDD reduced the sample preparation time and extraction solvents by 15% and 52%, respectively, as compared to extraction followed by an independent silica gel column cleanup [18]. Sulfuric acid impregnated silica, as a fat retainer, was also employed as improved PLE technique for the analysis of PCDD/Fs and dl-PCBs in food and feed samples [19]. Gomez-Ariza et al. reported an improved PLE technique, termed selective pressurized liquid extraction (SPLE),

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for measuring PCBs in ~2 g of biological samples, which incorporated florisil into the accelerated solvent extraction (ASE) cell [20]. This SPLE technique reduced the sample preparation time and extraction solvents by 94% and 84%, respectively, as compared to a conventional Soxhlet extraction. This SPLE study recommended a post-extraction cleanup step when incorporating only silica or alumina within ASE cell. Haglund et al. expanded the capacity and breadth of the SPLE technique to a higher capacity cell (66 mL) and included PCBs as well as PCDD/Fs in analysis of fish oil [21]. This increase in extraction cell volume resulted in an increase in sample capacity and robustness. Haglund et al. was also able to integrate carbon fractionation (carbopack/celite) with PLE, which allowed for the separation of PCDD/Fs from PCBs. However, this SPLE technique required an additional post-extraction cleanup step [21]. Post-extraction cleanup involves pre-conditioning, conditioning, and elution of analytes. A high-throughput analytical method incorporating all necessary column cleanup techniques such as silica gel, florisil, alumina, and carbopack/celite with PLE for the analysis of PCDD/Fs and dl-PCBs in fish tissue has not yet been reported.

The objective of this study was to develop and validate a high-throughput low-cost analytical method capable of analyzing PCDD/Fs and dl-PCBs in fish tissue by incorporating all cleanup adsorbents recommended by EPA 1613 with PLE. All the necessary cleanup adsorbents, including an integrated carbon fractionation, were incorporated into a single automated step. This enhanced pressurized liquid extraction (ePLE) technique eliminated an additional post-extraction cleanup(s) by combining all cleanup adsorbents with PLE. ePLE expands on historical PLE techniques by layering known sample preparative adsorbent(s) (silica gel, florisil, alumina, and carbopack/celite) beneath the sample homogenates within the accelerated solvent extractor cell. During ePLE, analytes and interferences were extracted from the sample and subsequently partition between the pressurized extraction solvent(s) and the adsorbents within a commercially available high-capacity 100 mL ASE cell at high pressure and high temperature. Significant analytical improvements were achieved by combining historical extraction solvents and cleanup adsorbents based on EPA 1613 with PLE, namely a reduction in the intrinsic costs associated with the sample preparation protocol: time (~95%), solvents (~65%), labor, laboratory space and training, and potential loss of analytes. As a result of these analytical improvements, laboratory capacity and preparedness was also increased for the analysis of PCDD/Fs and dl-PCBs in fish tissue. This analytical method was validated using a triplicate recovery study on fish fillet composites as well as certified reference material (CRM; CARP-2). The analytical method was employed to examine environmental concentration of PCDD/Fs and dl-PCBs in catfish from San Jacinto River Waste Pits, Houston, TX.

2. Experimental

2.1. Analytes and reagents

The analytes include the 7 PCDDs, 10 PCDFs and 12 dl-PCBs recommended by WHO/IPCS 2005 TEFs for human risk assessment [2]. Surrogates include $^{13}C_{12}$-labeled PCDD/Fs analogue of 7 PCDDs and 10 PCDFs congeners, $^{13}C_{12}$-PCB 77, $^{13}C_{12}$-PCB 81, $^{13}C_{12}$-PCB 126, and $^{13}C_{12}$-PCB 169. $^{13}C_{12}$-PCB 189 was used as an internal standard. The native and $^{13}C_{12}$-labeled PCDD/Fs and dl-PCBs were purchased from Wellington Laboratories (Wellington Laboratories Inc., ON, Canada). Perfluorokerosene (PFK), Florisil®, Carbopack™ C (80–100 mesh size), activated acidic and basic aluminum oxide (Brockmann-I), and Celite® 545RG resin were purchased from Sigma Aldrich (Aldrich Chemical Company, MO, USA). Ultra resi-analyzed® granular sodium sulfate (12–60 mesh size), toluene (TOL), and dichloromethane (DCM) were purchased from J. T. Baker (Mallinckrodt Baker Inc., NJ, USA). High purity silica Gel® 60 Å and ultra resi-analyzed® n-hexane (HEX) were purchased from VWR international (VWR International, PA, USA).

2.2. Fish samples

Farm-raised tilapia fillets were purchased from a local grocery store and used for method development and validation. As part of method validation, triplicate recovery experiment was performed on tilapia composites which have no background concentrations of PCDD/Fs and dl-PCBs. CRM, CARP-2, was purchased from National Research Council, Canada. Black drum (*Pogonias cromis*) and catfish (*Ictalurus punctatus*) were collected from San Jacinto River Waste Pits, Houston, TX. Collection and fish fillet tissue homogenization were according to the standard EPA protocols and have been previously described [22]. Method detection limits (MDLs) were determined on black drum, and the developed methodology was employed to examine the concerned contaminants in catfish. Samples were stored at −85 °C prior to extraction.

2.3. Adsorbents optimization

Efficiencies of adsorbents were examined in terms of the analytes' recoveries and effectiveness of sample cleanup in removing interferences, i.e. sample cleanliness. Different adsorbent combinations and ratios were examined with analyte spike and recovery experiments (Section 3.1.1). Fish composites (~5 g) were fortified with analytes (continuous calibration verification (CCV) level; Section 2.6), extracted using ePLE, concentrated, fortified with surrogates and internal standard, and analyzed as described in Sections 2.4 and 2.5. ePLE was performed using an accelerated solvent extraction (ASE 350, Dionex, Sunnyvale, CA). All adsorbents were pre-cleaned as described in Section 2.4.

Sample cleanliness was examined utilizing gel permeation chromatography–ultraviolet (GPC–UV) and gas chromatography electron impact ionization mass spectrometry (GC–EI/MS). Fish composites (~5 g) were extracted using ePLE, concentrated, and followed by GPC cleanup (Section 3.1.2). Additional samples were extracted, concentrated, and analyzed with GC–EI/MS without GPC cleanup. Moreover, one of the triplicate recovery samples, which was processed through the finalized ePLE technique (Section 2.7.1), was analyzed using GC–ECNI/MS to examine the sample cleanliness in terms of repeatability on analyte recoveries ($n = 7$) and requirements of additional instrumental maintenance.

2.4. Enhanced pressurized liquid extraction

Adsorbents recommended by EPA method 1613, such as silica, florisil, carbopack/celite, and alumina, were layered in the ASE cell (Fig. 1). Fish tissue homogenate was placed at the top of pre-cleaned adsorbents layers. Potential matrix interferences were preferentially adsorbed after passing through the adsorbents layers beneath the fish tissue homogenate during the ePLE. Target analytes and potential matrix interferences not adsorbed were allowed to pass through the adsorbent layers and collected in an ASE bottle.

EPA method 1613, 1668, and 8290A used column chromatography that contained carbopack with celite to separate dl-PCBs from PCDD/Fs, thereby reducing potential molecular interferences associated with the PCDD/Fs analysis. In this study, DCM:HEX (1:1) and TOL were examined to separate PCDD/Fs from dl-PCBs using ePLE (Section 3.1.3). The carbopack/celite layer was specifically added to the ASE cell to preferentially adsorb PCDD/Fs during the first extraction with DCM:HEX (1:1). dl-PCBs were not preferentially adsorbed to carbopack/celite and eluted with DCM:HEX (1:1).