POLYCHLORINATED BIPHENYLS (PCBs) IN FISH OIL DIETARY SUPPLEMENTS BY GPC AUTOMATED CLEAN-UP METHOD

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Abstract
Due to the presence of omega-3 polyunsaturated fatty acids (PUFA), fish and derived products are important nutritional components of human diet. These PUFA, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential in human diet as they cannot be synthesized by vertebrates. To counteract low consumption of fish many people use dietary supplements containing fish oils. However, despite these health benefits, consumers have to ensure that the fish oil that they consume does not contain contaminants. Many studies suggest that there are concerns that some fish oil supplements may contain PCBs. In this paper we apply a new automated clean-up system based on gel permeation chromatography (AccuPrep MPS™, J2 Scientific) combined with an in-line concentration system (AccuVap™, J2 Scientific) to provide quick, cheap, and reliable results on the presence of those compounds in the fish oil supplements.
The recoveries of the automated clean-up are good thought lower than the manual clean-up but still the system is more repeatable.

Introduction
Adequate daily intake of fish oils, particularly EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), is associated with a wealth of health benefits including decreased risk of heart disease, lower blood pressure, improvement in rheumatoid arthritis, prevention of macular degeneration, and reduced risk of type 2 diabetes[1]. There is also evidence that fish oil consumption is beneficial for patients with depression, schizophrenia, and Parkinson's disease. Finally, several studies have confirmed that fish oils, especially DHA, are essential for optimum development of an infant's brain and visual acuity. The American Heart Association (AHA) also recommends that patients with heart disease consume at least 1000 mg of EPA + DHA every day. Thus, it is not surprising that health authorities promote the frequent consumption of fish: the AHA specifically recommend that healthy people eat fish at least twice a week (preferably oily). Considering that oily fish, such as sardines, mackerel, salmon and tuna contain anywhere between 800 and 1500 mg of EPA + DHA per 3-oz (85 grams) serving means that people eating two oily fish meals a week would obtain between 230 and 430 mg/day of EPA + DHA.
To counteract low consumption of fish many people use dietary supplements containing fish oils.
However, despite these health benefits, consumers have to ensure that the fish oil that they consume does not contain contaminants.
Many studies suggest that there are concerns that some fish oil supplements may contain polychorinated biphenyls (PCB's)[2,3,4,5,6,7]. Polychorinated biphenyls are lipophilic which means that they have an affinity towards lipids or fats and are able to dissolve in them–this is one reason for the concern for these compounds being found in fish oil supplements.
To reduce the presence of these pollutants the supplements producers clean the fish oils before being packaged, using molecular distillation or solid sorbents as activated carbon.
Other producers to limit production costs do not make this clean-up. It is therefore important to perform control tests on the amount of PCBs present in these dietary supplements.
To analyze in routinely manner a great number of samples and to provide quick, cheap, and reliable results on the presence of those compounds in the fish oil supplements, a new automated clean-up system based on gel permeation chromatography (AccuPrep MPS™, J2 Scientific) combined with an in-line concentration system (AccuVap™, J2 Scientific) have been developed.

Materials and methods

Samples

Samples analyzed in this study were Chinese capsules of fish oil supplements obtained from drug stores in USA. 20 grams of capsules were weighed and put into a beaker, spiked with PCB $^{13}C_{12}$-labelled extraction standards (WP-LCS, Wellington Laboratories) , then silica containing 44% sulfuric acid was added to obtain a slurry. The slurry was divided in 6 parts and cleaned-up three times in parallel by manual and automated method.

Automated clean-up

The automated clean-up system configuration (Accuprep) consists of a 2.5 cm x 45 cm glass column packed with BioBeads SX-3 resin (J2 Scientific) in 100% methylene chloride, a pump, an auto sampler and the evaporating chamber. The whole system is computer controlled and can be programmed as required (i.e. volume, flow-rates, direction of solvent flow, etc.). The system used a 5-mL sample loop and a flow rate of 5 mL/min with methylene chloride as the mobile phase[8].

The slurry was placed inside empty Teflon cartridges. The cartridges were inserted into the Solid Phase Extraction Modules. A flow of hexane eluted analytes present in the slurry, sending into the AccuVap where they were concentrated and taken through 3 consecutive washes with methylene chloride up to a final volume of 1.5 ml before being sent to the column GCP. Finally, after the GPC elution, the sample were concentrated up to a final volume of 0.5 ml before being sent to the SPE alumina column. Finally, the sample was sent first in the AccuVap where it was concentrated up to a volume of 1.5 ml and after manually under a nitrogen flow to a final volume of about 100 µl. After addition of PCB $^{13}C_{12}$-labelled internal standards (WP-IS, Wellington Laboratories) the samples were analyzed.

Manual clean-up

The slurry was placed on top of a multilayer silica column, composed of sequential layers (from bottom to top) silica, SiO2 – AgNO3, silica, SiO2 - H2SO4, silica and Na2SO4. The extract applied to the top of the silica column was eluted with 150 ml of n-hexane and then concentrated prior to basic alumina column. PCBs were eluted from this column with a n-hexane:dichloromethane (98:2) solvent mixture.

Analysis

Purified extracts were analysed by a Trace GC Ultra high resolution gas chromatograph equipped with an AS2000 autosampler and coupled by a heated transfer line to an ITQ 900 ion trap MS/MS spectrometer (ThermoFisher, Austin, TX). Chromatographic separation was achieved with a VF-Xms (Varian) fused-silica capillary column (60 m x 0.25 mm ID, 0.25 mm film thickness) with helium as carrier gas in PTV injection mode.

Results and Discussion

The most toxic PCB-congeners are the non-*ortho* but lateral chlorine substituted coplanar PCBs 3,3',4,4'-TetraCB (IUPAC-No. 77), PCBs 3,4,4',5-TetraCB (IUPAC-No. 81), 3,3',4,4',5-PentaCB (No. 126) and 3,3',4,4',5,5'-HexaCB (No. 169) being isostereomers to the 2,3,7,8-substituted PCDD/PCDFs. Even if these toxic non-ortho-PCBs are usually present in lower concentrations than other abundant PCB-congeners, their concentrations in food are generally higher than those of the PCDD/PCDFs. So these congeners have a significant contribution to the 2,3,7,8-TCDD toxic equivalents (TEQs). Due to the extremely low concentrations of the non-*ortho*-PCBs and PCDD/Fs and the presence of many other organic compounds at higher concentration levels interfering in their instrumental determination, the analysis of dl-PCBs and PCDD/PCDFs always involves extensive clean-up procedures. In order to simplify the clean-up step we developed an automated clean-up system based on gel permeation chromatography to perform the purification of co-extractable and potentially interfering compounds combined with an in-line concentration system, followed by a SPE alumina column to separate dl-PCBs from PCDD/Fs. In a previous paper we used this clean-up system to analyze micropollutants content in stack emission and waste solid samples from various thermal industrial processes[9]. In this paper we used the clean-up system to analyze the PCBs content in capsules of fish oil commercially available. We compared the new automated clean-up method and the reference method, based on successive manual clean-up steps on various chromatographic adsorbents. In table 1 are reported the results of three replicate clean-up.

The recoveries of the automated clean-up are good but lower than the manual clean-up (average value 12%). It could be due to reduced efficiency washing the vial and the evaporation chamber after the sample loading and after the automated concentration step, respectively. Probably also the transfer of the slurry on top of the

multilayer column is complete with washing by hexane the beaker containing the slurry: this washing is not possible when loading the SPE cartridges.
In Table 2 the relative standard deviation (RSD%) are reported: we observe that the automated clean-up is more repeatable than manual method.
The dl-PCB profiles of the sample extract cleaned-up by the automated and the manual methods do not show significant differences, which indicates the capability of the new automated procedure. The peaks are well resolved and not interferents are present that can disturb the analysis. PCB 118 was the most concentrated congener, followed by PCB 105, PCB 156 and PCB 167. However, the most important contributor to TEQ was in all the cases PCB 126, due to the high TEF value assigned (0.1). This pattern is similar to that found by other authors[2,7].
Regarding the values found in the fish oil, the average dl-PCBs sum was about 1 pg WHO TEQ/g: Martì et al.[2] in Spain found a range of 0.29-1.39 pg TEQ/g , Hasegawa et al.[4] in Japan found a range of 1.0-23.6 pg TEQ/g, Zennegg and Schmid[6] in Switzerland found a range of 0.04-2.0 pg TEQ/g, Fernandes et al. [7] in UK found a range of 1.12-41.5 pg TEQ/g.
European Union legislation[10] has set a action limits of 14 WHO-TEQ pg/g fat for dioxin-like PCBs in fish oil products for human consumption. Our results exceed this limit with the only contribution of dioxin-like PCBs.

	Automated clean-up		Manual clean-up	
Non-ortho PCBs	pg /g	Recoveries %	pg /g	Recoveries %
PCB-77	18	83.6	22	89.2
PCB-81	1.3	75.7	1.7	87.7
PCB-126	8.9	78.3	8.1	83.4
PCB-169	2.8	66.9	2.6	81.0
Mono-ortho PCBs				
PCB-105	210	84.8	260	91.2
PCB-114	18	68.2	15	86.4
PCB-118	710	75.2	507	87.2
PCB-123	29	81.1	17	97.2
PCB-156	110	75.4	123	89.4
PCB-157	24	90.2	37	101.1
PCB-167	61	79.2	70	93.3
PCB-189	17	68.2	23	90.4
DL-PCBs	1210		1086	

Table 1. PCB content (pg/g) and standard $^{13}C_{12}$ recoveries (%) in fish oil dietary supplements by automated and manual procedure (three replicate clean-up)

	Automated clean-up		Manual clean-up	
	pg WHO TEQ/Kg[11]	RSD %	pg WHO TEQ/Kg[11]	RSD %
Non-ortho PCBs				
PCB-77	1.8	17	2.2	21
PCB-81	0.39	26	0.51	32
PCB-126	890	7	810	12
PCB-169	84	10	78	16
Mono-ortho PCBs				
PCB-105	6.3	10	7.8	19
PCB-114	0.54	22	0.45	24
PCB-118	21.3	9	15.2	14
PCB-123	0.87	17	0.5	29
PCB-156	3.3	13	3.7	16
PCB-157	0.72	19	1.1	21
PCB-167	1.83	14	2.1	15
PCB-189	0.51	18	0.7	24
DL-PCBs	1011.56		922.26	

Table 2. PCB content (pg WHO TEQ/Kg) in fish oil dietary supplements by automated and manual procedure (three replicate clean-up)

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