DEVELOPMENT OF A GC-MS/MS APPROACH FOR MEASURING HEXACHLOROBENZENE (HCB) AND HEXACHLORO-1,3-BUTADIENE (HCBD) IN FISH

Cariou Ronan[1], Vincent Vaccher[1], Marchand Philippe[1], Antignac Jean-Philippe[1,2], Le Bizec Bruno[1]

[1]ONIRIS, USC 2013, LABERCA, Atlanpole-La Chantrerie, BP 50707, Nantes F-44307, France
[2]INRA, Nantes F-44307, France

Introduction

Hexachlorobenzene (HCB, C_6Cl_6, CAS 118-74-1) is an organochlorine pesticide (OCP) banned (production and use) through the Stockholm Convention in 2004. Its physical-chemical properties qualify it as a Perpistent Organic Pollutant (POP). HCB biomagnifies in fat fraction of tissues trough the food chain. Hexachloro-1,3-butadiene (HCBD, C_4Cl_6, CAS 87-68-1) is used in chemical industries as intermediary product, elastomer, hydrolic or heating fluid. Even if it seems that there are no current applications in Europe, it is not banned, and can be present as impurity in other formulations. HCBD maximum level of 0.1 $\mu g.L^{-1}$ is tolerated in surface water within European Union. Most analytical methods are based on GC-ECD, but improved specificities have been obtained by GC-MS/MS for HCB in liver[1] and egg[2]. In this work, we intended to work on fish and to include HCBD as analyte.

Materials and methods

Reagents and chemicals

n-Hexane, n-decane, methanol and were provided by LGC Promochem (Picograde®, Wesel, Germany). Dichloromethane was provided by Biosolve (Dioxin & PCB grade, Leenderweg, The Netherlands). Sulfuric acid 98% was purchased from Merck. Hydrochloric acid 37% was purchased from Panreac (131020, Castellar del Vallès, Spain). Florisil® 100-200 mesh (LGC Promochem) was heated (600 °C, 5 h) before use. Silica gel (G60) was provided by Fluka (Buchs, Switzerland). Reference ^{12}C-native and ^{13}C-labelled standard solutions were purchased from Cambridge Isotope Laboratories, Inc (Andover, MA, USA), Ultra Scientific (Kingstown, RI, USA) or Wellington Laboratories (Guelph, Ontario, Canada). ^{13}C-labelled compounds used as internal standards for quantification included $^{13}C_6$-hexachlorobenzene and $^{13}C_4$-hexachloro-1,3-butadiene. $^{13}C_{12}$-PCB-111 was used as external standard.

Sample preparation

Fish filets were freeze-dried, powdered and homogenized. After addition of labeled internal standards to a 1 g dried matter sample size, 16 mL hydrochloric acid 37% was added for matrix digestion during 12 h. Extraction was performed using a mixture of 20 mL of dichloromethane/n-hexane 10:90 (v/v). The sample was vortexed and allowed to stand overnight for complete separation of the liquid phases. 8 mL of the extract was loaded onto a glass column (1 cm i.d.) filled with 4 g of Florisil® between two layers of anhydrous sodium sulfate and pre-conditioned with n-hexane. The eluate, constituted of the 8 mL loading and 30 mL n-hexane elution volume, was collected in a flask containing the external standard in 30 µL of n-decane. Extracts were concentrated and transferred to vials using a Rotavapor® device and a gentle stream of pure nitrogen (Class I, Air Liquid, Paris La Défense, France) at 35 °C, taking care of preventing of any evaporation to dryness.

GC-MS/MS measurements

Separation and detection of target compounds was achieved using an HP-9890 gas chrotomatograph (Palo Alto, CA, USA) coupled to a Quattromicro triple quadrupole mass spectrometer (Waters, Milford, MA, USA). Volumes of 2 µL were injected in the splitless mode (265 °C). The gas chromatograph was fitted with a capillary column, 30 m x 0.25 mm id., 0.25 µm film thickness, coated with a

diphenyl(5%)-dimethylpolysiloxane(95%) stationary phase (ZB-5MS, Phenomenex, Torrance, CA, USA). Helium was used as carrier gas at 1 mL.min^{-1}. The temperature gradient started at 120 °C (held for 2 min), rose to 140 °C (3 °C.min^{-1}), to 200 °C (20 °C.min^{-1}) and then to 320 °C (30 °C.min^{-1}, held for 6.3 min). Source temperature was set at 250 °C and the electronic beam energy was set at 70 eV. The apparatus was operating in the Multiple Reaction Monitoring mode (MRM) and the monitored transitions are detailed in Table 1.

Table 1: Acquisition parameters and diagnostic signals use for measuring HCB and HCBD by GC-MS/MS. EE: external standard ($^{13}C_{12}$-PCB-111).

Win. (min)	Analyte	Diagnostic MRM transitions	Coll. (eV)	Diagnostic Ions
5.0	HCBD	222.8>187.9 & 224.8>189.9	15	$[C_4{}^{35}Cl_5]^+ > [C_4{}^{35}Cl_4]^{+\bullet}$ & $[C_4{}^{35}Cl_4{}^{37}Cl]^+ > [C_4{}^{35}Cl_3{}^{37}Cl]^{+\bullet}$
	$^{13}C_6$-HCBD	232.8>197.9 & 234.8>197.9	15	$[^{13}C_4{}^{35}Cl_2{}^{37}Cl_3]^+ > [^{13}C_4{}^{35}Cl_1{}^{37}Cl_3]^{+\bullet}$ & $[^{13}C_4{}^{35}Cl_1{}^{37}Cl_4]^+ > [^{13}C_4{}^{35}Cl_1{}^{37}Cl_3]^{+\bullet}$
9.0	HCB	283.8>248.8 & 281.8>246.8	15	$[C_6{}^{35}Cl_5{}^{37}Cl]^{+\bullet} > [C_6{}^{35}Cl_4{}^{37}Cl]^+$ & $[C_6{}^{35}Cl_6]^{+\bullet} > [C_6{}^{35}Cl_5]^+$
	$^{13}C_6$-HCB	293.8>221.9 & 295.8>221.9	15	$[^{13}C_6{}^{35}Cl_3{}^{37}Cl_3]^{+\bullet} > [^{13}C_6{}^{35}Cl_2{}^{37}Cl_2]^{+\bullet}$ & $[^{13}C_6{}^{35}Cl_2{}^{37}Cl_4]^{+\bullet} > [^{13}C_6{}^{35}Cl_2{}^{37}Cl_2]^{+\bullet}$
13.5	EE	268.0>198.1 & 270.0>200.1	17	$[^{13}C_{12}H_7{}^{35}Cl_3]^{+\bullet} > [^{13}C_{12}H_3{}^{35}Cl_1]^{+\bullet}$ & $[^{13}C_{12}H_7{}^{35}Cl_2{}^{37}Cl]^{+\bullet} > [^{13}C_{12}H_3{}^{37}Cl_1]^{+\bullet}$

Reference samples

Table 2 describes seven samples used for the method performances evaluation.

Table 2: Nature and origin of the analysed samples. All samples are fish filets. In-house pools are made of 20 samples each, including eel samples. *: freeze-dried sample.

Identification	Provider	Origin
WMF-01	Wellington Laboratories (Guelph, Canada)	-
CARP-2*	Wellington Laboratories (Guelph, Canada)	-
SRM 1947	National Institute of Standards & Technology (NIST, USA)	Trout, Lake Michigan, USA
Laberca 7.327	In-house	*Silurus sp.*, Rhône river, France
Laberca 10.483-1	In-house pool	14 species, French rivers
Laberca 10.483-2	In-house pool	13 species, French rivers
Laberca 10.483-3	In-house pool	8 species, French rivers

Results and discussion

Choice of diagnostic signals

Two transitions in the SRM mode have been considered for each compound, including ^{13}C-labeled internal standards. The most intense transition was used for quantification and the second one was used for identification confirmation. Given the overlapping of native and labeled isotopic clusters, due to the relatively high number of chlorine atoms compared to the carbon atoms, the choice of specific transitions among the isotopic clusters was important. Table 3 shows that chosen transitions are not necessarily the most intense ones.

Table 3: Relative signal abundances (based on ^{35}Cl and ^{37}Cl contributions) for native and labeled HCB and HCBD, and chosen transitions (bold). Precursor ion C_6Cl_6 $[M]^{+\bullet}$ for HCB and C_4Cl_5 $[M\text{-}Cl]^+$ for HCBD.

HCB					HCBD			
Loss of ^{35}Cl		Loss of ^{37}Cl		Precursor	Loss of ^{35}Cl		Loss of ^{37}Cl	
Native	$^{13}C_6$-Lab.	Native	$^{13}C_6$-Lab.	Ion	Native	$^{13}C_4$-Lab.	Native	$^{13}C_4$-Lab.
51%	-	0%		m	**62%**	-	0%	
83%	-	17%		m+2	**80%**	-	20%	
54%	-	27%		m+4	39%	62%	26%	0%
17%	51%	17%	0%	m+6	8%	80%	13%	20%
3%	83%	6%	17%	m+8	0.7%	39%	3%	26%
0.2%	54%	0.9%	27%	m+10	0%	**8%**	0.2%	13%
0%	**17%**	0.06%	17%	m+12	-	0.7%	-	**3%**
-	3%	-	**6%**	m+14	-	0%	-	0.2%
-	0.2%	-	0.9%	m+16				
-	0%	-	0.06%	m+18				

Specificity

HCB and HCBD are relatively polar compared to other organochlorine pesticides. As illustrated on Figure 1, diagnostic transitions are highly specific. No interferences have been observed on the signals and ion ratios were always acceptable

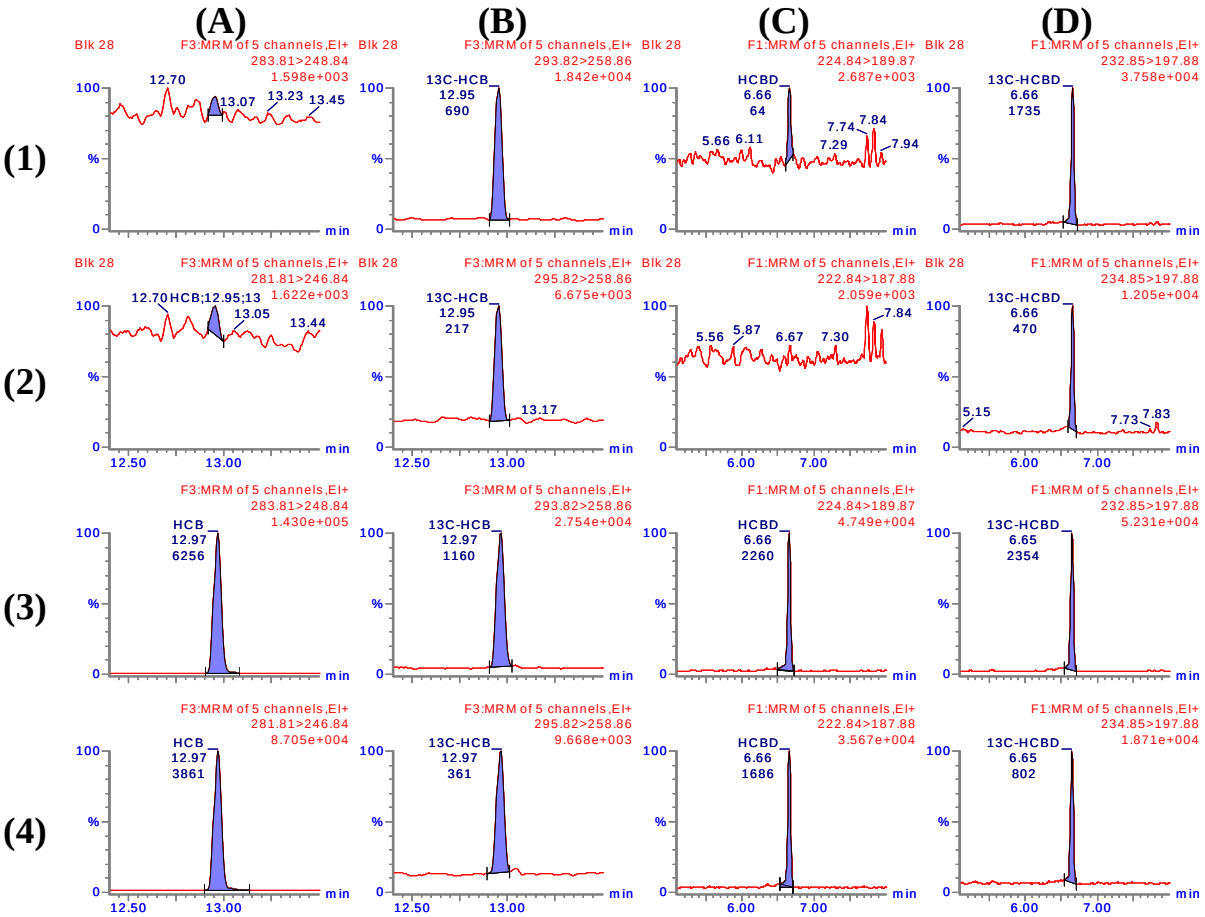

Figure 1: Example of diagnostic ion chromatograms obtained for a blank sample (1,2) and WMF-01 sample (3,4). HCB (A,B) or HCBD (C,D), native (A,C) or labeled (B,D), quantification trace (1,3) or identification confirmation trace (2,4).

Blank levels and Limits of Detection (LOD)

Although some HCl grades have been found to contain traces of target compounds, analytical contamination have been minimized to almost non-undetectable levels. Internal standards impurities (^{13}C purity) also slightly contribute to background signals measured for blank samples, particularly for the HCBD quantification trace. Even if ^{13}C-purity is higher than 98%, elevated spiked quantities added to compensate relatively low abundances of selected transitions make contribution of impurities non negligible. Indeed, like for numerous environmental contaminants (*e.g.* some brominated flame retardants or some phthalates for example), blank signals were always integrated (S/N≥3). Obtained blank levels (n=31) were always lower than 30 $pg.g^{-1}$ (considering a sample size of 4 g fresh weight - f.w.), with averages at 6.9 and 15.1 $pg.g^{-1}$ f.w. for HCB and HCBD, respectively. Limits of detection were considered to correspond to 3 times the blank level, being in the range of 0.01-0.1$ng.g^{-1}$ f.w (base on a 25% dried matter).

Precision uncertainty

Five samples have been analyzed in inter-day replicates, 7 to 32 times each. The observed signal variability (RSD) were below 10% (Table 4), and slightly better for HCB compared to HCBD. The global RSD for precision can be deduced from Equation 1[3], leading to 6.1% and 7.4% for HCB and HCBD, respectively.

Table 4: Obtained mean values (ng.g^{-1} f.w.) and relative standard deviations (RSD). *: assuming 25% dried matter (arbitrary value).

		HCB		HCBD	
Sample	**Replicates**	**Mean**	**RSD**	**Mean**	**RSD**
WMF-01*	n=7	1.01	6.4%	0.36	9.3%
Laberca 7.327	n=32	15.37	6.3%	0.57	7.3%
Laberca 10.483-1	n=10	1.73	8.1%	n.d.	-
Laberca 10.483-2	n=20	1.02	4.9%	0.15	6.6%
Laberca 10.483-3	n=10	9.63	5.5%	8.89	7.9%

Equation 1

$$RSD_{precision} = \sqrt{\frac{(n_1-1)*RSD_1^2+(n_2-1)*RSD_2^2+...}{(n_1-1)+(n_2-1)+....}}$$

Trueness

SRM 1947 sample is a Certified Reference Material for HCB (7.48±0.66 ng.g^{-1} f.w.). From a single analysis, the present method lead to 6.01 ng.g^{-1} f.w. Considering the precision uncertainty for this sample to be the $RSD_{precision}$ described above, the global trueness uncertainty for HCB was found to be 4.5%. An other way to address the trueness uncertainty was to consider results from the Northern Contaminants Interlaboratory Quality Assurance Program (NCP III–4, MOE, Ontario, Canada). Fifteen laboratories provided results for HCB in SRM 1947 and CARP-2 samples[4], with median values at 5.97±1.56 and 3.93±1.46 ng.g^{-1} f.w., respectively. From a single analysis, the present method lead to 6.01 and 3.34 ng.g^{-1} f.w., respectively, which appeared to be relatively satisfying. In order to go further on the performances evaluation of the method, our laboratory has been included as participant to the on-going NCP III–5.

Expanded uncertainty

Since no material was available to assess HCBD trueness uncertainty, expanded uncertainty was only evaluated for HCB. The third source of uncertainty which was considered was the standards purity (≥98%). Finally the combined relative uncertainty found for HCB was 7.7% and the expanded uncertainty (at 95%) was found to be 15.4%.

Acknowledgements

The authors want to express their special thanks to Eric Reiner and Rania Farag from the Laboratory Services Branch of Ministry Of the Environment (MOE), Ontario, Canada for providing WMF-01, CAPR-2 and SRM 1947 samples thought the Northern Contaminants Interlaboratory Quality Assurance Program (NCP III–4).

References

1. Garrido Frenich A, Plaza Bolanos P, Martinez Vidal JL (2007). *Journal of Chromatography A*, 1153: 194–202.
2. Plaza Bolanos P, Garrido Frenich A, Martinez Vidal JL (2007). *Journal of Chromatography A*, 1167: 9–17.
3. Eppe G, de Pauw E (2002). *Organohalogen Compounds* 59: 403-406.
4. Farag R, Selliah S, Reiner E, Ali B (2009). Final report on Northern Contaminants Interlaboratory Quality Assurance Program (NCP III – Phase 4). Laboratory Services Branch, Ministry Of the Environment (MOE), Ontario, Canada.