

## RAPID METHOD FOR SIMULTANEOUS DETERMINATION OF PCBs, PBDEs AND PAHs IN FISH SAMPLES

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### Abstract

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 that include 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. In this study, the fast extraction and clean-up procedure of all mentioned pollutants from a fish fillet by a pressurised liquid extraction (PLE) has been tested. The main goal was to optimize an appropriate extraction/clean up procedure and to demonstrate the separation power of comprehensive two-dimensional gas chromatography (GC×GC) coupled with a time-of-flight detector (TOF) for analysis of PCBs, PBDEs and PAHs in a single run. Repeatability of this simplified sample preparation procedure for all target 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 121%.

### Introduction

To protect consumers' health, maximum levels in feed and food have been set in Commission Regulation (EC) No 1881/2006 for various food contaminants including some persistent halogenated organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs). Although a lot of data has been generated in the EU countries, their reporting format as well as performance characteristics, such as limits of quantification (LOQs), of the method employed for analysis are largely un-harmonized and in some cases, poorly specified. With regards to the lack of credible data in some areas, legislative requirements and considering EFSA's request for more data on POPs occurrence in EU diets, representatives and hydroxylated metabolites of the following three groups are selected as target analytes: (i) dioxin-like PCBs - congeners that exhibit similar adverse health effects as PCDDs/PCDFs; (ii) brominated flame retardants (BFRs) such as polybrominated diphenylethers (PBDEs) and hexabromocyclododecane isomers (HBCD) – these “emerging” halogenated POPs are suspected for various toxic effects including endocrine disruption; (iii) polycyclic aromatic hydrocarbons (PAHs) – ubiquitous environmental/ processing contaminants with carcinogenic potency. This study is a part of the 7FP EU project CONFIDENCE (CONtaminants in Food and Feed: Inexpensive DETectioN for Control of Exposure), its aim is to support the needs that were identified by the Scientific Committee on the Food of the European Commission in the area of POPs. For the application of two novel complementary bioanalytical screening and the GC×GC/TOFMS comprehensive profiling a simple and fast sample preparation strategy is needed.

Classical approaches for the determination of organic pollutants (including PCBs, PBDEs and PAHs) in environmental and food matrices are usually laborious and time-consuming multistep procedures. These involve a number of treatments for extraction, usually a very long extraction in a Soxhlet apparatus and subsequent clean-up step, e.g. by gel permeation chromatography prior to final determination of target analytes using gas chromatography with an appropriate detector (ECD or MS) in case of organohalogen compounds and liquid chromatography with FLD for PAHs. Sample preparation and sample amount are critical steps in the analytical procedure of POPs.

A comprehensive profiling is a novel instrumental approach employing orthogonal gas chromatography-time-of-flight mass spectrometry. Thanks to the recent revival of TOFMS instruments several hundreds of analytes, belonging to different classes of organic pollutants such as dioxin-like PCBs, PAHs, BFRs, pesticides can be

theoretically measured in one run. The GC×GC TOFMS separation and detection parameters will be optimised aiming at reduced matrix interference and adequate sensitivity.

In this study the aim was to find a fast and user-friendly sample preparation procedure for PCBs, PBDEs and PAHs from fish fillet and to demonstrate the separation power of a GC×GC TOFMS.

### Materials and Methods

A pressurized liquid extraction system ASE 300 (Dionex, USA) was used for the combined extraction and clean-up. In short, a 5 g portion of lyophilized fish muscle tissue (1.5% lipids in fish fillet) was thoroughly mixed with fat retainer (15 g of alumina and 15 g florisil and/or silikagel) and placed into the PLE cell. The PLE cells were then spiked with a mixture of standards (PBDE congeners No. BDE 28; 47; 99; 100; 153; 153 and 183, PCB congeners No. 77; 81; 126; 169; 105; 114; 123; 156; 157; 167; 189; 28; 52; 101; 118; 138; 153 and 180; EU PAHs benzo(*a*)pyrene; benz(*a*)anthracene; benzo(*b*)fluoranthene; benzo(*j*)fluoranthene; benzo(*k*)fluoranthene; benzo(*g,h,i*)perylene; chrysene; cyclopenta(*c,d*)pyrene; dibenz(*a,h*)anthracene; dibenzo(*a,e*)pyrene; dibenzo(*a,h*)-pyrene; dibenzo(*a,i*)pyrene; dibenzo(*a,l*)pyrene; indeno(1,2,3-*cd*)pyrene; 5-methylchrysene; benzo(*c*)fluorene) and extracted three-times using a solvent mixture hexane:dichloromethane (1:1, *v/v*) at the temperature 100°C, heating time 5 min, pressure 15MPa, flush volume 60%, purge time 60s. The extract was evaporated to dryness and re-dissolved in 1 ml of isoctane.

The validation of sample handling method of PCBs and BFRs were performed using an Agilent 6890 GC system (Agilent Technologies, USA) coupled to a GCT (Micromass, Manchester, UK) high-resolution time-of-flight mass spectrometer for quantification of target analytes. The GC system was equipped with an electronic pressure control (EPC), a split/splitless injector, and a PAL Combi autosampler (CTC Analytics, Zwingen, Switzerland).

Gas chromatography conditions: DB-XLB (30 m x 0.18 mm x 0.18 μm) capillary column; helium flow rate: 1.0 mL/min; injection mode: pulsed splitless (4 mL/min for 1.5 min); injector temperature: 280°C; oven temperature: 60°C (1 min), @ 30°C/min to 220°C, @ 2°C/min to 240°C, @ 10°C/min to 340°C (6 min); mass spectrometry: NICI mode; ionisation gas: methane; acquisition rate: 2 spectra/s; Pusher interval: EI mode 40 μs (25000 raw spectra/second); *m/z* 45 – 500; ion source temperature: 220°C; transfer line temperature: 280°C; trap current: 200 μA; detector voltage: 2200 V.

The PAHs (within PLE experiments) were measured using an Agilent 6890 GC system coupled to a Agilent 5975 XL Inert MSD (Agilent Technologies, USA). Gas chromatography conditions: DB-17HT (30 m x 0.25 mm x 0.15 μm) capillary column; injection mode: solvent vent, 4 × 4 μl; vent time 3.4 min; vent flow 50 ml/min; vent pressure 34.4 kPa; splitless period 2 min; helium flow rate: 2.0 mL/min; injector temperature: 50°C (3.4 min) @ 400°C/min to 350°C; oven temperature: 110°C (5.4 min), @ 50°C/min to 350°C (5.8 min); mass spectrometry: EI/SIM mode; MSD transfer line temperature: 320°C; ion source temperature: 230°C; quadrupole temperature: 150°C.

An optimisation of comprehensive profiling an Agilent 6890N for comprehensive two-dimensional GC (GC×GC) with high speed TOFMS detector (Pegasus III, LECO Corp.); 1<sup>st</sup> column dimension: BPX-5 (30 m x 0.25 mm x 0.25 μm; SGE Analytical), Science; 2<sup>nd</sup> column dimension: Rt-LC35 (1 m x 0.15 mm x 0.1 μm; Restek) was used. GC parameters: injector temperature: 280°C; injection mode: pulsed splitless 30 psi (1 min); injected volume: 1 μL; column flow: 1.3 mL/min; oven temperature: 1st column: 60°C (1 min), @ 30°C/min to 220°C, @ 2°C/min to 240°C, @ 10°C/min to 340°C (6 min); 2nd column: 65°C (1 min), @ 30°C/min to 225°C, @ 2°C/min to 245°C, @ 10°C/min to 345°C (6 min). MS parameters: modulation period: 2 s; acquisition rate: 100 spectra/s; mass range: 50-1000.

### Results and Discussion

Figure 1 shows the flow chart of methods for analysis of major environmental contaminants represented by PCBs, PBDEs and PAHs that have been developed and validated in our laboratory within the recent two decades. Several alternatives of clean-up steps as well as detection technologies are employed. On this account, a comprehensive examination of particular sample contamination is rather ineffective, labour and time demanding. As previously mentioned, PLE represents one of the challenging approaches enabling unification of sample handling step. Both extraction of target analytes from food / environmental matrices and simultaneous removing of co-isolated matrix interferences are feasible under optimised conditions. In situ clean-up step can be

accomplished either by placing the sample on the top of suitable fat retainer and/or by direct mixing with respective sorbent. Besides of particular matrix composition (mainly its moisture content), the key parameters which can affect the PLE performance are extraction solvent and temperature, number of extraction cycles, pressure. Based on our previous experiments dealing with optimization of PLE<sup>1,2</sup>, hexane:dichloromethane (1:1, v/v) solvent mixture and temperature 100°C were approach of the first choice employed for our experiments. Within this study, we focused mainly on the selection of most suitable fat retainers. Traditional adsorbents alumina, silica gel Florisil and / or their combination were tested for PCBs, PBDEs and PAHs analysis in fish muscle tissue. Finally, the optimized PLE method was validated. An experimental layout is summarised in Fig.2.

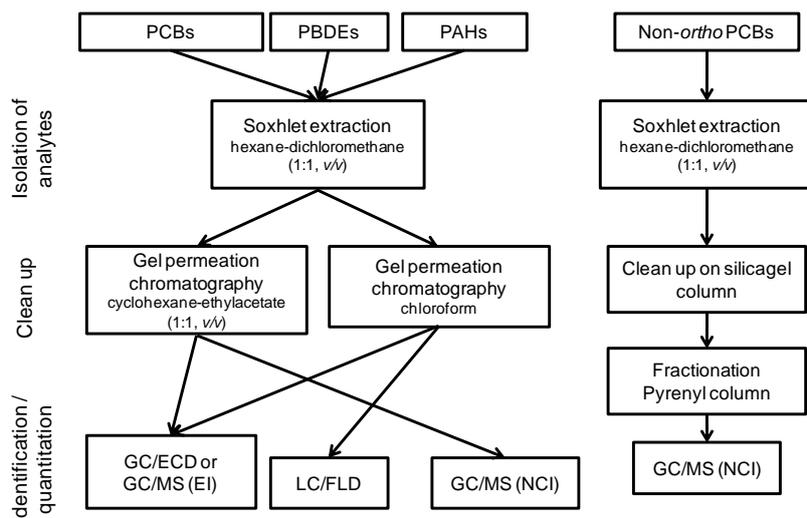


Figure 1: Example of a „conventional“ sample preparation method for analysis of PCBs, PBDEs and PAHs in fish fillet

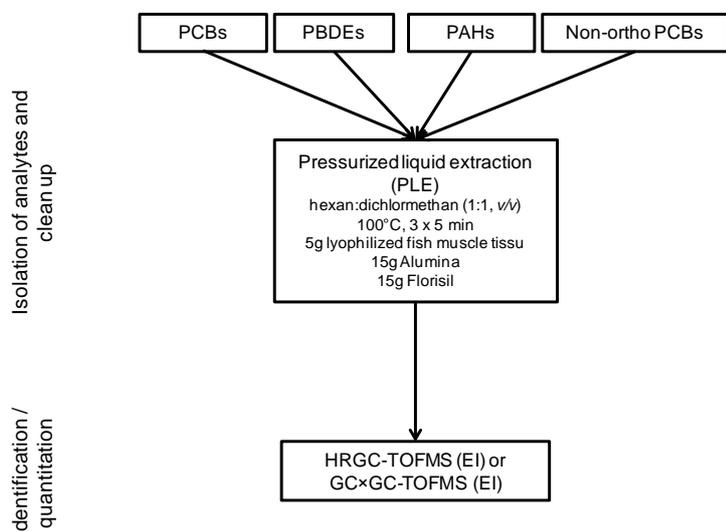


Figure 2: Example of a new sample preparation method for simultaneous analysis of PCBs, PBDEs and PAHs in fish fillet

Fillets prepared from cod, a relatively lean fish (1.5% of lipids in muscle tissue in particular case), were used for investigation of performance of several sorbents widely used for purification in POPs analysis - Florisil, silica gel and alumina. The recovery of target analytes and efficiency of lipids retaining was assessed. As documented

in Fig. 3, the highest amount of residual lipids was left in the extract from samples to which on silica gel was added, while the best purification effect was achieved by alumina / silica gel (1:1, w/w) and alumina / florisil mixtures (1:1, w/w). The later retainer was used for validation experiments, in which freeze dried fish tissue was spiked by 17 PCBs, 7 PBDEs and 16 PAHs (the contamination level, when expressed on wet weight, was 1 µg/kg) was processed by PLE. The final identification/ quantification of these contaminants in obtained extracts was carried out by GC-HRTOF MS or GC-MS under conditions described in the experimental section. Recoveries of all target analytes together with repeatability (expressed as relative standard deviation, RSD) ranged between 3–19 % (n=6) are summarised in Table I. Limit 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.

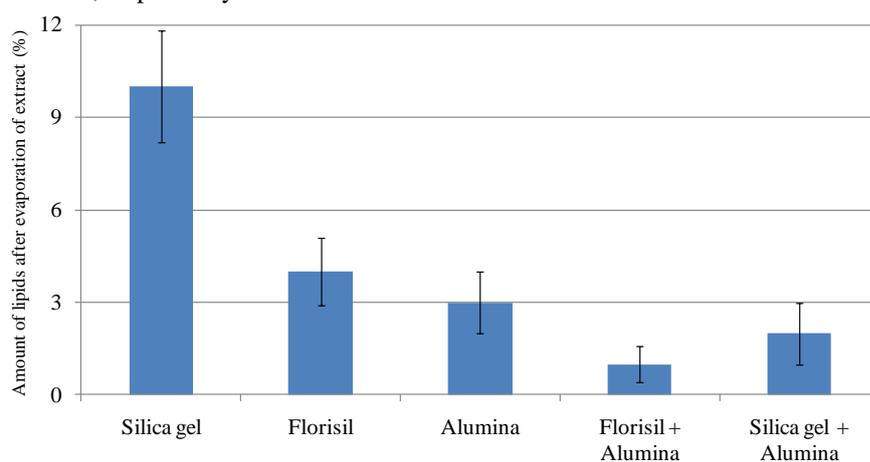


Figure 1 Effectiveness of lipid removal using different fat retainers, hexane-dichloromethane (1:1, v/v) used as extraction mixture

Table I Recoveries and repeatabilities of optimized analytical method; spiked fish fillet sample - 1 µg/kg each analyte; n = 6 (fat retainer Florisil+alumina)

	Analyte	Recovery [%]	RSD [%]
major PBDEs	BDE 28	90	4
	BDE 47	83	12
	BDE 99	83	10
	BDE 100	89	9
	BDE 153	80	10
	BDE 154	73	7
	BDE 183	71	9
non-ortho PCBs	CB 77	108	8
	CB 81	104	8
	CB 126	110	6
	CB 169	105	10
mono-ortho PCBs	CB 105	98	12
	CB 114	100	11
	CB 123	89	
	CB 156	89	12
	CB 157	90	12
	CB 167	106	11
	CB 189	87	13
major PCBs	CB 28	85	10
	CB 52	90	10
	CB 101	93	11
	CB 118	105	10
	CB 138	86	11
	CB 153	103	11
	CB 180	112	7

	Analyte	Recovery [%]	RSD [%]
EU PAHs	B[a]P	85	8
	B[a]A	98	5
	B[b]F+B[k]F	101	3
	B[j]F	97	5
	B[g,h,i]P	93	4
	Chr	11	8
	CP[c,d]P	93	7
	DB[a,h]A	100	4
	DB[a,e]P	93	3
	DB[a,h]P	95	3
	DB[a,i]P	89	4
	DB[a,l]P	95	5
	I[c,d]P	93	5
	5 MeChr	107	4
	B[c]Fln	90	10

In the second part of this study, a separation potential of a GC×GC TOFMS comprehensive profiling for the measurement of all groups of target analytes (representatives of BFRs, PCBs and PAHs) in a one single run was investigated. As mentioned earlier, for detection of PBDEs, various mass analyzers including single (low resolution) quadrupole and high resolution TOF operated either in electron ionization or electron capture negative ionization (NICI) mode have been routinely employed in our laboratory. The same instrumental techniques together with ECD have been used for PCBs analyses. On the other hand, PAHs were typically measured using HPLC-FLD, later on, as a response to the recommendation of the European Commission's Scientific Committee on Food (SCF) from, GC-MS method has been implemented to enable monitoring of 15 selected PAHs and benzo(c)fluorene.<sup>3</sup>

Within this very preliminary study conducted within the CONffIDENCE project, comprehensive gas chromatography (GC×GC) hyphenated with the time of flight mass spectrometry (TOFMS) was employed.

With regard to the complexity of POPs mixtures occurring in biota such fish, enhanced chromatographic resolution is required to avoid interferences due to chemical noise and co-eluting sample components and improve the quality of spectral information. In the first series of experiments, BPX-5ms column (30m×0.25mm×0.25µm) coated with a non-polar 95% methylpolysiloxane stationary phase, was used for the first-dimension separation based on volatility (generally recommended approach for the first dimension). To meet regulatory requirements, separation of target isomeric PAHs, such as benzo(a)fluoranthene, dibenzo(a,h)anthracene which cannot be deconvoluted, should be achieved. Unfortunately, this is, contrary to other POPs involved in our study, a rather difficult task when using this type of (short) column and relatively fast temperature programming up to 345°C to reduce heavy PAHs such as dibenzopyrenes. On this account, short capillary with liquid crystalline (smectic) stationary phase having a unique selectivity for separation of rigid planar molecule was put into second-dimension. Fig. 4–6 show obtained separation of model mixture (13 PBDE congeners, 16 recommended PAHs and 17 PCB congeners in a fish extract) in this system. In spite of the high separation power of GC×GC, it was not possible to resolve all target chemicals; the main problem was encountered with PAHs. Contrary to our expectation, BkF/BjF/BbF and IcdP/DBahA were almost totally overlapped, see Fig. 6. Under commonly used splitless injection mode, LOQs in range from 5 to 50 pg/injection were obtained. To improve the method detection limits the large volume injection mode and furthermore other types of capillary columns will be tested.

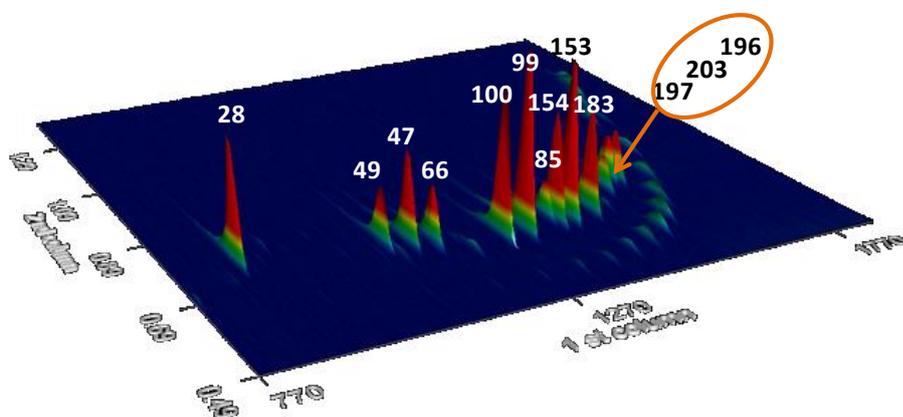


Figure 4 GC×GC –TOF MS (EI) analysis of PBDEs, ( $m/z$  246, 404, 484 and 562), fish extract prepared using PLE, 100 ng/ml isoctane

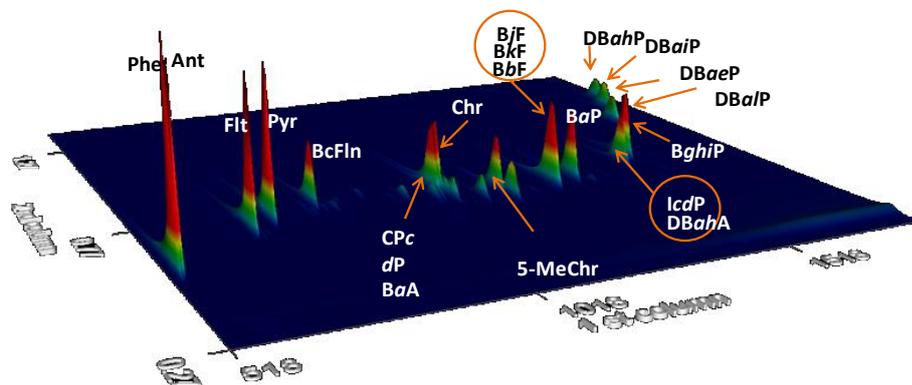


Figure 5 GC×GC–TOF MS (EI) analysis of PAHs, ( $m/z$  302, 276, 252, 242, 228, 178, 202, 216), fish extract prepared using PLE, 100 ng/ml isooctane

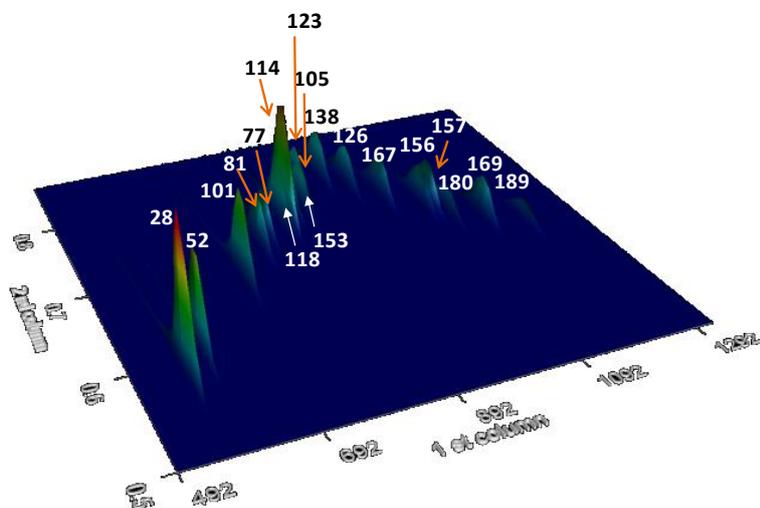


Figure 6 GC×GC–TOF MS (EI): PCBs mixture – 100 ng/ml isooctane ( $m/z$  256, 290, 324, 358, 390), fish extract prepared using PLE, 100 ng/ml isooctane

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