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ORIGINAL PAPER

Implementation of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the simultaneous determination of halogenated contaminants and polycyclic aromatic hydrocarbons in fish

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Abstract In the presented study, comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOFMS) was shown to be a powerful tool for the simultaneous determination of various groups of contaminants including 18 polychlorinated biphenyls (PCBs), seven polybrominated diphenyl ethers (PBDEs), and 16 polycyclic aromatic hydrocarbons (PAHs). Since different groups of analytes (traditionally analyzed separately) were included into one instrumental method, significant time savings were achieved. Following the development of an integrated sample preparation procedure for an effective and rapid isolation of several groups of contaminants from fish tissue, the GC×GC–TOFMS instrumental method was optimized to obtain the best chromatographic resolution and low quantification limits (LOQs) of all target analytes in a complex mixture. Using large-volume programmable temperature vaporization, the following LOQs were achieved—PCBs, 0.01–0.25 μg/kg; PBDEs, 0.025–5 μg/kg; PAHs 0.025–0.5 μg/kg. Furthermore, several capillary column combinations (BPX5, BPX50, and Rxi-17Sil-ms in the first dimension and BPX5, BPX50, Rt-LC35, and HT8 in the second dimension) were tested during the experiments, and the optimal separation of all target analytes even of critical groups of PAHs (group (a): benz[*a*]anthracene, cyclopenta[*cd*]pyrene and chrysene; group (b): benzo[*b*]fluoranthene, benzo[*j*]fluoranthene and benzo[*k*]fluoranthene; group (c): dibenz[*ah*]anthracene, indeno[*1,2,3–cd*]pyrene and benzo[*ghi*]perylene) was observed on BPX5×BPX50 column setup. Moreover, since the determination of target analytes was performed using TOFMS detector, further identification of other non-target compounds in real life samples was also feasible.

Keywords Fish · GC×GC–TOFMS · PAH · PBDE · PCB · PTV

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Introduction

Since the number of environmental contaminants which undergo the legislative control or are included in the monitoring programs of the European Food Safety Authority [1] and other international bodies (e.g., World Health Organization [2], US Environmental Protection Agency [3], and US Food and Drug Administration [4]) continuously increases, the demand for high-throughput, selective, sensitive, and non-expensive analytical methods is arising as well [5]. In response to the above-mentioned aspects, the European project CON*ff*IDENCE [6] was established to support respective food safety issues through the development and validation of simple, inexpensive, and rapid screening tools, which enable detection of selected food and environmental pollutants.

Polychlorinated biphenyl (PCBs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs) represent wide groups of important lipophilic environmental contaminants (PAHs may also originate from the improper

food processing) present not only in biotic and abiotic environmental matrices but also in feed, food, and human tissues and fluids [7–11].

Generally, although some steps in various "traditional" sample preparation approaches are rather similar, the final determination of PCBs and PBDEs is usually performed separately using one-dimensional gas chromatograph (1D–GC) coupled to an electron capture detector (ECD) or a mass spectrometer (MS) operated in different ionization modes (negative chemical ionization and/or electron ionization (EI)) [12–14]. PAHs are, on the other hand, routinely analyzed using a liquid chromatograph coupled to a fluorescence detector. However, as far as the non-fluorescence PAHs (e.g., cyclopenta[*cd*]pyrene and benzo[*c*]fluorene) are to be analyzed, then GC with a flame ionization detector (FID), a photoionization detector or MS in EI mode has to be used. Currently, MS is the dominating detection technique in GC analysis of PAHs providing, thanks to its selectivity, more accurate results compared with GC–FID [11, 15]. Simultaneous determination of a wide range of contaminants using 1D–GC–MS is often not possible, since a number of co-elutions and partial co-elutions among analytes and matrix components occur, especially when congeners and/or isomers with similar mass spectra, and thus the same quantification masses (*m/z*), had to be distinguished [5, 16–19].

Comprehensive two-dimensional gas chromatography (GC×GC) coupled to time-of-flight mass spectrometry (TOFMS) represents a powerful tool for the simultaneous determination of different types of contaminants. The separation efficiency and speed of GC analysis are increased not only among the target analytes but also from other non-target compounds and co-extracted matrix components [16–18, 20, 21]. Since the entire system comprises two capillary columns with different polarities, the total peak capacity of GC×GC setup is the product (multiplication) of two individual columns [7, 17, 20, 22], thus, GC×GC offers an alternative to 1D–GC with potentially greater measurement accuracy [23]. Moreover, a modulator-based focusing effect permits achieving lower limits of detection and quantification (LOQs) needed for the residual analysis as compared with 1D-GC [17, 20, 24].

Since a high number of narrow peaks are eluted from the GC×GC system, detectors with fast acquisition speeds are required to provide sufficient number of points per peak [16, 25]. In the past, FID or micro-ECD was commonly used in the environmental analysis employing GC×GC system for this purpose. However, none of them provides structural information of the kind needed for reliable compound identification. Recent progress in instrumentation design as well as the use of fast recording electronics led to introduction of a high-speed time-of-flight mass spectrometer. This detector allows collection of the data at acquisition rates >100 spectra/s, which is sufficient for reconstruction of very narrow peaks typically produced by GC×GC. In addition, employing of TOFMS permits the use of ^{13}C-isotopically labeled standards for quantification purposes [12, 16–18, 24, 25].

The applications of GC×GC–TOFMS can be divided into the group-type separation, fingerprinting, target-compound analyses, and non-target screening [26]. Until now, various applications of GC×GC coupled to TOFMS in food and environmental analyses have been reported [16, 17, 22, 23, 26, 27], but, according to the authors' best knowledge, simultaneous determination of halogenated compounds (PCBs and PBDEs) together with PAHs in fish matrix within a single analytical run has never been presented (the list of target analytes is primarily defined by the European project CON*ff*IDENCE [6]).

The main aim of the presented study was to develop and validate the GC×GC–TOFMS method for the simultaneous determination of PCBs, PBDEs, and PAHs in fish extracts prepared by the integral sample preparation procedure [28]. The focus of our experiments was to obtain the best chromatographic resolution and low LOQs for all target analytes. Therefore, several chromatographic capillary column combinations were tested, and also, the large-volume programmable temperature vaporization (LV-PTV) injection technique was optimized.

Experimental

Standards and chemicals

Certified standards of individual PBDE congeners # 28, 47, 99, 100, 153, 154, and 183 (all with declared purity >98 %) were supplied by Wellington Laboratories (Guelph, Ontario, Canada). PCB standards # 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189 (all with declared purity >97 %) and certified standards of individual PAHs: benz[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbFA), benzo[*c*]fluorene (BcFL), benzo[*j*]fluoranthene (BjFA), benzo[*k*]fluoranthene (BkFA), benzo[*ghi*]perylene (BghiP), chrysene (CHR), cyclopenta[*cd*]pyrene (CPP), dibenz[*ah*]anthracene (DBahA), dibenzo[*ae*]pyrene (DBaeP), dibenzo[*ah*]pyrene (DBahP), dibenzo[*ai*]pyrene (DBaiP), dibenzo[*al*]pyrene (DBalP), indeno[*1,2,3-cd*]pyrene (IP), 5-methylchrysene (5MC) (all with declared purity >98 %) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Calibration solutions prepared in isooctane containing all PCBs, PBDEs, and PAHs mentioned above at the concentration levels 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/mL were prepared in isooctane and stored at −18 °C.

Standards ^{13}C-chlorinated biphenyl (CB) 101, ^{13}C-CB 77 and brominated diphenyl ether (BDE) 37, BDE 77 (internal and syringe standards) were supplied by Cambridge Isotope Laboratories (Andover, MA, USA) and Wellington Laboratories (Guelph, Ontario, Canada), respectively. The certified standard solution of isotopically labeled PAHs used for quantification of target PAHs—US EPA 16 PAH cocktail ($^{13}C_6$-acenaphthene, $^{13}C_6$-acenaphthylene, $^{13}C_6$-anthracene, $^{13}C_6$-BaA, $^{13}C_4$-BaP, $^{13}C_6$-BbFA, $^{13}C_6$-BkFA, $^{13}C_{12}$-BghiP, $^{13}C_6$-DBahA, $^{13}C_6$-fluoranthene (FA), $^{13}C_6$-CHR, $^{13}C_6$-IP, $^{13}C_6$-naphthalene, $^{13}C_6$-phenanthrene, $^{13}C_3$-pyrene) and certified standards of $^{13}C_{12}$-DBaiP and $^{13}C_6$-DBaeP were supplied by Cambridge Isotope Laboratories Inc.

The standard reference material Lake Michigan Fish Tissue, SRM 1947 (10.4±0.5 % (*w/w*) of fat), for selected PCBs, organochlorine pesticides (OCPs), and PBDEs and standard reference material of mussel tissue, SRM 1974b (fat content not provided) for selected PAHs, PCBs, and OCPs were supplied by NIST (Gaithersburg, MD, USA).

Chemicals, reagents, and other material

n-Hexane, dichloromethane, and isooctane were supplied by Merck (Darmstadt, Germany). Ethyl acetate was purchased from Sigma-Aldrich (Steinheim, Germany). All solvents were of analytical grade. Silica (0.063–0.200 mm) supplied by Merck was activated by heating at 180 °C for 5 h, and then deactivated by adding 2 % of deionized water, shaking for 3 h, and finally, storing in a desiccator for 16 h before use. Magnesium sulfate and sodium chloride needed for the QuEChERS-like extraction were delivered from Sigma-Aldrich and Lach-ner (Neratovice, Czech Republic), respectively. A Pasteur pipette (D812, 230 mm length) and a glass wool were received from Poulten & Graf GmbH (Wertheim, Germany) and Merck, respectively.

Instruments

A tissue grinder was supplied by Retsch (Haan, Germany). A rotary vacuum evaporator Buchi Rotavapor R-114 and R-200 with a heating bath were obtained from Buchi Rotavapor (Flawil, Switzerland). A centrifugal machine Rotina 35R was supplied by Hettich Zentrifugen (Tuttlingen, Germany).

All GC–MS experiments were performed using a gas chromatograph Agilent 6890N (Agilent Technologies, Palo Alto, CA, USA) coupled to a high-speed time-of-flight mass spectrometer Pegasus III (LECO Corp, St Joseph, MI, USA). The GC system was equipped with an electronic pressure control, split/splitless and programmable temperature vaporization (PTV) injector, and an MPS 2 autosampler (Gerstel, Germany). During the experiments, several capillary columns (column combinations) with different polarities/selectivities were tested (see Table 1). All columns except for Rt-LC35 and Rxi-17Sil-ms (Restek, PA, USA) were purchased from SGE Analytical Science (Austin, TX, USA). Columns used in the first and second dimension had following parameters—30 m×0.25 mm i.d.×0.25 μm film thicknesses and 1 m×0.1 mm i.d.×0.1 μm film thickness, respectively. The ChromaTOF 4.24 software (LECO Corp.) was used for data processing.

Tested material

For the quality assurance/quality control of the entire method (sample preparation followed by GC×GC–TOFMS), trout (2 % (*w/w*) of fat) from the Czech retail market previously tested for the presence of PCBs, PBDEs, and PAHs was used.

Extraction and clean-up

The fish samples were prepared by a method described by Kalachova et al. [28]. Briefly, 10 g of fish tissue homogenate (with surrogate BDE 37 and ^{13}C-CB 77 – 10 ng absolutely) were mixed with 5 mL of distilled water and shaken vigorously with 10 mL of ethyl acetate in a polypropylene centrifuge tube for 1 min. Subsequently, 4 g of magnesium sulfate and 2 g of sodium chloride were added to the mixture. The tube was shaken for another 1 min and centrifuged, and an aliquot of 5 mL was removed from the organic layer. The crude extract (5 mL) was evaporated to the last drop, and residual solvent was carefully eliminated until dryness under the gentle stream of nitrogen.

An evaporated extract was re-dissolved in 1 mL of *n*-hexane and purified using a handmade silica minicolumn. The fat determination and the choice of the silica minicolumn size according to the fish muscle fat content are described elsewhere [28]. Collected eluates were carefully evaporated using a vacuum rotary evaporator, and the residual solvents were removed under the gentle stream of nitrogen. Residues were finally re-dissolved in 0.5 mL of isooctane containing BDE 77 (5 ng/mL), ^{13}C-CB 101 (40 ng/mL), and ^{13}C-PAHs (2 ng/mL) used as syringe standards.

Table 1 Column systems tested within the GC×GC–TOFMS experiments

Column system	First dimension	Second dimension	Modulation period, s
1	BPX5	BPX50	4.0
2	BPX5	Rt-LC35	2.0
3	BPX5	HT8	4.5
4	BPX50	BPX5	4.0
5	BPX50	HT8	4.0
6	Rxi-17Sil-ms	HT8	4.0

GC×GC–TOFMS analysis

All experiments were performed using an Agilent 6890N GC system coupled to a Pegasus III TOFMS operated in EI that allowed identification and quantification of all target PCBs, PBDEs, and PAHs within a single analytical run. Hot splitless (injected volume 1 μL) and LV-PTV injection in solvent vent mode (injected volume 1×5, 1×8, and 1×10 μL) with following parameters were tested—vent flow, 50 mL/min; vent pressure, 50 psi; initial temperature, 50 °C; inlet heating velocity, 400 °C/min; final inlet temperature, 300 °C. Both solvent vent time and purge time were changed according to the injected volume (see Table 2). Helium was used as a carrier gas using flow 1.3 mL/min. Target analytes were separated using several chromatographic capillary column combinations with different polarities of stationary phases (see Table 1). Oven temperature program was 80 °C (4.3 min), at 30 °C/min to 240 °C, at 2 °C/min to 270 °C, at 5 °C/min to 320 °C, and at 40 °C/min to 360 °C (12 min). Secondary oven was held 10 °C above the main oven.

The MS detector was operated under the following conditions—mass range, *m/z* 45–750; ion source temperature, 250 °C; transfer line temperature, 280 °C; detector voltage, 1650 V; acquisition rate, 100 spectra/s. The ions (*m/z*) selected for quantification and identity confirmation are shown in Electronic supplementary material Table S1. The modulation period was dependent on the column system (see Table 1) and was set down to avoid wrapping-around of the later-eluting analytes.

All target analytes were identified by comparison of their first and second dimension retention times with respective reference standard. For the quantification, the most selective/intensive ion of particular compounds was used. Quantification was performed according to their height, and four major modulated peaks for each compound were summed. The minor modulated peaks with S/N<6 were ignored by the software setting (the selected value S/N<6 represents a compromise between the number of particular modulated peaks that need to be re-integrated and the repeatability of the measurement). In case of peaks that were not baseline-separated, the manual integration was mainly used. An 11-point calibration was used.

Table 2 Inlet parameters tested within the LV-PTV optimization

Injection volume, μL	Purge time, s	Solvent vent time, s
5	190	70
8	260	140
	300	180
10	320	200
	370	250
	420	300

To eliminate the potential injection inaccuracies, syringe standards were used, as follows: BDE 77 for all PBDEs, ^{13}C-CB 101 for all PCBs, and corresponding ^{13}C-labeled analogues for PAHs. For those PAHs that do not have their own ^{13}C-labled standard, the following ^{13}C-PAHs were used for the quantification: $^{13}C_4$-BaP for BjFA, $^{13}C_{12}$-DBaiP for DBahP, and DBalP and $^{13}C_6$-CHR for CPP and 5MC.

Results and discussion

The optimization of a GC×GC–TOFMS analysis of a mixture of organohalogenated contaminants together with PAHs in fish tissue is an uneasy task, not only because a high number of analytes possessing different physico-chemical properties has to be included into a single analytical method but also due to wide and often very low (trace) concentration levels of these environmental contaminants present in real life samples [17, 20, 25]. Moreover, fish tissue represents a complex biotic matrix in which also similar non-target contaminants could be present, e.g., other congeners of PCBs and PBDEs, which may have the identical quantification (confirmation) *m/z* as target isomers; thus a good chromatographic separation is needed to avoid the overestimated results caused by potential co-elutions. Under these conditions, GC×GC–TOFMS represents a suitable approach since it provides both high separation power and possible deconvolution of compounds with close retention times. In the paragraphs below, the optimization and further validation of the multi-residue procedure employing this approach for the identification and quantification of the most important representatives of PCBs, PBDEs, and PAHs in fish tissue extracts within a single analytical run is presented for the first time, and the most difficult points of the method development are pointed out.

Optimization of injection technique

The injection parameters pose one of the most important tasks especially, when multi-residue instrumental method for various groups of analytes has to be developed. In this particular case, when PCBs, PBDEs, and PAHs have to be included into a single run, the attention must be paid mainly on the later group of analytes, as the injection conditions suitable for PCBs and PBDEs could lead to the losses of the highly volatile PAHs.

To reduce the size of generated data and time needed for their handling, in the first part of our experiments, when the setting of the injection step was tested, only 1D–GC–TOFMS system was employed. In the follow-up experiments, the second dimension was involved. Firstly, the most common hot splitless injection technique (1 μL) was tested mainly due to its ease of operation, robustness, and availability in routine

laboratories [29]. The injector temperature 280 °C was taken from the GC–MS method routinely used in our laboratory. Unfortunately, since this type of injection allows injection of only a limited volume of the solvent (1–2 μL) [12], the obtained LOQs were too high to meet the goal of this study defined in the European project CONffIDENCE (PBDEs ≤0.2 μg/kg, BaP 2 μg/kg) [6]. Moreover, degradation of thermo-labile compounds as higher brominated PBDE may occur [12, 19, 29]. Considering the desired LOQs, five to ten times higher injections were required. On this account, PTV injection in the solvent vent mode was further implemented. An additional benefit offered by this technique was the reduction of discrimination of heavy molecular analytes (e.g., dibenzopyrenes) compared with hot splitless injection (reported also in other studies [12, 29, 30]).

However, LV-PTV requires more operating parameters to be optimized as compared with a hot splitless injection [30]. The most critical step of LV-PTV in solvent vent mode was the solvent vaporization when solvent should be eliminated without losses of the analytes [30]. Tested injection volumes (1×5, 1×8, and 1×10 μL) were chosen based on previous experiences, and the related parameters (injection volume, purge time, and solvent vent) were calculated by the software according to the selected volumes (for more details, see Table 2). An 8-μL injection was seen as optimal since it allowed achieving the lowest LOQs; the higher injection (10 μL) did not provide significant improvement of LOQs. A multiple injection which allows repeated injection of lower volume represents another option to decrease the LOQs. Unfortunately, the setup of the autosampler coupled to GC×GC–TOFMS instrument did not allow the use of this type of injection. For the injection of 8 μL, purge time 260 s and solvent vent time 140 s were chosen as the best setup. The inlet temperature was set at 50 °C. Secondly, the influence of an injection speed (5, 10, 20, and 30 μL/s) was tested, and the best results were obtained by injecting the sample at a speed of 10 μL/s. Better results using a slower injection rate were in accordance with results of Gómez-Ruiz et al. who dealt with optimization of PTV injection in PAHs analysis [30]. The LOQs (see also the section "Method validation and performance characteristic specification") obtained by 1 and 8 μL injection volumes in 1D–GC system are compared in Table 3. Since all injected analytes were at the same concentration level, even for partly resolved compounds, the peak apexes could be identified, thus quantification in 1D was possible. The decrease of LOQs by more than one order of magnitude was observed. Moreover, further improvement was observed after the implementation of the second dimension that allowed, thanks to the modulation system, the detection even of trace levels of contaminants contained in the sample extracts. Slightly higher LOQ was obtained for heptabrominated BDE 183 caused by its lower sensitivity in EI mode [31] and also by rather slow temperature program which was necessary for separation of all PCB congeners (not only of target congeners but also of other potentially present PCBs in the sample extracts). However, this GC oven temperature program was not optimal for higher brominated compounds, for which faster program and shorter column (15 m) would lead to much lower LOQs [12].

Selection of the GC×GC capillary column combination and oven temperature program

Another crucial point when optimizing the GC×GC–TOFMS method is to find out a suitable separation system. The column combinations included in the presented experiments were selected on the basis of data previously reported in the literature [5, 12, 21–23, 25, 27] and on the orthogonal requirements of the GC×GC system [32]. However, PCBs, PBDEs, and PAHs have always been analyzed separately, and the system enabling separation of all these three groups of contaminants in a single run has not been published until now. Moreover, it is not only the appropriate column for the first and second dimensions that has to be found out but also the suitable oven temperature program enabling at the same time (1) rather slow separation of all target and non-target PCBs and PBDEs, (2) separation of critical pairs of isomeric PAHs, and (3) quite fast elution of thermo-labile higher-brominated PBDEs and late-eluting heavy PAHs not to prolong the total duration of the analysis more than it is necessary.

One-dimensional setup

For organic contaminant analysis, non-polar or slightly polar stationary phases are typically used in the first dimension, where analytes are separated mainly based on their volatility. Since low-volatile, late-eluting PAHs with high molecular weights were also involved in these experiments, only columns with a high upper temperature limit (at least 360 °C) were taken into the consideration. Selection of the column diameter depends on the desired separation, but, in most cases, columns 15–30 m×0.25–0.32 mm i.d.×0.1–1 μm film thickness are used [11, 12, 16–19, 21–23, 25, 26, 30, 31]. For PCBs, lower-brominated PBDEs and PAHs, usually 30-m-long columns, are applied. However, as mentioned above, when analyzing higher-brominated PBDEs, shorter columns (10–15 m) are typically involved [12]. Putting together all these considerations, three 30-m-long capillary columns with different polarities were tested within these experiments for the first-dimension separation: (1) BPX5 (5 % phenyl polysilphenylene–siloxane), non-polar stationary phase; (2) BPX50 (50 % phenyl polysilphenylene–siloxane), moderately polar stationary phase; and (3) Rxi-17Sil-ms (crossbond silarylene), moderately polar stationary phase. The separation and elution order of all analytes on BPX50 and Rxi-17Sil-ms in one-dimensional setup was

Table 3 Comparison of limits of quantification (LOQs, micrograms per kilogram fish muscle tissue) of 1D–GC and GC×GC setups

Analytes		LOQ, μg/kg			Analytes	LOQ, μg/kg		
		1D–GC		GC×GC		1D–GC		GC×GC
		1 μL[a]	8 μL[b]	8 μL[b]	PAHs	1 μL[a]	8 μL[b]	8 μL[b]
Mono-*ortho* PCBs	CB 105	5	0.1	0.01	BaA	1	0.1	0.01
	CB 114	5	0.1	0.01	BaP	1	0.25	0.05
	CB 118	5	0.1	0.01	BbFA	1	0.1	0.025
	CB 123	5	0.1	0.01	BcFL	1	0.1	0.025
	CB 156	5	0.25	0.01	BjFA	1	0.25	0.025
	CB 157	5	0.25	0.01	BkFA	1	0.1	0.025
	CB 167	5	0.25	0.025	BghiP	1	0.1	0.025
	CB 189	0.5	0.25	0.025	CHR	1	0.05	0.01
Major PCBs	CB 28	0.5	0.01	0.01	CPP	1	0.1	0.01
	CB 52	1	0.05	0.01	DBahA	1	0.25	0.25
	CB 101	5	0.025	0.01	DBaeP	5	0.25	0.25
	CB 138	5	0.1	0.01	DBahP	5	0.5	0.1
	CB 153	5	0.1	0.01	DBaiP	5	1	0.5
	CB 180	5	0.25	0.025	DBalP	5	0.1	0.1
Non-*ortho* PCBs	CB 77	1	0.05	0.01	IP	10	0.25	0.1
	CB 81	5	0.05	0.01	5MC	1	0.1	0.1
	CB 126	5	0.1	0.01				
	CB 169	5	0.25	0.025				
PBDEs	BDE 28	10	0.5	0.025				
	BDE 47	10	0.5	0.025				
	BDE 99	10	1	0.25				
	BDE 100	10	0.5	0.1				
	BDE 153	10	0.5	0.25				
	BDE 154	10	1	1				
	BDE 183	>10	10	5				

[a]Hot splitless
[b]LV-PTV

completely identical. In case of BPX5, different elution order of CB 114 and 153 (CB 114 eluted prior CB 153), CB 157 and 180 (CB 157 eluted prior CB 180), and CPP and CHR (CPP eluted prior CHR) occurred.

The development of the oven temperature program was based on the combination of temperature programs used for individual separation of each target group (PCBs, PBDEs, and PAHs). A relatively slow ramping had to be used for the separation of all target and non-target PCBs that could also be present in the sample extracts. However, as already mentioned above, this oven temperature program was not optimal for the separation of higher PBDEs, and therefore faster programming had to be included in the second part of the analysis. Final heating up to 340 °C was then used for the elution of a group of heavier PAHs (dibenzopyrenes).

Two-dimensional setup

During the measurements in one-dimensional setting, the problematic groups of analytes were identified. All target PCBs (except for isomeric pairs CB 28 and 31, and CB 118 and 123) and PBDEs were separated from other target as well as non-target congeners. The only separation difficulties were with the following groups of PAHs: (a) BaA, CPP, and CHR; (b) BbFA, BjFA, and BkFA; and (c) DBahA, IP, and BghiP. While the chromatographic separation was the only possibility in case of three isomeric fluoranthenes, as they have the same quantification mass (*m/z*), groups A and C could be partially resolved according to their different ion masses (*m/z*). Indeed, the presence of small fragment ion at *m/z* 276 originated from DBahA (quantification *m/z* 278; confirmation *m/z* 276 forms approximately 20 % of the abundance *m/z* 278) may interfere with IP quantification (quantification *m/z* 276; confirmation *m/z* 278 forms approximately 5 % of the abundance *m/z* 276). While in the analysis of the standard mixture (all compounds at the same concentration level), the ratio of ion abundances of these two compounds can be calculated, their concentrations in the real-life samples are different, thus this estimation is not possible. However, the levels of DBahA in real-life samples

are usually present at the concentration levels ten times lower than IP (as in SRM 1974b used in our experiments), thus only negligible overestimation by less than 1.8 % for IP may be obtained. On the other hand, since the concentration of IP is ten times higher than that of DBahA, more significant overestimation (approximately 20 %) of this analyte may occur (as can be seen also in our results), even though the contribution of *m/z* 278 from IP to DBahA in the standard mixture at the equal ratio is approximately 1.2 %.

The stationary phases in the second dimension typically used for organic contaminants are 35–50 % phenylene/65–50 % dimethylpolysiloxane, polyethyleneglycol, carbonare (HT8), and cyanopropyl–phenyl–dimethylpolysiloxane. Since the separation in the second column has to be completed in a few seconds, narrow-bore columns are commonly used, for instance, 0.5–2 m×0.1 mm i.d.×0.1 μm film thickness [12, 16, 17, 25, 26, 32]. During GC×GC optimization, following capillary columns with different stationary phases for the second dimension were tested: (1) BPX50 (50 % phenyl polysilphenylene–siloxane), (2) Rt-LC35 (dimethyl (50 % liquid crystal) polysiloxane), (3) BPX5 (5 % phenyl polysilphenylene–siloxane), and (4) HT8 (8 % phenylpolysiloxane–carbonare). Comparable column combinations for the analysis of several organohalogenated compounds (not including PAHs) to those applied in our experiments were used by Bordajandi et al. who tested among the others also ZB5×BPX50 and ZB5×HT8 [22]. Nevertheless, the non-polar×(medium)–polar column setting is the widely used option worldwide [32]; the reversed-type column combination was also tested within our experiments (BPX50 and Rxi-17Sil-ms columns in the first dimension). All target PCBs and PBDEs were separated, as mentioned above, on all column systems tested within the experiments except for (BPX50×HT8) and (Rxi-17Sil-ms×HT8) where CB 118 and 123 were co-eluted. Selection of the best column combination was therefore mainly influenced by its ability to separate three critical groups of PAHs. The best separation for each critical group was achieved on the following column systems: (a) BaA, CPP, and CHR–BPX5×BPX50; (b) BbFA, BjFA, and BkFA–BPX50×HT8; (c) DBahA, IP, and BghiP–BPX5×HT8. As shown in Table 4, in which the capability of all tested column systems to separate the critical groups of PAHs is summarized, only BPX5×BPX50 setup was able to resolve all critical groups as well as all PCBs and PBDEs. The separation of PAHs using the final column setup BPX5×BPX50 with optimized oven temperature program is shown in Fig. 1. It should be emphasized that following groups of PAHs were not baseline-separated using BPX5×BPX50 column setup: (a) BaA and CPP, (b) benzofluoranthenes (BbFA, BjFA, and BkFA), and (c) DBahA and IP. The calculated chromatographic resolutions (R_{1D} and R_{2D} for the first and second dimensions, respectively) of these closely eluted analytes (isomers) in both dimensions were as follows: BaA and CPP R_{1D}=0.25, R_{2D}=1.25; BbFA and BkFA R_{1D}=0.19, R_{2D}=0.25; BkFA and BjFA R_{1D}=0.75, R_{2D}=0.40; DBahA and IP R_{1D}=1.41, R_{2D}=0.96. Not fully achieved separation of BbFA with BkFA and DBahA with IP in composite diesel fuels using similar stationary phases in the first and second dimensions has been presented by Cavagnino et al. who dealt with a single analysis of several PAHs in complex abiotic matrices [21]. Although the column setup BPX5 with liquid crystals (LC50) in the second dimension has already been successfully used in previous studies for the separation of PAHs [23], in our study, complete co-elution of all critical groups of PAHs using liquid crystals in the second dimension was observed. It is worth noticing, that liquid crystalline columns are mainly dedicated for good separation of PAHs; these columns have somewhat lower upper temperature limit (270 °C), thus, elution of the heavier PAHs is not feasible. Moreover, a high column bleed background was observed using this type of stationary phase [12]. On the contrary, the use of non-orthogonal column setting (BPX50 and Rxi-17Sil-ms columns in the first dimension) led to a good separation of the second critical group of PAHs.

The development of first-dimension oven temperature program is described in section "One-dimensional setup." An offset temperature of 10 °C of the second dimension was maintained relatively to the primary oven to allow the separation of target analytes as well as other (matrix) components but also to avoid wrap-around phenomenon (with the exception of later eluting PAHs (DBaeP, DBahP, DBaiP, DBalP)). In this case, both modulation period (4 s) and temperature offset (10 °C) were insufficient for their proper elution. However, longer modulation period (7 s) was not used since this might lead to recombination of some isomeric compounds during the modulation process, thus, lost of chromatographic resolution. In addition, the 2D-separation space of these later-eluting PAHs was not occupied by matrix interferences; thus, shorter modulation period was justified in this case.

Table 4 Capability of different column systems to separate critical groups of PAHs ((a) BaA, CPP and CHR; (b) BbFA, BjFA, and BkFA; (c) DBahA, IP, and BghiP)

First dim. Second dim.	BPX5 BPX50	BPX5 Rt-LC35	BPX5 HT8	BPX50 BPX5	BPX50 HT8	Rxi-17Sil-ms HT8
Group (a)	Yes	No	Yes	No	No	No
Group (b)	Yes	No	No	Yes	Yes	Yes
Group (c)	Yes	No	Yes	No	No	No

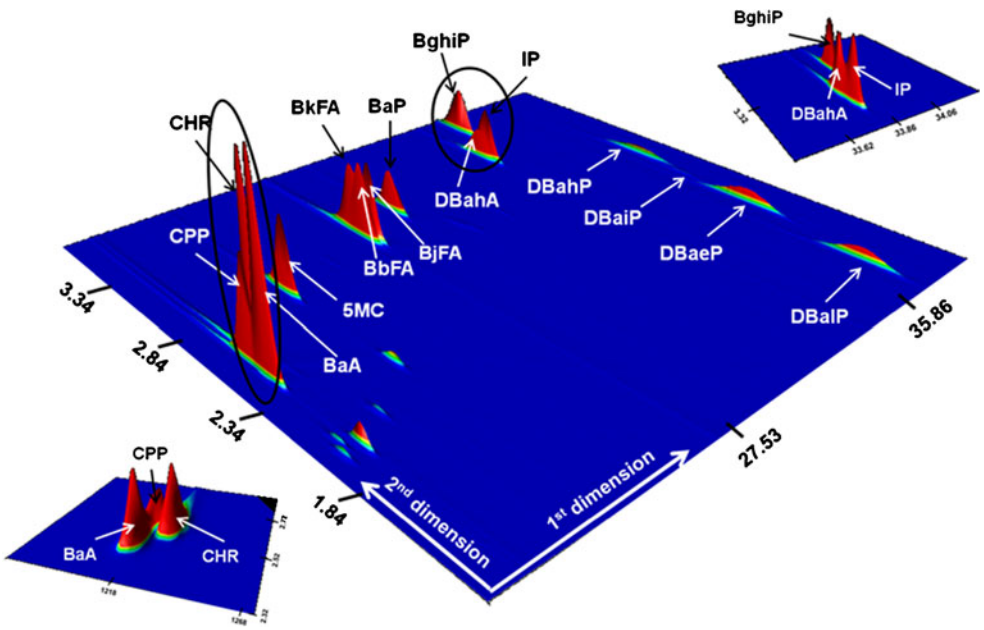

Fig. 1 An example of a GC×GC–TOFMS chromatogram of PAHs (concentration 100 ng/mL, corresponding to 10 μg/kg fish muscle tissue) separated on BPX5×BPX50 column system. Sum of *m/z* 226, 228, 242, 252, 276, 278, and 302 is displayed

Method validation and performance characteristic specification

Using the final injector setup, the repeatability of instrumental measurement was tested on the standard mixture of all target compounds in isooctane at the concentration 100 ng/mL (corresponding to 10 μg/kg fish muscle tissue). The volume of 8 μL of standard that corresponds to 800 pg of each standard was injected onto the column system BPX5×BPX50. The repeatability of instrumental measurement for all target compounds, calculated from 15 repeated analyses, and expressed as a relative standard deviation (RSD, percent), was in the range 7–17 %.

The overview of validation data (recovery, repeatability, LOQ, and linearity of the system) obtained by the optimized sample preparation and instrumental method for fresh fish tissue (trout) is summarized in Table 5. To validate the entire analytical method, the fish muscle tissue spiked with all target analytes was prepared and analyzed. With each batch of samples, the procedural blank (i.e., sample processed in a common way, but without the use of test matrix) was prepared. Only traces of some major CB 138, 153 and 180, and BDE 47 were determined in the blank sample. The recovery (percent) was calculated as an "absolute" recovery (not corrected to the recovery of surrogate standard) and repeatability (percent) was expressed as a RSD from six replicate analyses of blank trout fortified 15 min prior to extraction with a mixture of target analytes at two different concentration levels (1 and 5 μg/kg). Since some trace levels of major CB 138, 153, and 180, and BDE 47 in blank (trout) samples were found, the concentration levels of 5 and 25 μg/kg were applied in case of these four analytes. The recoveries (percent) and RSD (percent) were in the range 75–120 % (RSD 4–19 %) for PCBs, 91–117 % (RSD 3–18 %) for PBDEs, and 78–97 % (RSD 4–14 %) for PAHs. The key performance characteristics documented through the validation protocol met the criteria applied in the European Union in food contaminants control (SANCO document no. 12495/2011 [33] originally designed for pesticide residue analysis but commonly applied also for other organic food contaminants): recoveries in the range 70–120 % and the repeatability less than 20 %. Moreover, for BaP the method, performance criteria were also tested against Commission Regulation (EC) No 333/2007 (LOQ< 0.9 μg/kg; recovery, 50–120 %; precision expressed as HORRATr<2) [34].

To control the entire sample preparation of real-life samples, the recovery of surrogate standards ^{13}C-CB 77 and BDE 37 added to the sample prior the extraction was monitored.

The LOQ was defined as the lowest concentration of each analyte in matrix at which the quantification and identity confirmation ions are intensive enough to meet pre-defined criteria (signal-to-noise ratio at least 6:1; defined in SANCO document no. 12495/2011 [33]). Based on preliminary GC–TOFMS measurements using matrix samples contaminated at low concentrations, the LOQs in fish muscle tissue were in the following ranges—PCBs (0.01–0.25 μg/kg), PBDEs (0.025–5 μg/kg), and PAHs (0.025–0.5 μg/kg). An example of the GC×GC–TOFMS chromatogram of spiked fish muscle tissue is shown in Fig. 2.

With regard to a wide concentration range of target analytes to be analyzed in fresh fish tissue (e.g., trace levels of dioxin-like PCBs as compared with relatively high abundant indicator PCBs (non-dioxin-like) [18, 21, 26]), it is necessary to use an extensive scale of working standard solutions for calibration (0.05–100 ng/mL). Weighted linear

Table 5 Recoveries (percent), repeatabilities (expressed as RSD, percent) calculated from six replicate analysis of trout (2 % fat) spiked with target analytes at two concentration levels (level 1, 1 μg/kg for three major PCBs, eight mono-*ortho* PCBs, four non-*ortho* PCBs, six PBDEs, and 16 PAHs and 5 μg/kg for major PCBs 138, 153, and 180 and PBDE 47; level 2, 5 μg/kg for three major PCBs, eight mono-*ortho* PCBs, four non-*ortho* PCBs, six PBDEs, and 16 PAHs and 25 μg/kg for major PCBs 138, 153, and 180 and PBDE 47), LOQ (micrograms per kilogram) and linearity (R^2)

Analytes		Level 1		Level 2		LOQ, μg/kg	Linearity (R^2)[a]	No. of calibration points
		Rec, %	RSD, %	Rec, %	RSD, %			
Mono-*ortho* PCBs	CB 105	119	13	77	9	0.01	0.99010	10
	CB 114	114	8	94	10	0.01	0.99779	10
	CB 118	98	11	106	8	0.01	0.99385	10
	CB 123	118	12	92	8	0.01	0.99977	10
	CB 156	85	19	95	6	0.01	0.99846	10
	CB 157	118	12	88	6	0.01	0.99892	10
	CB 167	106	4	100	6	0.025	0.99837	9
	CB 189	116	19	98	5	0.025	0.99910	9
Major PCBs	CB 28	76	15	84	5	0.01	0.99984	10
	CB 52	98	14	86	7	0.01	0.99432	10
	CB 101	111	12	102	13	0.01	0.99758	10
	CB 138	101	12	83	13	0.01	0.99791	10
	CB 153	75	11	75	10	0.01	0.99798	10
	CB 180	99	11	79	16	0.025	0.99898	9
Non-*ortho* PCBs	CB 77	111	18	97	4	0.01	0.99705	10
	CB 81	120	9	98	8	0.01	0.99646	10
	CB 126	115	9	79	6	0.01	0.99753	10
	CB 169	101	16	96	8	0.025	0.99922	9
PBDEs	BDE 28	117	18	94	18	0.025	0.99752	9
	BDE 47	99	10	106	8	0.025	0.99132	9
	BDE 99	110	15	112	16	0.25	0.99201	6
	BDE 100	116	12	96	3	0.1	0.99742	7
	BDE 153	105	11	91	12	0.25	0.99795	6
	BDE 154	95	18	99	9	1	0.99156	4
	BDE 183	N/A	N/A	113	9	5	0.99008	2
PAHs	BaA	84	8	92	6	0.01	0.99805	10
	BaP	92	6	91	6	0.05	0.99939	8
	BbFA	93	9	90	6	0.025	0.99877	9
	BcFL	78	13	80	5	0.025	0.99968	9
	BjFA	85	10	90	9	0.025	0.99862	9
	BkFA	91	7	86	8	0.025	0.99625	9
	BghiP	96	6	94	9	0.025	0.99922	9
	CHR	79	12	86	11	0.01	0.99846	10
	CPP	78	5	82	4	0.01	0.99931	10
	DBahA	89	14	93	9	0.25	0.99778	6
	DBaeP	85	5	89	10	0.25	0.99690	6
	DBahP	83	9	85	6	0.1	0.99928	7
	DBaiP	83	11	86	5	0.5	0.99782	5
	DBalP	89	7	91	6	0.1	0.99800	7
	IP	95	8	97	6	0.1	0.99887	7
	5MC	85	4	87	8	0.1	0.99950	7

[a] The regression coefficient (R^2) was calculated for the calibration curve from the LOQ up to the highest calibration point (100 ng/mL=10 μg/kg)

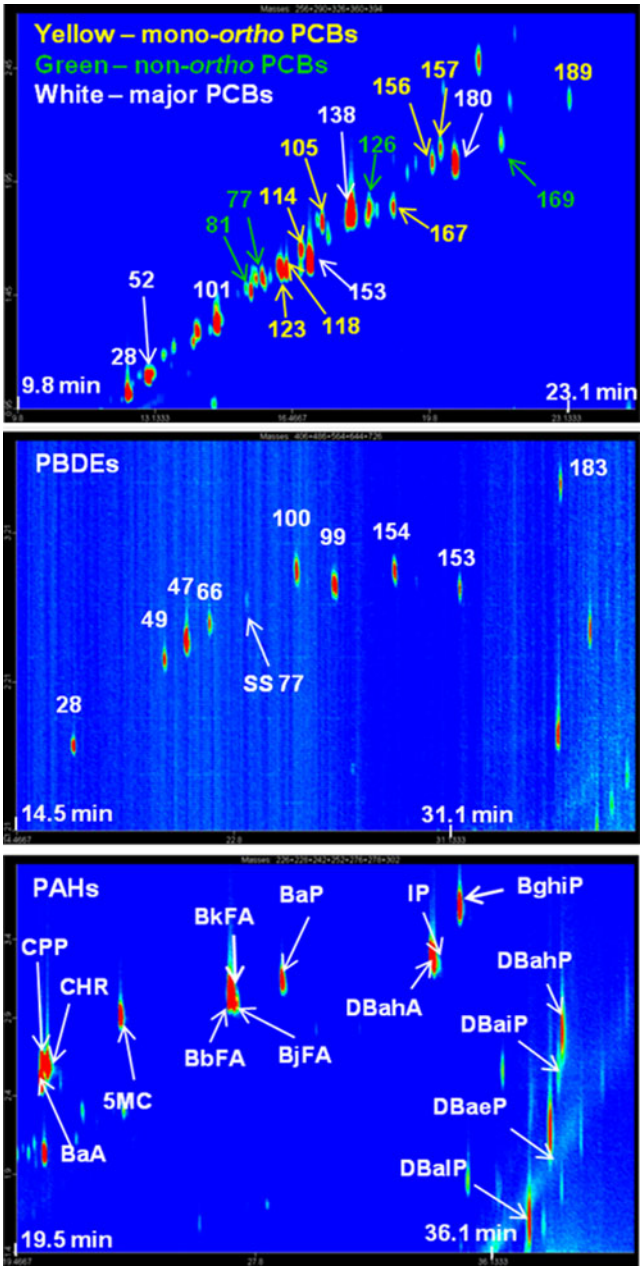

Fig. 2 An example of a GC×GC–TOFMS chromatogram of fish muscle tissue spiked with target PCBs, PBDEs, and PAHs at level 1 and separated in BPX5×BPX50 system. Sums of *m/z* 256, 290, 326, 360, and 394 (PCBs); *m/z* 406, 486, 564, 644, and 726 (PBDEs); and *m/z* 226, 228, 242, 252, 276, 278, and 302 (PAHs) are displayed

regression ($1/x$) was used, and regression coefficient (R^2) was calculated for the calibration curve from the LOQ up to the highest calibration point (100 ng/mL). Within our experiments, all target analytes fulfill the linearity in calibration range mentioned above with R^2 higher than 0.99.

To prove the trueness of the method, the US National Institute of Standards and Technology (NIST) SRM 1947 Lake Michigan Fish Tissue reference material for selected PCBs and PBDEs and SRM 1974b Mussel Tissue for PAHs and PCBs were finally analyzed (for details see Electronic supplementary material Tables S2 and S3). The determined values for all analytes were in accordance with certified/reference values except for CB 28, CB 105, and DBahA in SRM 1974b for which the determined values slightly differ compared with that stated in the certificate. The examples of chromatograms of PCB and PAHs in fish and mussel matrices are shown in Figs. 3 and 4.

Non-target screening

Since the determination of target analytes was performed using a TOFMS detector, further identification of other non-target compounds in real-life samples (in our case in SRM) which were not primarily included in the study (the list of analytes was pre-defined in the description of work of the European project CON*ff*IDENCE [6]) was feasible. The identification of non-target compounds was based on comparison of obtained deconvoluted mass spectra with those from the NIST2008 library. In the case of PCBs, the standard mixture of 52 most abundant PCBs was measured during the optimization of chromatography separation (to verify that no co-elution with other non-target PCB congeners occurs), thus the confirmation of their presence was additionally done by comparison of their retention times in the first and second dimensions with those in the standard mixture. It should be noted that, due to the similar physico-chemical properties with target congeners, satisfactory recoveries of non-target PCBs can be expected when using sample preparation method presented in this paper. An example of additionally identified PCBs is shown in Fig. 3.

Another group of persistent contaminants that may occur in fish tissue at relatively high concentration are OCPs. This group involves many compounds differing in physico-chemical properties what might cause rather limited compatibility with sample preparation employed in this study. In our previous experiments, we found low recoveries of, e.g., dieldrine, endrine, β-endosulfane, and endosulfan sulfate, due to their relatively high affinity to silica sorbent used for the purification of the crude extract. The data obtained by the retrospective analysis of fish samples enabled detection of several representatives of this group: hexachlorocyclohexane (α-, β-, and γ-isomer), hexachlorobenzene, chlordane (*cis*-, *trans*-), *o*,*p*′-DDE, *p*,*p*′-DDE, *o*,*p*′-DDD, *p*,*p*′-DDD, *o*,*p*′-DDT, *p*,*p*′-DDT, aldrine, and nonachlor in SRM 1947 and chloradne (*cis*-, *trans*-), *p*,*p*′-DDE, and *o*,*p*′-DDT in SRM 1974b.

However, since above-mentioned non-target analytes identified in standard reference materials were not included into the validation process of the entire method, the identification and potential quantification of these compounds can be considered only as a preliminary data, further confirmation is needed.

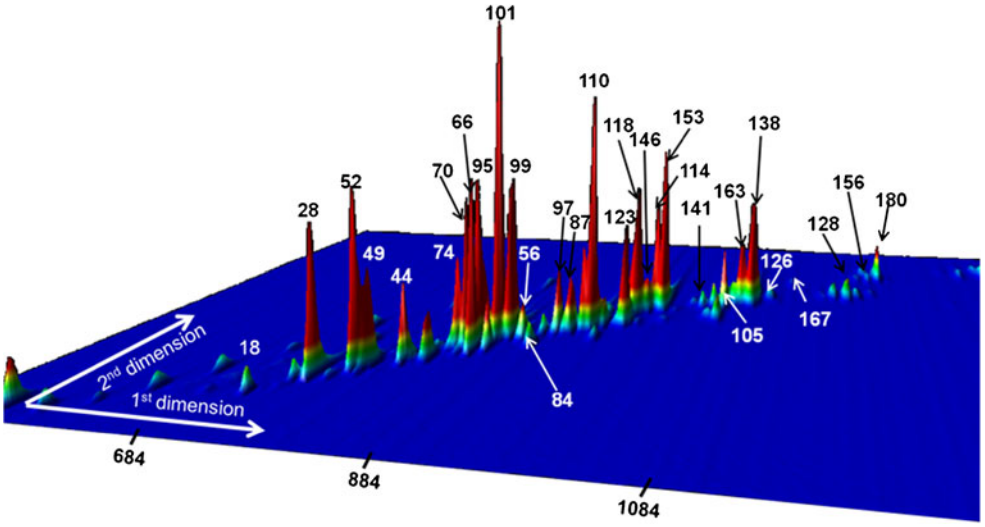

Fig. 3 An example of a GC×GC–TOFMS chromatogram of SRM 1947 Lake Michigan Fish Tissue of selected PCBs separated in BPX5×BPX50 system. Sum of *m/z* 256, 290, 326, 360, and 394 is displayed

Conclusion

The experience obtained within our investigation on the GC×GC–TOFMS of POPs/PAHs analysis of fish extracts prepared by fast and simple sample preparation procedure [28] can be summarized as follows:

1. LV-PTV–GC×GC–TOFMS employing EI was shown to be a powerful tool for the simultaneous identification and quantification of various groups of contaminants including PCBs, PBDEs, and PAHs as well as for a potential non-target screening of similar compounds and retrospective data mining in a complex matrix. Since different groups of analytes (traditionally analyzed separately) were included into one instrumental method, significant time savings were achieved.
2. All target PCBs, PBDEs, and PAHs were separated in GC×GC system consisting of BPX5 in the first dimension and BPX50 in the second dimension. The most critical analytes for the column selection and oven temperature program optimization were mainly isomeric groups of PAHs ((a) BaA, CPP, and CHR; (b) BbFA, BjFA, and BkFA; (c) DBahA, IP, and BghiP). All target PCB and PBDE congeners, including mono- and non-*ortho* dioxin-like PCBs, were separated on all tested column combinations except for BPX50×HT8 and Rxi-17Sil-ms×HT8 where CB 118 and 123 were co-eluted.

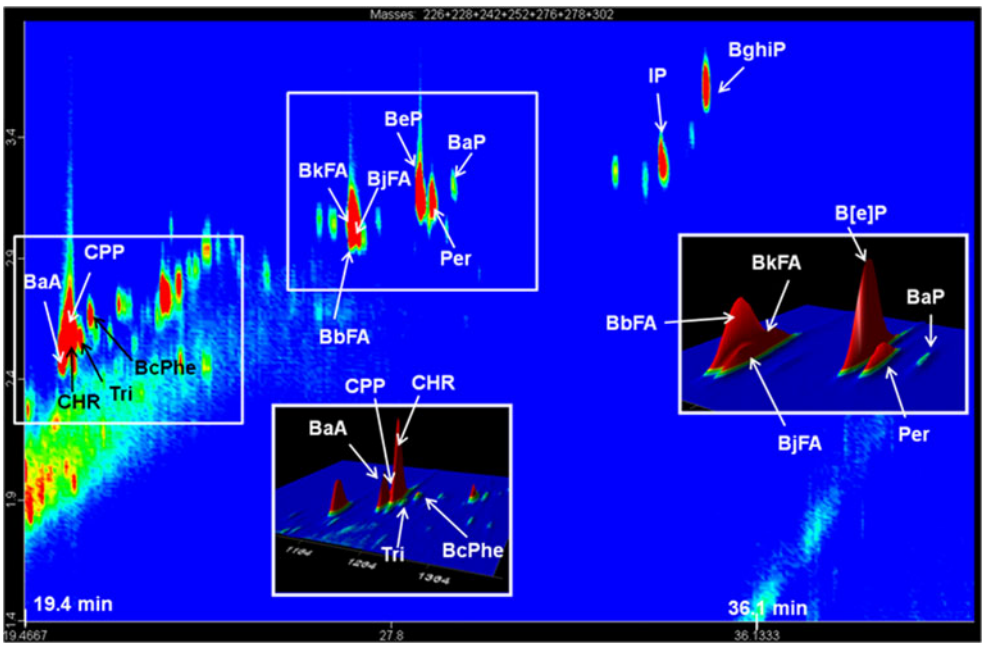

Fig. 4 An example of a GC×GC–TOFMS chromatogram of SRM 1974b Mussel Tissue of PAHs separated in BPX5×BPX50 system. Sum of *m/z* 226, 228, 242, 252, 276, 278, and 302 is displayed. *Tri* triphenylene, *Per* perylene, *BeP* benzo[e]pyrene), *BcPhe* benzo[c]phenanthrene)

3. LV-PTV injection technique enabled to achieve very low LOQs of all target analytes which are necessary for the control even of background levels of contaminants in fish tissue. The LOQs of the new method were in the following ranges: PCBs (0.01–0.25 μg/kg), PBDEs (0.025–5 μg/kg), and PAHs (0.025–0.5 μg/kg).

Acknowledgment The financial support by the European Commission through the 7th Framework Program (contract no FP7–211326–CP–CON*ff*IDENCE) and the Ministry of Education, Youth and Sports of the Czech Republic (MSM6046137305; contract no IDS 7E08068) is gratefully acknowledged.

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Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Implementation of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the simultaneous determination of halogenated contaminants and polycyclic aromatic hydrocarbons in fish

Kamila Kalachova, Jana Pulkrabova, Tomas Cajka, Lucie Drabova, Jana Hajslova

Table S1 Elution order (1st and 2nd dimension retention time) and ions selected for identification and identity confirmation of target PCBs, PBDEs, PAHs, and respective syringe and internal standards.

Analyte	Retention time (s)		Quantification ion (*m/z*)	Confirmation ion (*m/z*)
	1st dimension	2nd dimension		
CB 28	748	1.010	256	254, 258
CB 52	776	1.090	290	292, 294
CB 101	876	1.320	326	324, 328
CB 81	924	1.470	290	292, 294
CB 77	940	1.300	290	292, 294
CB 123	968	1.551	326	324, 328
CB 118	976	1.560	326	324, 328
BcFL	992	1.870	216	215
CB 114	996	1.650	326	324, 328
BDE 28	996	1.790	406	246, 408
CB 153	1008	1.600	360	358, 362
CB 105	1028	1.770	326	324, 328
CB 138	1068	1.820	360	358, 362
CB 126	1096	1.850	326	324, 328
CB 167	1128	1.840	360	358, 362
CB 156	1188	2.040	360	358, 362
CB 157	1200	2.100	360	358, 362
BaA	1212	2.510	228	226
CPP	1216	2.630	226	228
CB 180	1220	2.040	394	392, 396
CHR	1224	2.610	228	226
BDE 47	1256	2.480	486	326, 484
CB 169	1288	2.130	360	358, 362
5MC	1380	2.930	242	241
CB 189	1388	2.310	394	392, 396
BDE 100	1508	2.980	564	404, 566
BDE 99	1592	2.880	564	404, 566
BbFA	1604	3.070	252	253
BkFA	1608	3.130	252	253
BjFA	1616	3.050	252	253
BaP	1716	3.180	252	253
BDE 154	1784	2.900	644	484, 642
BDE 153	1880	2.870	644	484, 642
IP	2028	3.340	276	278
DBahA	2036	3.310	278	276
BghiP	2092	3.620	276	278
BDE 183	2116	3.523	726	641, 724
DBalP	2236	1.650	302	303
DBaeP	2280	2.400	302	303
DBaiP	2302	2.640	302	303
DBahP	2308	2.880	302	303
BDE 37	1044	1.920	406	246, 408
BDE 77	1392	2.770	486	326, 484
^{13}C-CB 77	940	1.300	302	304
^{13}C-CB 101	876	1.320	336	338
$^{13}C_6$-BaA	1212	2.510	234	232

Analyte	Retention time (s)		Quantification ion (*m/z*)	Confirmation ion (*m/z*)
	1st dimension	2nd dimension		
$^{13}C_6$-CHR	1224	2.610	234	232
$^{13}C_6$-BbFA	1604	3.070	258	259
$^{13}C_6$-BkFA	1616	3.050	258	259
$^{13}C_4$-BaP	1716	3.180	256	257
$^{13}C_6$-IP	2028	3.340	282	283
$^{13}C_6$-DBahA	2036	3.310	284	282
$^{13}C_{12}$-BghiP	2092	3.620	288	289
$^{13}C_6$-DBaeP	2280	2.240	308	307
$^{13}C_{12}$-DBaiP	2302	2.640	314	313

Table S2 Verification of trueness of generated data: Analysis of selected PCBs, PBDEs and PAHs in standard reference material – Lake Michigan fish tissue (SRM 1947, NIST, USA).

Analyte		Determined value (μg/kg) [1]	Certified/reference value (μg/kg)	Agreement Yes/No
Mono-*ortho* PCBs	CB 105	49.8 ± 13.6	50.3 ± 3.7	Yes
	CB 114	8.3 ± 2.5	N/A	N/A
	CB 118	111 ± 27	112 ± 6	Yes
	CB 123	21.9 ± 5.3	N/A	N/A
	CB 156	15.1 ± 2.7	13.3 ± 0.9	Yes
	CB 157	4.9 ± 0.9	4.1 ± 0.8	Yes
	CB 167	10.2 ± 1.9	N/A	N/A
	CB 189	2.8 ± 0.4	N/A	N/A
Major PCBs	CB 28	15.8 ± 2.4	14.1 ± 1.0	Yes
	CB 52	34.6 ± 7.3	36.4 ± 4.3	Yes
	CB 101	93.6 ± 36.9	90.8 ± 0.3	Yes
	CB 138	159 ± 63	162 ± 7	Yes
	CB 153	207 ± 63	201 ± 3	Yes
	CB 180	80.6 ± 39.1	80.8 ± 5.0	Yes
Non-*ortho* PCBs	CB 77	1.6 ± 0.9	N/A	N/A
	CB 81	n.d.	N/A	N/A
	CB 126	0.9 ± 0.2	N/A	N/A
	CB 169	1.9 ± 0.9	N/A	N/A
PBDEs	BDE 28	1.89 ± 1.03	2.26 ± 0.46 [2]	Yes
	BDE 47	75.3 ± 18.3	73.3 ± 2.9	Yes
	BDE 99	17.6 ± 8.5	19.2 ± 0.8	Yes
	BDE 100	16.5 ± 1.5	17.1 ± 0.6	Yes
	BDE 153	4.2 ± 1.5	3.8 ± 0.04	Yes
	BDE 154	5.7 ± 1.6	6.9 ± 0.5	Yes
	BDE 183	< 5	N/A	N/A

Yes/No – result is/not in agreement with the certified value

N/A – not available

n.d. – not detected

1 The uncertainty was estimated by the "top-down" approach combining standard uncertainties calculated for both precision and trueness. The estimation of the relative standard deviation for reproducibility conditions was based on the empiric equation between repeatability (RSD) and reproducibility (RSD_R) ($RSD = 0.66 * RSD_R$).

[2] Certified value is a sum of BDE 28 and 33

Note: None of target PAHs was detected in the SRM 1947.

Table S3 Verification of trueness of generated data: Analysis of selected PCBs, PBDEs and PAHs in standard reference material – Mussel Tissue (SRM 1974b, NIST, USA).

Analyte		Determined value (μg/kg) [1]	Certified/reference value (μg/kg)	Agreement Yes/No
Mono-*ortho* PCBs	CB 105	2.96 ± 0.81	4.00 ± 0.18	No
	CB 114	0.26 ± 0.06	N/A	N/A
	CB 118	9.7 ± 2.3	10.3 ± 0.4	Yes
	CB 123	1.23 ± 0.45	N/A	N/A
	CB 156	0.96 ± 0.55	0.718 ± 0.080	Yes
	CB 157	0.32 ± 0.12	0.236 ± 0.024	Yes
	CB 167	0.13 ± 0.02	N/A	N/A
	CB 189	< 0.025	N/A	N/A
Major PCBs	CB 28	4.69 ± 0.71	3.43 ± 0.25	No
	CB 52	6.23 ± 1.32	6.26 ± 0.37	Yes
	CB 101	12.5 ± 4.9	10.7 ± 1.1	Yes
	CB 138	8.6 ± 3.1	9.2 ± 1.4	Yes
	CB 153	14.6 ± 4.4	12.3 ± 0.8	Yes
	CB 180	1.23 ± 0.41	1.17 ± 0.10	Yes
Non-*ortho* PCBs	CB 77	0.69 ± 0.38	N/A	N/A
	CB 81	n.d.	N/A	N/A
	CB 126	<0.01	N/A	N/A
	CB 169	n.d.	N/A	N/A
PBDEs	BDE 28	<0.025	N/A	N/A
	BDE 47	2.98 ± 0.72	N/A	N/A
	BDE 99	1.03 ± 0.47	N/A	N/A
	BDE 100	0.89 ± 0.32	N/A	N/A
	BDE 153	<0.25	N/A	N/A
	BDE 154	<1	N/A	N/A
	BDE 183	n.d.	N/A	N/A
PAHs	BaP	3.01 ± 0.55	2.80 ± 0.38	Yes
	BaA	5.96 ± 1.08	4.74 ± 0.53	Yes
	BbFA	6.23 ± 1.13	6.46 ± 0.59	Yes
	BkFA	3.76 ± 0.57	3.16 ± 0.18	Yes
	BjFA	2.69 ± 0.73	2.99 ± 0.29	Yes
	BghiP	4.02 ± 0.97	3.12 ± 0.33	Yes
	ChR	5.98 ± 1.63	6.3 ± 1.0	Yes
	CPP	0.96 ± 0.35	N/A	N/A
	DBahA	0.45 ± 0.07	0.327 ± 0.031	No
	DBaeP	n.d.	N/A	N/A
	DBahP	n.d.	N/A	N/A
	DBaiP	n.d.	N/A	N/A
	DBalP	n.d.	N/A	N/A
	IP	1.98 ± 0.42	2.14 ± 0.11	Yes
	5-MC	n.d.	N/A	N/A
	BcFL	n.d.	N/A	N/A

Yes/No – result is/not in agreement with the certified value

N/A – not available

n.d. – not detected

[1] *The uncertainty was estimated by the "top-down" approach combining standard uncertainties calculated for both precision and trueness. The estimation of the relative standard deviation for reproducibility conditions was based on the empiric equation between repeatability (RSD) and reproducibility (RSD_R) ($RSD = 0.66 * RSD_R$).*