Development of cleanup method of polychlorinated naphthalenes (PCNs) in beef by freezing-lipid filtration

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Introduction

Polychlorinated naphthalenes (PCNs) comprise a sub-group of 75 congeners, some with recognised toxic, bio-accumulative and persistence properties. As an industrial chemical, commercial PCN mixtures (e.g. Halowaxes) were mass produced over much of the last century and were commonly used in electrical equipment due to their physical properties of hydrophobicity, high chemical and thermal stability, good weather resistance, good electrical insulating properties and low flammability. Apart from the environmental release associated with this commercial use, PCNs are also reported to be produced in small amounts as combustion products[1] .

The structural similarity of PCNs to the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin molecule, indicates an aryl hydrocarbon (Ah) receptor-mediated mechanism of toxicity, and a number of toxic responses such as mortality, embryotoxicity, hepatotoxicity, immunotoxicity, dermal lesions, teratogenicity.

The development of analytical methods to analyze PCNs has been. New extraction techniques for analysis of PCNs have been developed involving extraction with organic solvents, supercritical fluid extraction (SFE)[2], microwave-assisted extraction(MAE)[3] or pressurized liquid extraction (PLE)[4] and further cleanup by adsorption chromatography[5] or by SPE[6]. However, a simple and rapid method for the ultra-trace quantification of individual congeners is required to determine the extent of environmental exposure. PCNs in biological samples are largely associated with the lipid fraction.

Recent reports have recommended the freezing-lipid filtration method to eliminate large amounts of lipids in biological samples for an efficient cleanup. This method has been applied successfully for the determination of chlorinated pesticides, anabolic steroids, synthetic hormones, PCDD/Fs, PBDEs in biological matrices[7-10]. The most of lipids extracted from biological samples can be removed through the freezing-lipid. For further extract purification, via impregnated multi-layer silica gel columns with neutral silica, acidified silica and basic silica[11] were applied.

In the present study, It is to apply the new method of freezing lipid filtration for the congener-specific determination of 11 different PCNs for the monitoring of biological samples.

Materials and methods

Mackerel samples were purchased from a grocery store in Seoul. A pooled sample, weighing approximately 100g, was homogenized and stored below -20°C pending analysis. Aliquots of the samples that did not contain PCNs were used for spiking experiments. The spiked samples were then stored in a tightly closed amber glass bottle at room temperature for 24 h. The lipid content in 10 g of the fish samples ranged from 10 to 15 % according to gravimetric determination.

A 10 g ground sample was added to 30 g of powered anhydrous sodium sulfate, 10 g of fish samples was spiked with 0.2ng and 20ng of the mixed 11 native PCNs standard solutionand was added prior to extraction respectively. The extraction was performed by sonicating twice for 10 min with 100 mL of n-hexane. Both freezing-lipid filtration and sulfuric acid treatment were used to remove any lipids. During freezing-lipid filtration, the extract was dissolved in 50 mL of acetonitrile and stored at -24 °C for 40 min to freeze the lipids. Most of the lipids were suspended as clusters and easily removed via filtration. The lipid removal (%) was calculated from the lipid residue after freezing-lipid filtration. The detailed freezing-lipid filtration procedure has been described previously[2-5]. Sulfuric acid treatment was performed following EPA method 1614 for comparison with the freezing-lipid filtration method. After eliminating the lipids, the sample was concentrated for addition to the multilayered silica gel column.

A Hewlett-Packard GC 7890A equipped with HP MSD 5975 and DB-5MS capillary columns (length, 60 m; column i.d., 0.25 mm; film thickness, 0.25um) was used to analyze the following PCN congeners: PCN52

(1,2,3,5,7-pentaCN); PCN 53 (1,2,3,5,8-pentaCN); PCN 66/67 (1,2,3,4,6,7-hexaCN/1,2,3,5,6,7-hexaCN); PCN 68 (1,2,3,5,6,8-hexaCN); PCN 69 (1,2,3,5,7,8-hexaCN); PCN71/72 (1,2,4,5,6,8-hexaCN/1,2,4,5,7,8- hexaCN); PCN73(1,2,3,4,5,6,7-heptaCN); PCN74(1,2,3,4,5,6,8- heptaCN); and PCN 75 (octachloro-CN). The MS was run in the single ion monitoring (SIM) mode The temperature programme consisted of an isothermal period (60°C, 5 min), a rise at 24°C/min to 180°C(5min), then at 5°C/min to 250°C(2min), a rise at 10°C/min to 300°C(8 min). The instrument was operated in electron ionisation (EI) mode at a mass spectrometer. Selected ion monitoring (SIM) was used to record the two most intense ions in the molecular ion cluster for each homologue.

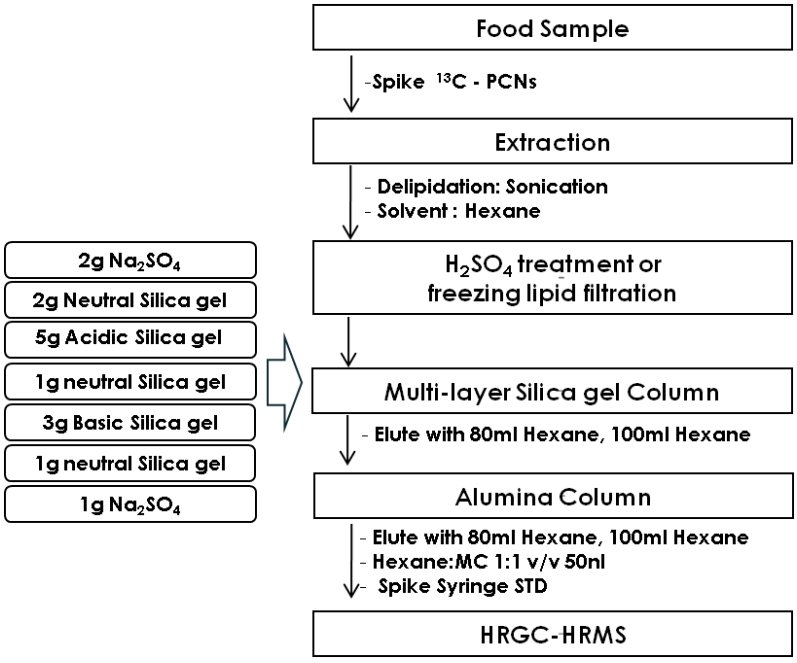

Fig. 1 Schematic diagram for analytical procedure

Results and discussion

The crude extract requires cleaning as many compounds (e.g., humic acids, lipids) can be coextracted with the analytes. Biota extracts usually contain high lipid concentrations that should be removed by either destructive or nondestructive methods prior to gas chromatographic separation of the PCNs. Sulfuric acid treatment, applied either directly to the extract or via an impregnated silica column, is the most common destructive lipid removal method.

To compare the performance of sulfuric acid treatment and freezing-lipid filtration, 100ul of the PCNs mixture standard (0.2 ng/mL, 20ng/ml), which contains the 11 different PCNs congeners, was added to separate 10 g beef tissue samples for an equivalent concentration of 20pg/g and 200pg/g in the tissue samples, respectively, and analyzed using both the sulfuric acid treatment and freezing-lipid filtration cleanup methods. Table 1 and Table2 present the recoveries for each of the spiked native congeners from 20pg/g and 200pg/g. The recoveries obtained using freezing-lipid filtration were nearly equivalent to those for sulfuric acid.

The overall time required for the freezing-lipid filtration procedure was about 3 h; oppositely, 1 day is required for the sulfuric acid treatment. Freezing-lipid filtration also possesses the advantage of requiring a smaller quantity of reagents than required for sulfuric acid treatment.

Table 1. Recoveries and RSD of PCNs in beef spiked with 2pg/g obtained via sulfuric acid treatment and freezing-lipid filtration

Isomer	IUPAC No.	Conc. (pg/g)	Sulfuric acid treatment recovery(RSD%)	Freezing-lipid filtration recovery(RSD%)
1,2,3,5,7-PentaCN	PCN 52	2	111.3(10.1)	76.8(6.3)
1,2,3,5,8-PentaCN	PCN 53	2	110.6(9.5)	90.3(2.6)
1,2,3,4,6,7-HexaCN/1,2,3,5,6,7-HexaCN	PCN 66/67	2	101.4(10.9)	88.4(8.6)
1,2,3,5,6,8-HexaCN	PCN 68	2	107.1(10.1)	89.4(8.6)
1,2,3,5,7,8-HexaCN	PCN 69	2	116.7(2.8)	87.8(13.9)
1,2,4,5,6,8-HexaCN/1,2,4,5,7,8-HexaCN	PCN71/72	2	118.5(2.4)	80.6(0.3)
1,2,3,4,5,6,7-HeptaCN	PCN 73	2	83.2(8.6)	85.0(6.6)
1,2,3,4,5,6,8-HeptaCN	PCN 74	2	92.8(6.0)	90.1(13.7)
Octachloro-CN	PCN 75	2	90.5(10.2)	89.8(14.2)

Table 2. Recoveries and RSD of PCNs in beef spiked with 200pg/g obtained via sulfuric acid treatment and freezing-lipid filtration

Isomer	IUPAC No.	Conc. (pg/g)	Sulfuric acid treatment recovery(RSD%)	Freezing-lipid filtration recovery(RSD%)
1,2,3,5,7-PentaCN	PCN 52	200	80.9(3.6)	84.4(6.6)
1,2,3,5,8-PentaCN	PCN 53	200	100.9(9.5)	93.0(7.0)
1,2,3,4,6,7-HexaCN/1,2,3,5,6,7-HexaCN	PCN 66/67	200	84.1(4.1)	94.7(9.5)
1,2,3,5,6,8-HexaCN	PCN 68	200	89.3(10.1)	92.3(8.8)
1,2,3,5,7,8-HexaCN	PCN 69	200	90.5(7.6)	91.8(9.3)
1,2,4,5,6,8-HexaCN/1,2,4,5,7,8-HexaCN	PCN71/72	200	97.5(7.9)	77.1(11.8)
1,2,3,4,5,6,7-HeptaCN	PCN 73	200	87.5(5.3)	86.1(16.9)
1,2,3,4,5,6,8-HeptaCN	PCN 74	200	96.5(4.1)	91.2(7.8)
Octachloro-CN	PCN 75	200	89.1(3.5)	94.4(9.1)

The freezing-lipid filtration method was evaluated for the efficient removal of lipids extracted from beef samples for the analysis of PCNs. This method was rapid, simple and economically cleanup that had several advantages over conventional sample treatments. The analytical results for the elimination of lipids from biological samples obtained after freezing-lipid filtration were nearly equivalent to those for others, official methods. This method can be applied as a rapid screening tool for the determination of PCNs in biological samples, especially in samples containing high levels of lipids and as it allows for the treatment of larger sample sizes to effectively eliminate lipids.

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