Implemetation of GC×GC-TOF MS for the Simultaneous Determination of PCBs, PBDEs and PAHs in Environmental Samples

Kamila Kalachová, Jana Pulkrabová, Tomáš Čajka, Lucie Drábová, Jana Hajšlová✉

Department of Food Chemistry and Analysis, Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Technická 3, 166 28 Prague 6, Czech Republic, ✉ jana.hajslova@vscht.cz

Abstract

In this study, comprehensive two-dimensional gas chromatography coupled to time-of--flight mass spectrometry (GC×GC-TOFMS) for the simultaneous determination of eighteen polychlorinated biphenyls (PCBs), seven polybrominated diphenyl ethers (PBDEs) and sixteen polycyclic aromatic hydrocarbons (PAHs) was optimized to obtain the best chromatographic resolution and quantification limits (LOQs) of target analytes. Two injection techniques, pulsed splitless and large volume programmable temperature vaporization (LV-PTV), and several capillary column systems were tested within the experiments (BPX-5 and BPX-50 in the 1st dimension and BPX-50, Rt-LC35 and HT-8 in 2nd dimension). All tested combinations of columns were able to separate all target PCBs and PBDEs. Selection of the column system was therefore mainly influenced by its ability to separate the critical groups of PAHs (1st group: B[*a*]A, CP[*cd*]P, and Chr; 2nd group: B[*f*]F, B[*k*]F, and B[*b*]F; 3rd group: DB[*ah*]A, I[*1,2,3-cd*]P, and B[*ghi*]P). Although none of the column combinations evaluated in the present study allowed a complete separation of all target PAHs, a combination of BPX-50 × HT-8 columns showed the best ability to separate the 2nd group of PAHs. Application of PTV injection technique decreased LOQs of almost all target analytes approximately by one order of magnitude. The LOQs achieved using LV-PTV-GC×GC were as follows: PCBs 0.1–0.25 μg kg^{-1}, PBDEs 0.5–2.5 μg kg^{-1}, PAHs 0.05–0.25 μg kg^{-1}.

Keywords
fish
GC×GC-TOF MS
PAH
PBDE
PCB
PTV

1. Introduction

To protect consumers' health against undesirable effects originating from food contaminants, several legislative steps have been accepted until now [1]. However, considering the lack of reliable information in some areas, the European Food Safety Authority (EFSA) continually announces the calls to collect information on the occurrence of selected persistent organic pollutants (POPs) in food to assess their potential human exposure [2]. Regarding these requirements, quick, inexpensive and high throughput analytical approaches have to be developed.

Comprehensive two-dimensional gas chromatography (GC×GC) coupled to time-of-flight mass spectrometry (TOF MS) represents a powerful tool for the simultaneous determination of different types of contaminants that considerably increase the separation efficiency of GC analysis [3, 4]. Since the whole system comprises two capillary columns with different polarities, the total peak capacity for GC×GC is the outcome of the individual column capacities [5]. Moreover, focusing effects allows achieving lower limits of detection needed for the residue analysis as compared with one-dimensional GC [5]. Until now, various applications of GC×GC coupled to TOFMS in food and environmental analyses have been reported [3, 6–11], but according to the authors' best knowledge simultaneous determination of PCBs, PBDEs and PAHs within a single run has never been presented until now.

The main aim of this study was to develop and optimize the GC×GC-TOFMS method for the simultaneous determination of PCBs, PBDEs and PAHs to obtain the best chromatographic resolution and detection limits for all target analytes. Therefore, several chromatographic capillary column combinations were tested. In the subsequent experiments, the large volume programmable temperature vaporization (LV-PTV) injection technique was optimized. To confirm the application of this newly developed method, several real-life contaminated fish samples were analyzed.

2. Experimental

2.1. Test Material

Since fish contamination by halogenated pollutants such as PCBs and PBDEs has different sources from PAHs, which originate mainly from incomplete combustion or heat-induced decomposition of organic matter, it is very difficult to obtain fish contaminated with all these groups of contaminants. Because of that, two different naturally contaminated fish and one blank fish sample were used for the experiments. Trout (blank sample) previously tested for the absence of PCBs, PBDEs and PAHs obtained from the Czech retail market was used for QA/QC experiments. All samples were kept at –18°C after pooling and homogenization.

2.2. GC×GC-TOF MS Analysis

All experiments were performed using an Agilent 6890N GC system (Agilent, Palo Alto, USA) coupled to a Pegasus III (LECO Corp, USA) high-speed time--of-flight mass spectrometer (GC-TOF MS) operated in electron ionization mode (EI). Target analytes were separated using several chromatographic capillary column combinations with different polarities (see Table 1). All columns except for Rt-LC35 (Restek, USA) were purchased from SGE Analytical Science (Australia). Columns used in the 1st and 2nd dimension were of following parameters: 30 m × × 0.25 mm i.d. × 0.25 μm film thickness and 1 m × 0.1 mm i.d. × 0.1 μm film thickness, respectively. Different first/second-dimension oven temperature programmes depending on the column used were tested. Pulsed splitless (injected volume 1 μL) and LV-PTV injection in solvent vent mode (injected volume 1 × 5 μL, 1 × 8 μL and 1 × 10 μL) were tested. Carrier gas helium flow was 1.3 mL min^{-1} during all experiments. The TOFMS detector was operated under the following conditions: mass range: *m/z* = = 45–750; ion source temperature: 250°C; transfer line temperature: 280°C; acquisition speed: 100 spectra s^{-1}. The modulation period was dependant on the column system (see Table 1). The ChromaTOF 4.24 software (LECO Corp, USA) was used for data processing.

Table 1
The GC column set-ups tested within the experiments.

Column system	1st dimension	2nd dimension	Modulation period [s]
1	BPX-5	BPX-50	4.0
2	BPX-5	Rt-LC35	2.0
3	BPX-5	HT-8	3.5
4	BPX-50	BPX-5	2.5
5	BPX-50	HT-8	4.5

Table 2
Large volume programmable temperature vaporization inlet parameters within the method optimization.

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

3. Results and Discussion

3.1. Optimization of Injection Technique

In the initial part of our experiments, hot splitless injection (1 μL) was used mainly due to its ease of operation. Unfortunately, the obtained LOQs were too high to meet the goal of the study; therefore, LV-PTV injection in solvent vent mode (up to 10 μL) was optimized. Using this approach desired LOQs were achieved. As an additional benefit, discrimination of certain analytes was reduced as compared to hot splitless injection.

However, LV-PTV requires more operating parameters to be optimized as compared to hot splitless injection [7]. Firstly, different injection volume (5, 8 and 10 μL) was tested. An 8-μL injection was seen as optimal since it allowed to achieve the lowest LOQs; the higher injection tested provided worse performance, probably due to its lower capacity. Simultaneously with the injection volume, purge time and solvent vent time were optimized (for more details see Table 2). Secondly, the influence of an injection speed (10 and 30 μL s^{-1}) was tested and the best results were obtained by injecting the sample at a speed of 10 μL s^{-1}. Better results using slower injection rate are in accordance with results of Gómez-Ruiz *et al.* who deals with optimization of PTV injection in PAHs analysis [7]. The initial inlet temperature 50 °C was set on the basis of previous experiments and data reported in literature [7]. The comparison of LOQs obtained by 1 and 8 μL injection volume in one dimensional system is summarized in Table 3 (see on next page).

Further decrease of LOQs was achieved using GC×GC thanks to the modulator focusing effect (Table 3) [5].

Table 3
Limits of quantification using one-dimension GC (1D-GC) and GC×GC obtained by the optimized methods.

Analytes		LOQ [μg kg^{-1}]		
		1D-GC		GC×GC
		1 μL[a]	8 μL[b]	8 μL[b]
Mono-*ortho* PCBs	PCB 105	2.5	0.25	0.1
	PCB 114	2.5	0.25	0.1
	PCB 118	2.5	0.25	0.1
	PCB 123	2.5	0.25	0.1
	PCB 156	5	0.5	0.25
	PCB 157	5	0.5	0.25
	PCB 167	5	0.5	0.25
	PCB 189	5	1	0.5
Major PCBs	PCB 28	2.5	0.1	0.1
	PCB 52	2.5	0.25	0.1
	PCB 101	2.5	0.25	0.1
	PCB 138	2.5	0.5	0.1
	PCB 153	2.5	0.5	0.1
	PCB 180	5	1	0.25
Non-*ortho* PCBs	PCB 77	2.5	0.25	0.1
	PCB 81	2.5	0.25	0.1
	PCB 126	2.5	0.5	0.1
	PCB 169	5	0.5	0.25
PBDEs	PBDE 28	10	2.5	0.5
	PBDE47	10	1	0.5
	PBDE 99	10	1	0.5
	PBDE 100	10	1	0.5
	PBDE 153	>10	1	0.5
	PBDE 154	>10	1	0.5
	PBDE 183	>10	2.5	2.5
EU PAHs	B[*a*]A	1	0.1	0.05
	B[*a*]P	1	0.1	0.05
	B[*b*]F	1	0.1	0.05
	B[*c*]Fln	1	0.1	0.05
	B[*j*]F	1	0.1	0.05
	B[*k*]F	1	0.1	0.05
	B[*ghi*]P	1	0.25	0.25
	Chr	1	0.1	0.05
	CP[*cd*]P	1	0.1	0.05
	DB[*ah*]A	1	0.25	0.25
	DB[*ae*]P	2.5	0.5	0.5
	DB[*ah*]P	2.5	0.5	0.5
	DB[*ai*]P	2.5	0.5	0.5
	DB[*al*]P	2.5	0.5	0.5
	I[*cd*]P	1	0.25	0.25
	5MeChr	1	0.25	0.05

[a] Hot splitless injection.
[b] Large volume programmable temperature vaporization.

3.2. Selection of GC×GC Capillary Column Combination

The column combinations included in the presented study were selected on the basis of data previously reported in the literature and on the orthogonal requirements of GC×GC system. Since the PAHs with a high molecular weight were involved in these experiments, only columns with a high upper temperature limit (more than 360 °C) could be chosen. During GC×GC optimization, two capillary columns with different polarities were used for the first-dimension separation: *(i)* non-polar BPX-5 (5% phenyl polysilphenylene-siloxane) and *(ii)* medium-polar BPX-50 (50% phenyl polysilphenylene-siloxane). (Note: Different elution order of PCB 114 and 153 and of CP[*cd*]P with B[*a*]A and Chr using these two columns in one dimensional setting was observed.)

In the second dimension, following capillary columns with different stationary phases were tested: *(i)* BPX-50 (50% phenyl polysilphenylene-siloxane), *(ii)* Rt-LC35 (dimethyl (50% liquid crystal) polysiloxane), *(iii)* BPX-5 (5% phenyl polysilphenylene-siloxane) and *(iv)* HT-8 (8% phenylpolysiloxane-carbonare). All column set-ups tested within the experiments were able to separate all target PCBs and PBDEs. Figure 1 (on next page) shows an example of the chromatogram of PCBs and PBDEs using BPX-50 × HT-8 column set-up. Therefore, selection of "best" column combination was mainly influenced by its ability to separate the critical groups of PAHs (1st group: B[*a*]A, CP[*cd*]P, and Chr; 2nd group: B[*f*]F, B[*k*]F, and B[*b*]F; 3rd group: DB[*ah*]A, I[*1,2,3-cd*]P, and B[*ghi*]P). Unfortunately, none of the column set-ups tested within this study was able to separate the 1st and 3rd critical group. The best separation of the 2nd critical group formed by three isomers of benzofluoranthene was achieved using BPX-50 × HT-8 column set-up (Figure 2; see on next page).

3.3. QA/QC

All experiments were performed in an accredited laboratory (No. 1316.2) in the Czech Republic; currently operating in compliance with EN ISO/IEC 17025. Recoveries (%) and repeatabilities (expressed as RSD, %) of all target analytes calculated from six replicates were as follows: PCBs 82–118% (RSD 3–15%), PBDEs 79–118% (RSD 5–14%), and PAHs 83–102% (RSD 3–9%). The LOQs are summarized in Table 3.

4. Conclusions

GC×GC-TOF MS employing EI represents a powerful tool for the simultaneous identification and quantification of various groups of chemicals including PCBs, PBDEs and PAHs as well as for a potential non-target screening. All target PCB and PBDE congeners were separated on all tested column combinations. Therefore selection of the column system was mainly influenced by its ability to separate the critical

Fig. 1. An example of GC×GC chromatogram of PCBs and PBDEs (200 pg injected) using BPX-50×HT-8 capillary column system; *m/z* = 256, 290, 324, 358, 390, 404, 484, 564, 644 (yellow mono-ortho PCBs, green non-ortho PCBs, white major PCBs, red PBDEs).

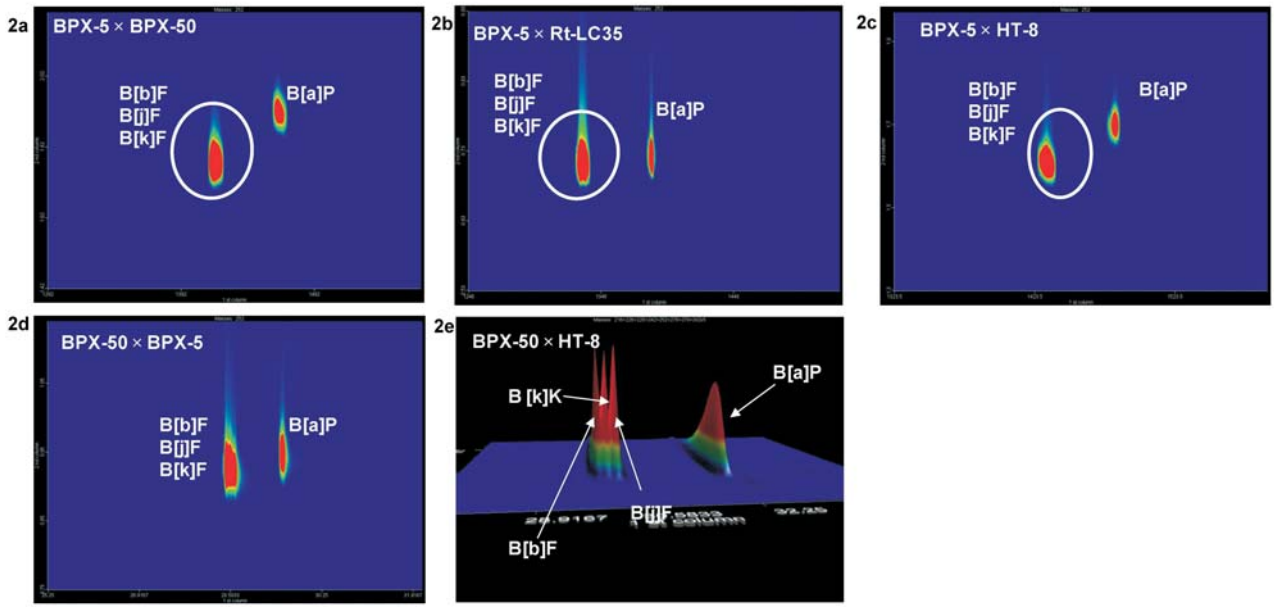

Fig. 2. Separation of B[*b*]F, B[*j*]F, B[*k*]F and B[*a*]P; *m/z* = 252 (200 pg injected) using different capillary column systems.

groups of PAHs (1st group: B[*a*]A, CP[*cd*]P, and Chr; 2nd group B[*f*]F, B[*k*]F, and B[*b*]F; 3rd group: DB[*ah*]A, I[*1,2,3-cd*]P, and B[*ghi*]P). Although none of the column combination evaluated in the presented study allowed a complete separation of all target PAHs, BPX-50 × HT-8 column set-up showed the ability to separate the 2nd group of PAHs. Other two groups remained still unseparated. To achieve desired LOQs, the LV-PTV injection (8 µL) was implemented allowing to obtain LOQs for almost all target analytes by approximately one order of magnitude lower as compared to hot sptliless injection (1 µL).

Acknowledgements

The financial support by the European Commission through the 7th Framework Programme (contract no FP7-211326-CP – CONffIDENCE) and the Ministry of Education, Youth and Sports of the Czech Republic (MSM6046137305) is gratefully acknowledged.

References

[1] *Directive 2003/11/EC of 6 February 2003 amending for the 24th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether).*

[2] *Request for data on Brominated Flame Retardants Levels in Foodstuffs, European Food Safety Authority* http://www.efsa.europa.eu/en/data/call/datex091215.htm (23.3.2010).

[3] Borajandi L. R., Ramos J. J., Sanz J., González M. J., Ramos L.: *J. Chromatogr. A* **1186** (2008), 312–324.

[4] Korytár P., Leonards P. E. G., de Boer J., Brinkman U. A. Th.: *J. Chromatogr. A* **1086** (2005), 29–44.

[5] Marriott P. J., Haglund P., Ong R. C. Y.: *Clin. Chim. Acta* **328** (2003), 1–19.

[6] Leeuwen S.P.J., Boer J.: *J. Chromatogr. A* **1186** (2008), 161–182.

[7] Gómez-Ruiz J. A., Cordeiro F., López P, Wenzl T.: *Talanta* **80** (2009), 643–650.

[8] Adahchour M., Beens J., Vreuls R. J. J., Brinkman U. A. Th.: *Trend Anal. Chem.* **25** (2006), 726–740.

[9] Adahchour M., Beens J., Vreuls R. J. J., Brinkman U. A. Th.: *Trend Anal. Chem.* **25** (2006), 821–840.

[10] Korytár P., Haglung P., Boer J., Brinkamn U. A. Th.: *Trend Anal. Chem.* **25** (2006), 373–396.

[11] Dasputa S., Banerjee K., Patil S. H., Ghaste M., Dhumal K. N., Adsule P. G.: *J. Chromatogr. A* **1217** (2010), 3881–3889.