SIMPLIFIED SAMPLE PREPARATION PROCEDURE FOR SIMULTANEOUS ISOLATION OF ORGANOHALOGEN POLLUTANTS AND PAHs FROM FISH SAMPLES

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Introduction

Analytical methods for determination of various organic contaminants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and polyaromatic hydrocarbons (PAHs) in environmental and food matrices are typically based on multistep procedures. Routinely used sample preparation procedure includes Soxhlet extraction with a subsequent clean up and fractionation steps prior to relatively slow gas chromatography (GC) runs using either an electron capture (ECD) or a mass spectrometric (MS) detection in case of halogenated analytes. PAHs are commonly analysed separately using a liquid chromatography coupled to a fluorescence detector (LC-FLD), but for several non-fluorescence PAHs including in the EFSA opinion, a GC-MS analysis is needed. Pressurized liquid extraction (PLE) is a rapid sample preparation method that can be fully automatized. Moreover, extraction and clean up can be performed in one fully automated step operation.

Aim of the study

- **The main goal was to optimize an appropriate extraction/clean up procedure for analysis of PCBs, PBDEs and PAHs in fish fillets in a single run.**

Experimental

Target analytes

- **Dioxin-like PCBs**
  - non-*ortho* congeners #77, 81, 126, 169
  - mono-*ortho* congeners #105, 114, 118, 123, 156, 157, 167, 189
- **Brominated flame retardants**
  - Polybrominated diphenylethers: congeners #28, 47, 99, 100, 153, 154, 183
  - Hexabromocyclododecane
  - Polybrominated biphenyl: congener #153
- **Polycyclic aromatic hydrocarbons**
  - Benz(a)anthracene - B[*a*]A
  - Benzo(a)pyrene - B[*a*]P
  - Benzo(b)fluoranthene - B[*b*]F
  - Benzo(c)fluoren - B[*c*]Fln
  - Benzo(j)fluoranthene - B[*j*]F
  - Benzo(k)fluoranthene - B[*k*]F
  - Benzo(g,h,i)perylene - B[*ghi*]P
  - Chrysene - Chr
  - Cyclopenta(c,d)pyrene - CP[*cd*]P
  - Dibenz(a,h)anthracene - DB[*ah*]A
  - Dibenzo(a,h)-pyrene - DB[*ah*]P
  - Dibenzo(a,l)pyrene - DB[*al*]P
  - Indeno(1,2,3-cd)pyrene - I[*cd*]P
  - 5-Methylchrysene - 5 MeChr

Instruments

ASE 300 (Dionex, USA)

Agilent 6890 with a high-resolution TOF-MS detector (GCT Premier, Waters)

Results

Analytical strategies

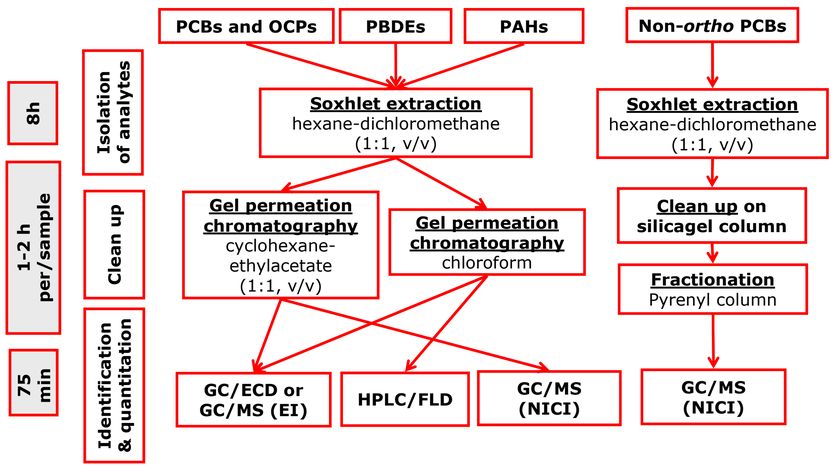

Figure 1: An example of a „conventional" sample preparation method for analysis of PCBs, PBDEs and PAHs in fish fillet

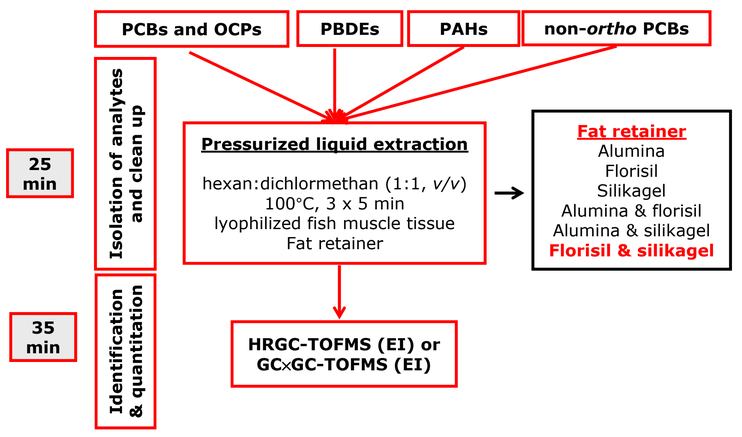

Figure 2: An example of a new sample preparation method for simultaneous analysis of PCBs, PBDEs and PAHs in fish fillet

Table I: Recovery and repeatability of optimized analytical method; spiked fish fillet sample – 1 µg/kg each analyte; n = 6 (fat retainer florisil + alumina)

	Analyte	Recovery [%]	RSD [%]		Analyte	Recovery [%]	RSD [%]
Non-*ortho* PCB	CB77	108	8	Major PBDE	BDE28	90	4
	CB81	104	8		BDE47	83	12
	CB126	110	6		BDE99	83	10
	CB169	105	10		BDE100	89	9
Mono-*ortho* PCB	CB105	98	12		BDE153	80	10
	CB114	100	11		BDE154	73	7
	CB123	89	12		BDE183	71	9
	CB156	89	12	EU PAHs	B[a]P	85	8
	CB157	90	12		B[a]A	98	5
	CB167	106	11		B[b]F+B[k]F	101	3
	CB189	87	13		B[j]F	97	5
Major PCB	CB28	85	10		B[ghi]P	93	4
	CB52	90	10		Chr	110	8
	CB101	93	11		CP[cd]P	93	7
	CB118	105	10		DB[ah]A	100	4
	CB138	86	11		DB[ae]P	93	3
	CB153	103	11		DB[ah]P	95	3
	CB180	112	7		DB[ai]P	89	4
					DB[al]P	95	5
					I[cd]P	93	5
					5MeChr	107	4
					B[c]Fln	90	10

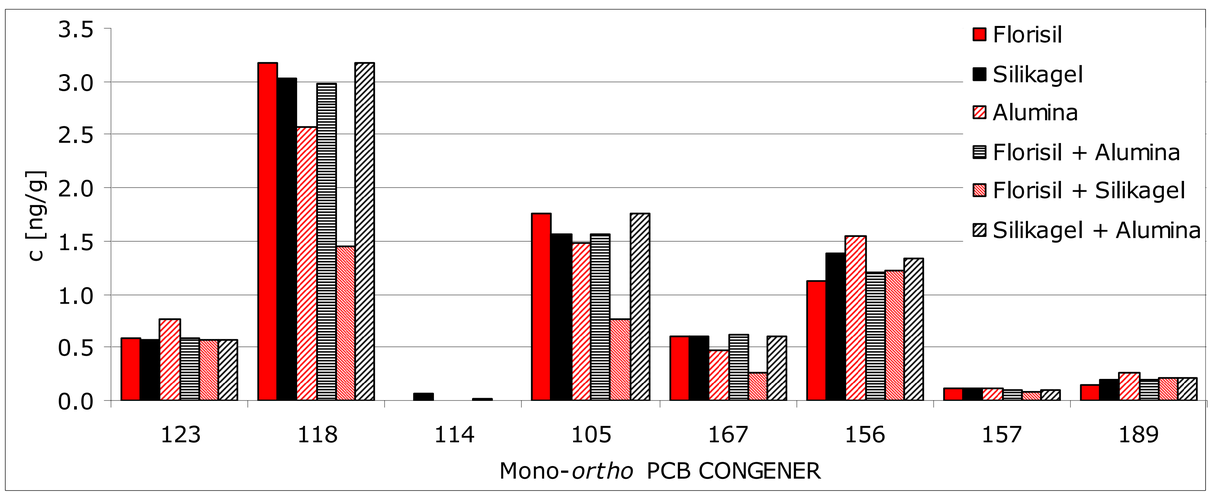

Figure 3: Levels of mono-*ortho* PCBs in naturally contaminated fish fillet (Bream – *Abramis brama*) obtained by PLE with different fat retainers

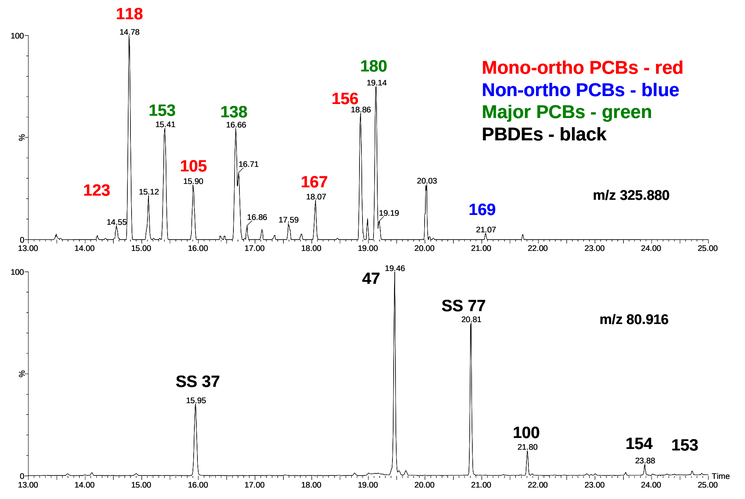

Figure 4: An example of chromatogram of dioxin-like PCB and PBDEs in naturaly contaminated fish fillet (Bream – *Abramis brama*) obtained by PLE with florisil and alumina used as fat retainer

- Recoveries of all target analytes together with repeatability (expressed as relative standard deviation, RSD) for fish fillets prepared from cod, a relatively lean fish (1.5% of lipids in muscle tissue in particular case) ranged between 3–19 % (n=6).
- Limits of detection (LOD) of this method were between 0.03–0.1 µg/kg fresh weight, 0.02–0.1 µg/kg fresh weight and 0.05–0.3 µg/kg fresh weight for PAHs, PBDEs and PCBs, respectively.

Conclusions

- **Traditional adsorbents alumina, silica gel, Florisil and / or their combinations were tested for PCBs, PBDEs and PAHs analysis in fish muscle tissue. Finally, the optimized PLE method using florisil and alumina as a fat retainer was validated. An experimental layout is summarized in Fig. 2.**
- **Repetability of simplified sample preparation procedure for all analytes, expressed as relative standard deviation (RSD, n=6) ranged from 3 to 19% and recovery on the level 1 µg/kg ranged from 71 to 112%.**

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