WP1a

Multiplex Screening of Persistent Organic Pollutants in Fish Using Spectrally Encoded Microspheres

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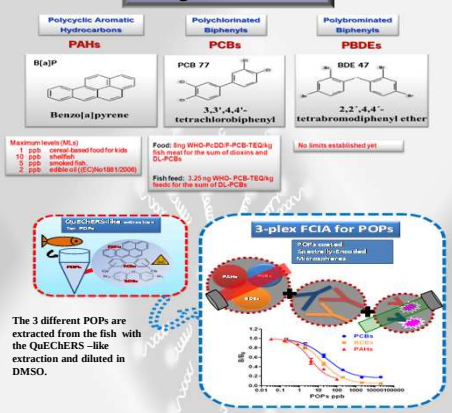
Objectives



The 3 different POPs are extracted from the fish with the QuEChERS –like extraction and diluted in DMSO.

Figure 1: The 3 different microbead sets coated with the protein conjugates of BaP-BSA, PCB77-OVA and PBDE47-BSA are combined to capture the mixture of a monoclonal antibody against BaP (MabBaP) and the two polyclonal antibodies against the PCB77 (PAbPCB77) and PBDE47 (PabPBDE47). The binding of the mixture of antibodies to the mixture of the three different coated microbeads was inhibited by the presence of the respective free POPs in the fish extract. Detection and quantification of the immune complexes were obtained via a combination of secondary antibodies against mouse or rabbit immunoglobulin (IgG) conjugated to the fluorescent protein R-phycoerythrin (PE).

3-plex FCIA

- Apart from the 3 markers, can detect at least 14 other POPs
- Can simultaneously detect BDEs, PCBs and PAHs in fish.
- Meets the regulatory requirements of the EU and US food safety authorities for PCBs and PAHs.
- After further validation, can be a valuable screening tool for POPs in fish and other food and environmental samples prior to GC-MS.

Three-plex FCIA development

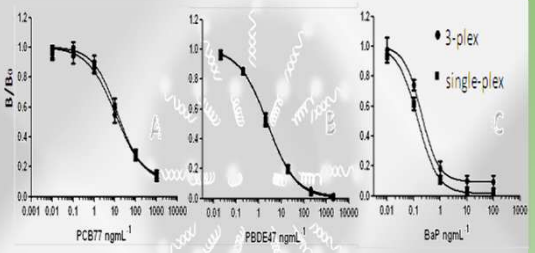


Figure 2: Dose-response curves obtained with the FCIA in 3-plex (●) and singleplex (■) formats in buffer for the three main POPs representatives analysed in this current study: (A) PCB77, (B) (BDE47) and (C) (BaP). The relative binding (B/B0) was calculated by dividing the response (B) of each concentration by the maximum response obtained in a solution without the analyte (B0). Solid lines show curves fitted with the four-parameters (4P) model. Each point represents the average of six replicates ± SD.

3-plex FCIA to fish samples & Aroclors

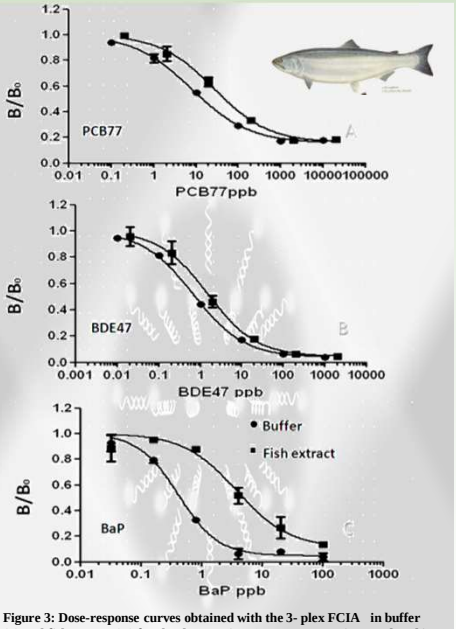


Figure 3: Dose-response curves obtained with the 3- plex FCIA in buffer (●) and fish extract (■) for the three main POPs representatives analysed in this current study: (A) PCB77 (fish extract (-) clean-up), (B) BDE47 (fish extract (+) clean-up) and (C) BaP (fish extract (-) clean-up). The relative binding (B/B0) was calculated by dividing the MFI response (B) of each concentration by the MFI obtained in a solution without the analyte (B0). Solid lines show curves fitted with the four-parameters (4P) model. Each point represents the average of six replicates ± SD.

Table 1 :3-plex FCIA characteristics in buffer and fish extracts and comparison to previously reported ELISAs for the detection of the three target POPs in buffer.

Target POPs	Matrix	Goodness of the 4P R^2 [a]	Curve steepness (mlng^{-1}) [b]	IC_{10} ppb in the 3plex FCIA[c]	IC_{50} ppb in ELISAs
PCB77 (A)	Buffer	0.9968	-0.8	20±2	2-15 [34]
	Fish extract	0.9927	-0.6	55±5	Not measured
BDE47 (B)	Buffer	0.9992	-0.7	2±0.1	0.135 [30]
	Fish extract	0.9902	-0.7	2±0.4	Not measured
BaP (C)	Buffer	0.9857	-1.3	0.4±0.1	0.3 [32]
	Fish extract	0.9435	-1	4±0.5	Not measured

[a] Goodness of the four-parameter model fit to the calibration curve. [b] Calculated from the four-parameter fitted calibration curve. [c] The average half-maximal inhibitory concentration (IC_{50}) for each analyte extrapolated from six standard curves as the concentration of the analyte that provokes 50% inhibition of the maximum response.

Table 2: Contaminated fish samples with their fat contents, levels of the target POPs (BaP, PCB77 and BDE47), as measured with GC-MS, and the percentages of inhibition of the maximum responses as was measured in the 3-plex FCIA with (+) or without (-) the clean-up.

Fishes	Target POPs measured	Fat content %	µgkg-1 as measured in GC-MS	Clean-up	% of inhibition of maximum response in 3plexFCIA
Smoked trout	BaP	10	0.06	-	0
Smoked trout	BaP	11	1	-	80±2
Smoked trout	BaP	14	5	-	80±5
Smoked trout	BaP	13	14.7	-	80±3
Trout	PCBs/BDEs	2	n.d.	+	0±0.1
Chub	BDE47	1.5	0.43	+	45±2
Chub	BDE47	2	4.93	+	56±5
Chub	BDE47	2	9	+	50±4
Chub	PCB77	1.5	1.95	-	22±2

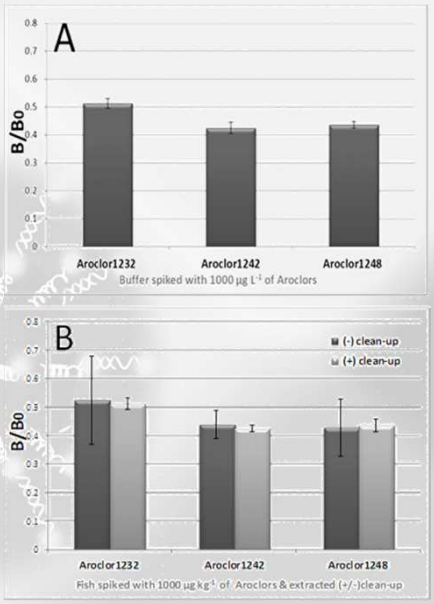


Figure 4: Relative inhibition (B/B0) of the maximum MFIs (B0) caused by the addition of 1000 ppb of Aroclor 1232, 1242 and 1248 respectively, to buffer (µg L-1) (A) and fish (µg kg-1) (B), applying a concentration step of 2.5 (2.5 g of fish mL-1 of extract), as measured in the 3-plex FCIA. The different Aroclors spiked fish samples were extracted with (+) or without (-) clean-up using the simplified extraction procedure described in this paper.

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