Supplementary materials:

Polyhalogenated Compounds (Halogenated Natural Products and POPs) in Sardine (*Sardinops sagax*) from the South Atlantic and Indian Oceans

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1. Sample blank

A blank sample contained no noticeable impurities except trace amounts of PCBs (typically between LOD and LOQ). Quantifiable levels were subtracted from the calculated contents of the samples. To find the source, blank values of the individual preparation steps (i) ASE, (ii) GPC, (iii) adsorptive chromatography and (iv) all solvents were examined separately. No relevant impurities could be detected in the examined solvents, including redistilled cyclohexane/ethyl acetate (46:54, w/w). Yet, all other subsamples showed trace amounts of PCBs which could not be excluded. In addition, traces of TBP appeared in the blank values of the GPC (S/N ~3). This prompted the setup of a new GPC column (see section 3.).

1.1 Analysis scheme and method design

One batch consisted of 5-7 samples which were prepared at the same time. To each batch, a blank value was prepared to correct for minor contaminations. In all batches, PCBs in the blank were much lower than in the sardine samples. Only in batch 2 (samples SiteA-1A, SiteA-3A, SiteA-4A, SiteA-5A and SiteA-8A&B), peaks of PCBs and BC-2 and BC-3 in the GC/NCI-MS-SIM chromatogram of the blank value were higher than in the samples which were prepared at the same time (**Figure 1.1**). Even after correction with the syringe standard, the blank value showed higher contents of these polyhalogenated compounds than the sample.

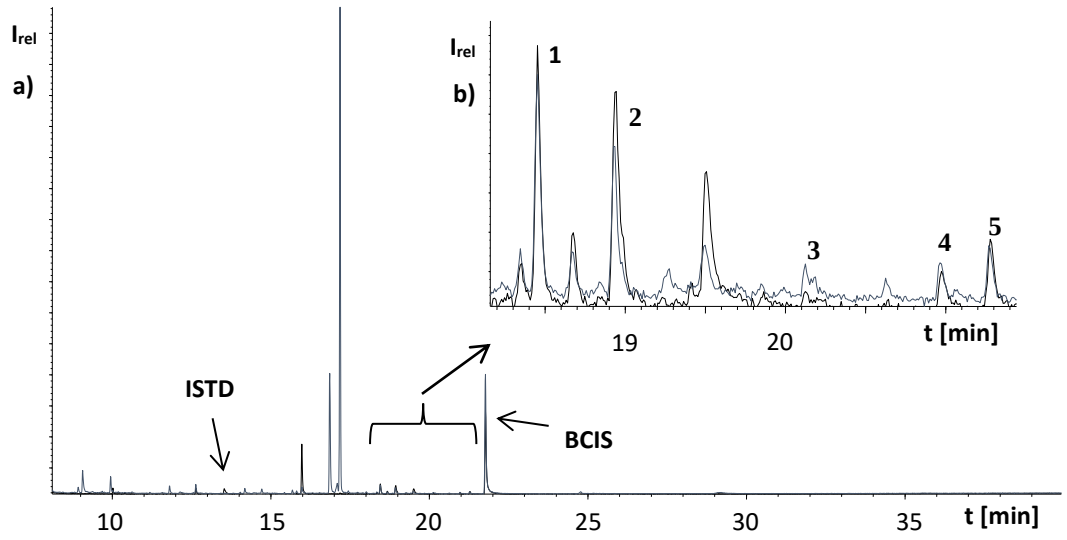

Figure 1.1: a) GC/ECNI-MS-SIM-chromatogram (SIM 7, TIC) of blank (black) and sardine sample Site A-8B (blue) b) enlarged section with 1) PCB 153, 2) PCB 138, 3) PCB 180, 4) BC-2, 5) BC-3

Discoloration of the GPC column suggested that the abnormal results were associated with an aged GPC column, which was re-newed (see section 3). Later sample preparations with the new GPC column were free of relevant impurities in the blank values. Therefore, samples

of batch two were reanalyzed with the new GPC column. It could be shown that PCBs, BC-2 and BC-3 were present in all five samples of batch 2 (samples SiteA-1A, SiteA-3A, SiteA-4A, SiteA-5A and SiteA-8A&B). For this reason, only the second run with the new GPC column (samples SiteA-1B, SiteA-3B, SiteA-4B, SiteA-5B and SiteA-8C) was considered when evaluating PCBs, BC-2 and BC-3. Since other compounds were not affected (Q1, MHC-1, 2,4,6-TBP etc.) by the initial blank value problem, the mean value was formed for all samples (samples SiteA-1A&B, SiteA-3A&B, SiteA-4A&B, SiteA-5A&B and SiteA-8A&B&C).

2. Investigation of precipitation in ASE extracts by thin layer chromatography

Some ASE extracts showed precipitating matter in the sample tubes of ASE extracts after cooling. The precipitate could not be dissolved by ultra-sonication with n-hexane. It was therefore concluded that the residue did not contain nonpolar (lipid) components. Using methanol, a share could be dissolved and a weak yellow color of the solution was obtained. This and other sample fractions were analyzed by TLC.

Reference standards were run to determine R_f values of triacylglycerols (TAG), sterols, free fatty acids (FFA), fatty acid methyl esters (FAME) and polar lipids such as phospholipids (which remained on the starting zone).[1] Substance spots were made visible by derivatization with 10% sulfuric acid methanol.[1, 2]

The ASE extract of sardines showed typical lipid classes of fish.[3] As expected, TAG showed an intense bond. A compound (U1) not present in the standard showed an R_f value of 0.9 (**Figure 2.1**), hence this very non-polar compound had a similar R_f value as squalene. A very weak spot at 366 nm at the starting (polar, not moved) had a different coloration than phospholipids in the standard. Phospholipids could be identified in the methanol extract of the ASE residue by their plain spot and definite color (presumably the polar phospholipids were not dissolved in the ASE solvent). The methanol extracts did not show any lipid components. Hence, the precipitate was removed by centrifugation.

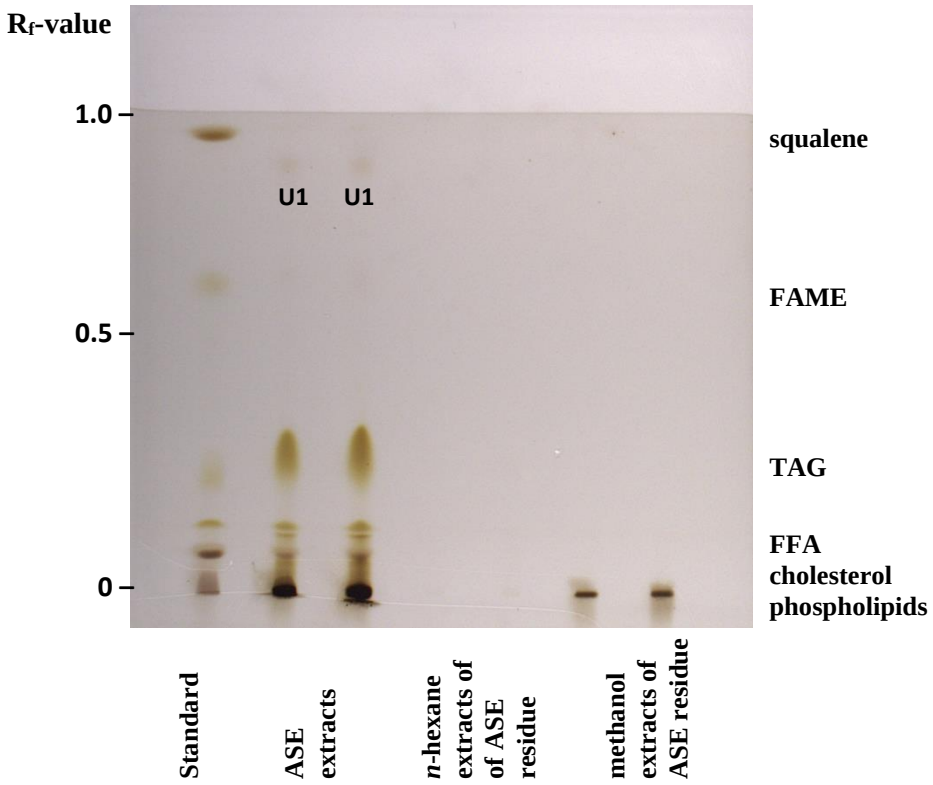

Figure 2.1: Photo of the TLC plate at white light after derivatization with 10% sulfuric methanol using lipid standards and different fractions of a sardine sample

3. Validation of the gel permeation chromatography (GPC) method

The in-house GPC method was validated by Vetter *et al*.[4] At the beginning of the study, the (old) GPC column showed an unusually strong discoloration (**Figure 3.1**), and initial tests indicated the presence of traces of BC-2, BC-3 as well as PCB 153, 138 and 180. Hence, a new GPC column was packed and validated.

On the newly-packed GPC column, GPC elution times of polyhalogenated compounds are shown in **Table 3.1**.[5] In order to target BDE 209, which elutes notably late from the GPC column,[6] the complete removal of sulfur has to be avoided.[6]

After GPC, a further purification step is required to remove all lipid-related substances (e.g. sterols).[7,8]

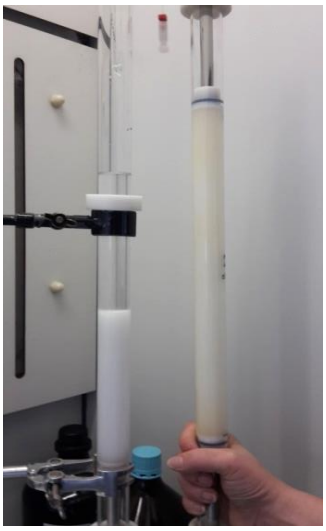

Figure 3.1: Photo of the new (left side) and the old (right side) GPC column

Table 3.1: Elution times [min] of the analyzed compounds on the validated GPC column as well as literature values

compound	elution time	literature	source
β-HCH	20 – 26	20 – 26	[5]
hexachlorobenzene	26 – 32	28 – 33	[5]
sulfur	–	43 – 49	[5]
trans-chlordane	20 – 26	20 – 26	[5]
hexabromobiphenyl	–	32 – 38	[5]
BDE-209	–	35 – 52	[6]
PBB 209	32 – 40	–	–
OCN	32 – 38	–	–

3.1 Validation of lipid separation

At first salmon oil and sunflower oil (0.9 g each) were used to determine the elution volume of the lipids. Ten individual 1 mL fractions (B1-B10) were taken using GPC method B (**Table 3.2**) and the weight was determined after the solvent had evaporated. No lipids were detected after 24 min (salmon oils, fraction B5) or 22 min (sunflower oil, fraction B3) (**Figure 3.2a,b**). Negative weights in later fractions (B5-B10) were most likely due to the inaccuracies in the weighing. The check was carried out by using method C (**Table 3.2**) for the next two samples to collect six 1 mL fractions of 20-26 min (fractions C1-C6) in which only traces of oils should appear. Values similar to those in the first determination were obtained for both oils.

Table 3.2: GPC methods used during the validation

number	method lab name	discard time	collecting time	fractions
A	CH_KS_fractionation 20 + 16 + 16 min 4-5 mL	0 – 20 min	20 – 36 min 36 – 52 min	1 1
B	KS_validation_Fat1	0 – 20 min	20 – 30 min	10
C	KS_validation_Fat2	0 – 20 min	20 – 26 min	6
D	KS_validation_Fat3	0 – 18 min	18 – 26 min	8
E	KS_validation_POP1	0 – 18 min	18 – 52 min	17
F	KS_fractionnation 19 + 17 +16 min 4-5 mL	0 – 19 min	19 – 36 min 36– 52 min	1 1

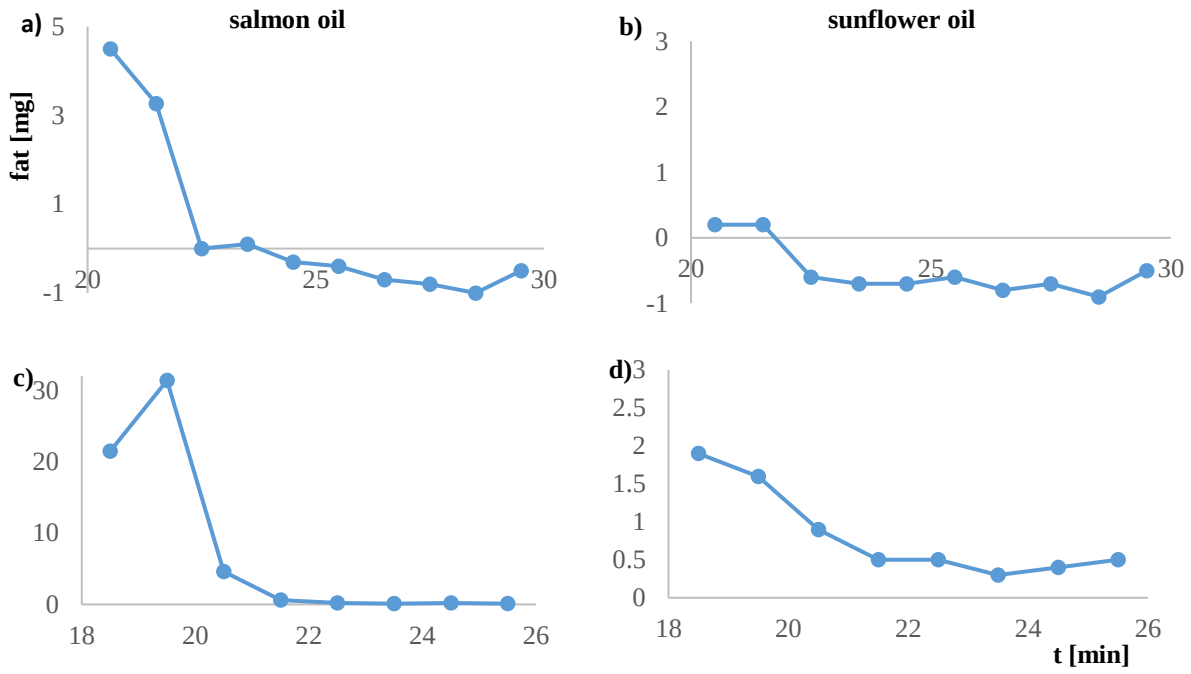

Figure 3.2: Oil weight [mg] on the y-axis depending on the elution time t [min] on the x-axis: a) salmon oil and b) sunflower oil (method B); c) salmon oil and d) sunflower oil (method D)

The total amount of collected lipids was less than 10 mg for salmon oil and even less than 4 mg for sunflower oil. Since up to 100 mg of oil can be separated by means of adsorptive chromatography,[9] it was considered to reduce the discard time of 0-20 min.

A further GPC fractionation was carried out for this purpose, in which the fraction was additionally examined for 18-20 min (method D). The amount of lipids in fractions 18-26 min (D1-D8) was well below 100 mg even after normalizing the values to an oil weight of 1 g (**Table 3.3**). Therefore, the rejection time was reduced by 1 min to 0-19 min.

Table 3.3: Sum of the oil weight, normalized to 1 g sample weight (method D)

Fraction	D3-D8 (20-26 min)	D2-D8 (19-26 min)	D1-D8 (18-26 min)
salmon oil	6,8 mg	43,3 mg	68,4 mg
sunflower oil	3,4 mg	5,1 mg	7,2 mg

In contrast to methods B and C, lipids were detected until fraction D8 (25-26 min, **Figure 3.2c,d**). Lipid analysis of the first three fractions (D1-D3. 18-21 min) were performed by high-temperature GC, TAGs could be detected in 100 ng injected quantity of fraction D1 (18-19 min) of the sunflower oil, but much smaller amounts than in the unfractionated oil (**Figure 3.3**). In the other two fractions, the TAGs were below the detection limit. In contrast, sterols (**Figure 3.3**), which could not be detected in the unfractionated oil, were already clearly

visible in the first fraction (18-19 min). The same observation was made for salmon oil, but here TAGs were also detectable in fraction D2.

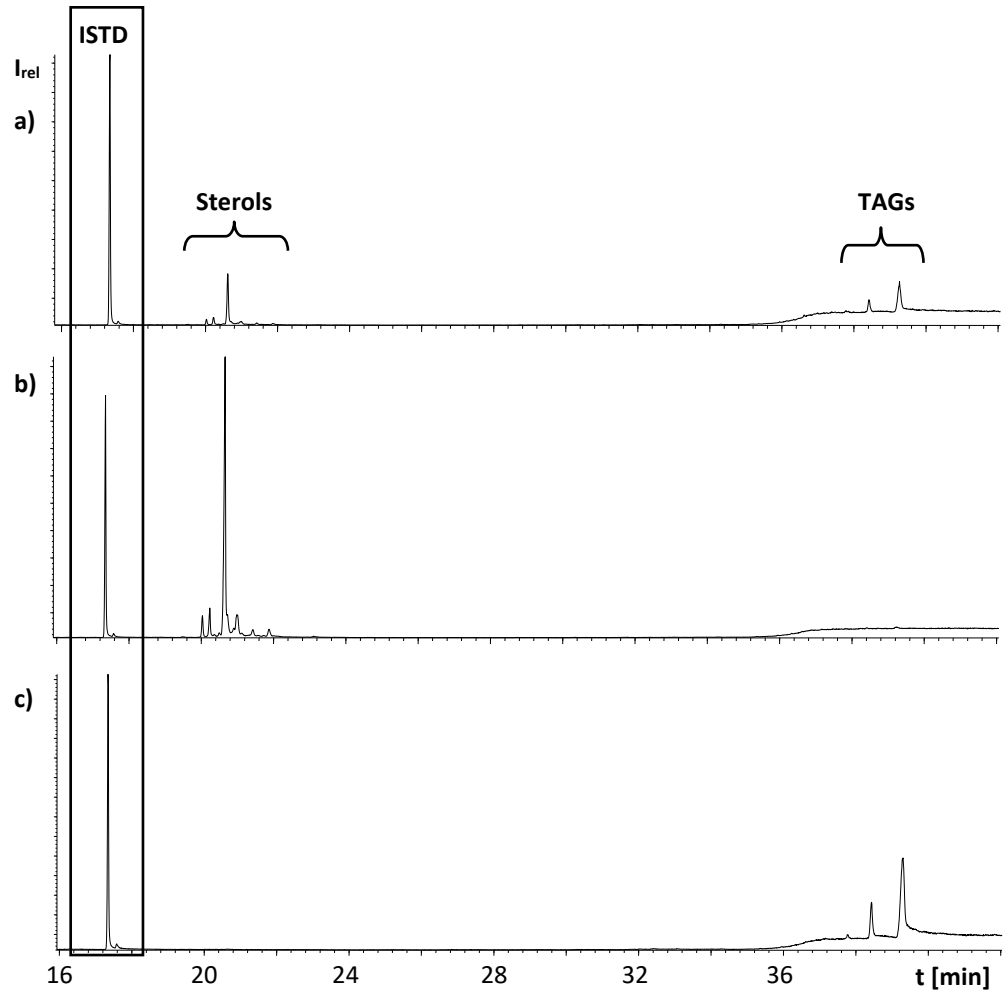

Figure 3.3 GC/EI-MS-*full-scan*-chromatograms of a) fraction D1 (18-19 min) (95 ng), b) fraction D2 (19-20 min) (96 ng) and c) unfractionated sunflower oil (76 ng), measured with high temperature GC

The proportion of sterols increased with increasing GPC elution time, the maximum being 20-22 min (fractions D3 and D4). In fraction D5 (22-23 min) the sterol content decreased again (**Figure 3.4a, b**) and after 23 min (fraction D6) no more sterols could be detected.

Furthermore, bound and free fatty acids were examined together after transesterification to FAMEs in the same way. Typical fatty acid patterns were determined for both oils.[10,11] Overall, there was a continuous decrease in FAMEs (**Figure 3.4c,d**), but free fatty acids were still detectable even in the last fraction. Free fatty acids were present in the oil before transesterification as verified by high-temperature GC of silylated extracts. Since these are very small molecules, they penetrate deeper into the pores of the GPC column and have a longer elution time due to the longer path.[12] By contrast, TAGs were present in no more than the first two fractions (18-20 min).

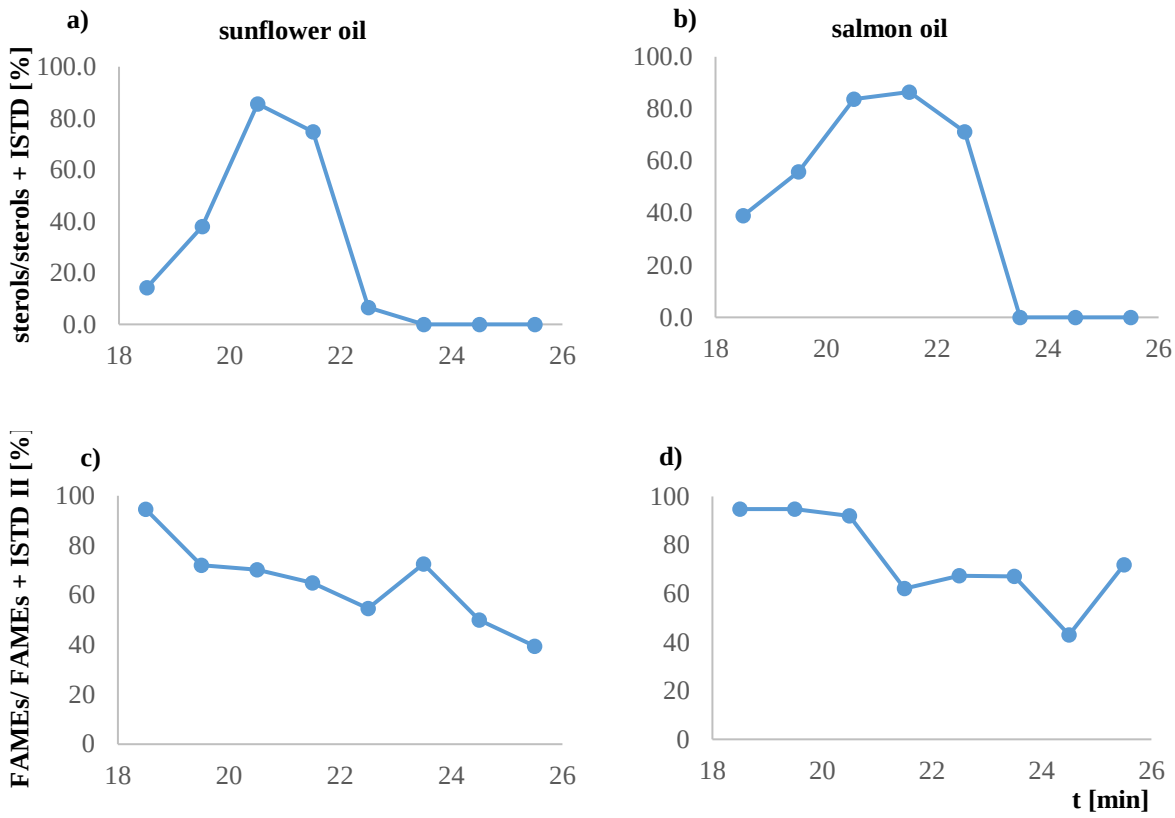

Figure 3.4: Percentage of sterols in the total peak area of a) sunflower oil and b) salmon oil, as well as percentage of FAMEs in the total peak area of c) sunflower oil and d) salmon oil dependent on the elution time t [min]

The results showed that the lipids were fractionated according to molecular size, since the significantly smaller sterols eluted after the TAGs. Although the later GPC fractions still contained traces of fatty acids, the amount of oil remaining in the GPC column after the first 18 min was clearly below 100 mg. Almost no TAG remained in the remaining oil residue, but from 20 min most of the sterols eluted, which could not be separated from the polyhalogenated compounds in this step.

3.2 Validation of the elution times of halogenated compounds

GPC elution profiles (method E) determined with POPs were used that eluted either early (trans-chlordane and β-HCH), late (PBB 209 and OCN) or in the middle (HCB).[5] The elution range of 18-52 min was divided into 17 fractions of 2 min each (E1-E17). The recoveries were between 78% and 128%, although it should be noted that the evaluation was carried out without a syringe standard and therefore only semi-quantitative. The first eluting POPs *trans*-chlordane and β-HCH could not be detected in fraction E1 (18-20 min) and no analytes were detectable from fraction E12 on (40-42 min) (**Figure 3.5**).

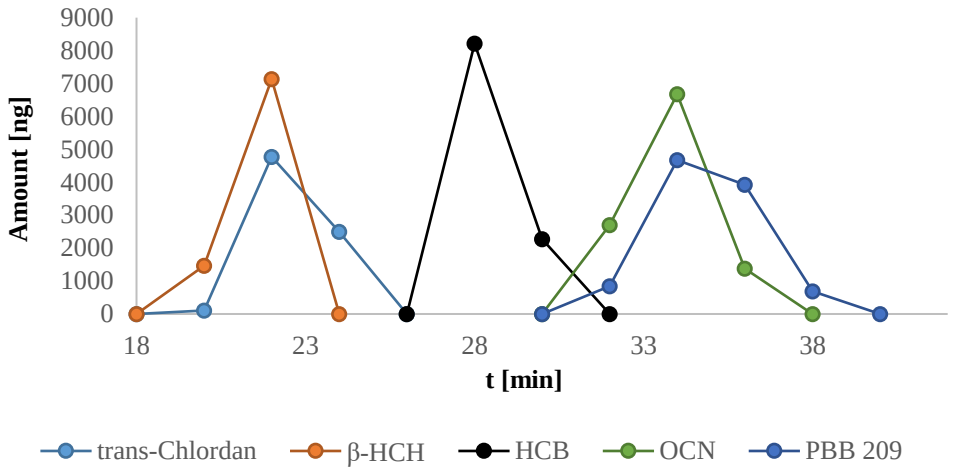

Figure 3.5: Absolute amount [ng] determined by GC/NCI-MS-SIM (SIM 1) depending on the elution time t [min]

The elution time of the POPs was in good agreement with the literature values (**Table 3.1**), only HCB eluted somewhat earlier than was described in literature. No literature values could be found for the last two eluting compounds, which is why PBB 209 was compared with the homologous hexabromobiphenyl, which both had almost identical elution profiles (**Table 3.1**).

Since a large proportion of β-HCH eluted into fraction E2 (20-22 min), the fractionation was started 1 min earlier (i.e. from 19 min on), and the end was set at 52 min so that the late-eluting BDE 209 was also recorded. For sediment samples, however, the elution profile for the complete elution of BDE 209 should be checked again.

3.3 Validation of the complete GPC-method F

Double or triple determination (salmon oil, five standards) resulted in recovery rates of 85-108% after both GPC and silica gel purification. Hence, method F was suitable for sample preparation. Only OCN had a recovery of <90%; however, this was attributed to the compound's very poor response.

Validation of the recovery rates of HNPs after GPC and silica gel purification showed that the recovery rates of major quantitative HNPs were >90%. The salmon oil sample featured MHC-1 and BC-3 (**Figure 3.6a**). This could be confirmed by repeating the GPC fractionation with POPs and sunflower oil (**Figure 3.6b**). For this reason, all other samples were prepared using GPC method F.

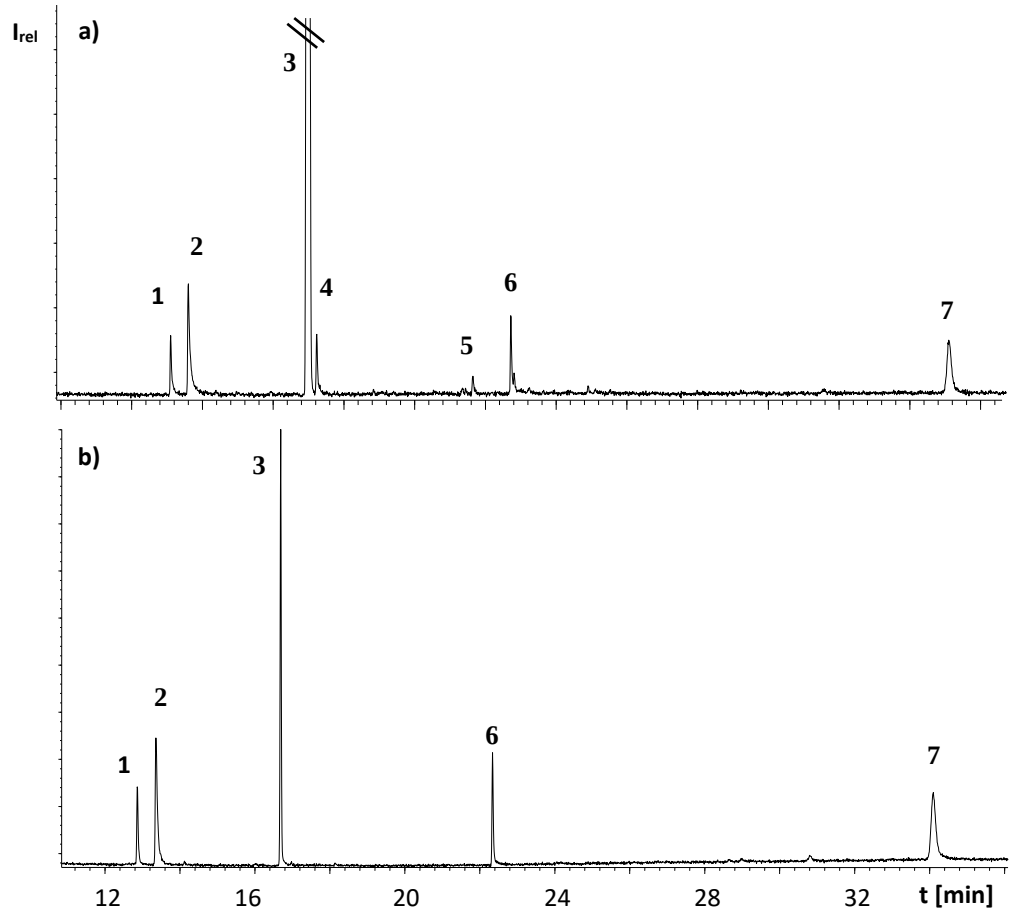

Figure 3.6: GC/NCI-MS-SIM-chromatograms (SIM 1, TIC) of a) salmon oil and b) sunflower oil; with 1) HCB, 2) β-HCH, 3) *trans*-chlordane, 4) MHC-1, 5) BC-3, 6) OCN and 7) PBB 209

4. Recovery rates of internal standards and reproducibility

4.1 Test sample salmon fillet (Salmo salar) from Norway and method design for sardine samples

To test the method, the test sample (*Salmo salar*) was first purified and analyzed in triplicate. Unfortunately, the water bath temperature of the rotary evaporator of sample A was erroneously set to 55 °C instead of 35 °C. This sample (A) had the lowest recovery rate and lowest levels and was thus excluded. Without sample A, the recovery rate of the internal standard α-PDHCH was >90% (**Table 4.1**). The recovery rates were thus above the threshold of 70% recovery, from which the workup in the working group is considered successful. This threshold was also used for sardine samples (see below).

The reproducibility of GC/NCI-MS quantification of polyhalogenated compounds in the salmon fillet test sample duplicate was generally very good (**Table 4.2**).

Table 4.1: Overview of the recoveries [%] of the internal standard α-PDHCH after clean-up and lipid contents in dry matter [%] of the salmon (*Salmo salar*) fillet test sample

sample	Sample number	recovery α-PDHCH [%]	Lipid content in dry matter [%]
Salmo	B	92	28
salar	C	91	29

Table 4.2: Contents [ng/g lm] in *Salmo salar* from aquaculture in Norway

	TBA	MHC-1	BC-3	Q1	BDE 47	PCB 153	PCB 138	PCB 180	HCB	α-HCH	*p.p´*-DDE	DDD
B	8.2	7.0	4.6	3.4	5.6	9.1	10	3.0	29	0.7	17	13
C	10	7.6	3.8	3.0	5.7	8.5	9.1	2.8	34	0.7	17	11

4.2 Analysis of Sardinops sagax from the Atlantic and Indian Oceans

Only limited freeze-dried sample material was available from the sardine samples, and duplicate samples could only be prepared in the case of six samples from the Indian Ocean (site A) (one in triplicate) and two from the South Atlantic (site B). An error occurred during the ASE extraction of sample Site B-9, so that the extraction was stopped automatically due to a leaky cell and the sample was lost. Since no further material was available from sample Site B-9, this sample could then only be evaluated individually. Two other samples (# Site B-5, Site A-6) had recovery rates <70% (**Table 4.3**).

In sample Site B-5 levels of TBA, Q1 and MHC-1 (**Table 4.4**) were lower, consistent with the lower recovery rate. Thus, only sample Site B-5B was used for the quantitative assessment (no mean formed). Likewise, sample Site B-9 could not be taken at all into the evaluation because one sample failed and the other showed an unusual and unacceptable recovery rate.

All other samples processed without duplicate sample were taken into account when the recovery rate was >70%. This was the case except for sample Site A-6 and Site B-15.

Table 4.3: Recovery rates [%] of the internal standard α-PDHCH after preparation and subsequent concentration of the sample of sardines

sample	Determination	recovery rate α-PDHCH [%]	lipid content in dry matter [%]
Site A-1	A	99	8.8
	B	94	8.4
Site A-2	-	88	16
Site A-3	A	88	2.6
	B	90	2.8
Site A-4	A	94	6.8
	B	92	6.5
Site A-5	A	91	8.0
	B	87	8.2
Site A-6	-	62	9.0
Site A-7	-	91	16
Site A-8	A	97	7.9
	B	93	7.7
	C	79	7.8
Site A-9	A	87	11
	B	90	11
Site A-10	-	91	13
Site A-11	-	77	7.2
Site A-12	-	74	7.1
Site A-13	-	108	8.8
Site A-14	-	97	8.3
Site A-15	-	100	17
Site B-1	-	96	6.7
Site B-2	-	92	2.6
Site B-3	-	92	5.4
Site B-4	-	87	11
Site B-5	A	~~61~~	4.6
	B	80	4.6
Site B-6	-	92	4.7
Site B-7	-	97	5.1
Site B-8	-	85	5.7
Site B-9	A	~~18~~	4.0
	B	~~Error~~	Error
Site B-10	-	110	8.6
Site B-11	-	98	6.0
Site B-12	-	95	13
Site B-13	-	81	4.0
Site B-14	-	84	4.5
Site B-15	-	60	3.6

Table 4.4: Contents of TBA, MHC-1 and Q1 [ng/g lm] and deviations [%] of the duplicate determination of Site B-5 and Site A-9

	TBA		MHC-1		Q1	
	content	deviation	content	deviation	content	deviation
Site B-5A*	2.7	27	53	31	42	23
Site B-5B	3.7		77		54	
Site A-9A	1.2	0	28	1.0	55	1.0
Site A-9B	1.2		29		56	

*low recovery

5. Lipid contents of the salmon samples

As the samples were already dried and fresh weights were not available, lipid contents were derived from dry matter. The lipid contents of the duplicate samples were in very good agreement. The sardine samples from the Indian Ocean were slightly higher in lipids than the nine samples from the Atlantic Ocean (**Table 5.1**). Fluctuations in the lipid content of fish have already been found in different seasons,[13–15] mostly due to lipid mobilization during spawning, but also to other factors such as the availability of food or water temperature.[13–15] Since the sardines examined in this work were caught shortly after the end of the spawning phase this was considered as a possible reason along with the age and gender of the sardines.[16]

Table 5.1: Lipid content (mean, lowest and highest value) in dry matter [%] of sardines from the Indian and South Atlantic Oceans

	mean	lowest content	highest content
Indian Ocean	9.9	2.6	16
Atlantic Ocean	6.5	2.6	13

6. Limits of detection (LOD) and limits of quantification (LOQ)

The limits of detection (LOD) and limits of quantification (LOQ) of quantitative compounds were listed in Table S2.

The calibration lines of standards were available at https://doi.org/10.1016/j.envpol.2019.113282.

B)

Table S1. GC/ECNI-MS retention time and characteristic ions screened for *o,p´*-DDT, *p,p´*-DDT, *o,p´*-DDE *p,p´*-DDE and *p,p´*-DDD.

	compounds	retention time [min]	characteristic ion [*m/z*]
1	*o,p´*-DDT	21.63	246
2	*p,p´*-DDT	22.23	318
3	*o,p´*-DDE	20.19	246
4	*p,p´*-DDE	20.77	318
5	*p,p´*-DDD	21.54	248

C)

Table S2. Limits of detection (LOD) and limits of quantification (LOQ) (ng/g lm) for compounds listed in Table 1 (manuscript file).

	compounds	LOD	LOQ
1	MHC-1	0.03	0.10
2	Q1	0.03	0.09
3	2,4,6-TBP	0.08	0.26
4	2,4,6-TBA	0.01	0.02
5	2,4-DBA	0.02	0.08
6	2,6-DBP	0.02	0.07
7	BC-3	0.07	0.23
8	BC-2	0.02	0.07
9	*p,p*'-DDE	0.03	0.10
10	PCB 153	0.02	0.05
11	PCB 138	0.01	0.05
12	BDE 47	0.06	0.20
13	HCB	0.01	0.02

D)

Table S3. Detailed range and (mean) [median] of PCB and PBDE congeners (ng/g lm) in sardine (*Sardinops sagax*) from the Indian Ocean and the South Atlantic.

#		**Indian Ocean (Site A)**	**South Atlantic (Site B)**
	PCBs		
1	PCB 118	0.3-0.7 (0.5) [0.5]	0.3-0.8 (0.6) [0.7]
2	PCB 138	0.3-1.7 (0.9) [1.0]	ND-2.3 (1.0) [0.8]
3	PCB 153	0.4-2.1 (1.0) [1.0]	0.1-1.9 (0.9) [0.7]
4	PCB 180	0.1-1.0 (0.4) [0.3]	ND-0.5 (0.2) [0.2]
	PBDEs		
1	BDE 47	ND-1.5 (0.5) [0.3]	ND-2.1 (0.8) [0.5]
2	BDE 71	ND	ND-1.5 (0.5) [0.2]
3	BDE 75	0.5-0.7 (0.6) [0.6]	ND

ND= not detected.

E)

Table S4. Estimated daily intake (EDI) (ng) of halogenated compounds through 100 g fresh fillets with different trophic levels, current daily intake of halogenated compounds in the Europe (ng/kg body weight -day) and Reference Dose for Oral Exposure (RfD) (ng/kg body weight -day) of major halogenated compounds.

	Compounds	EDI (sardines)	EDI (chokka squid)[17]	EDI (albacore tuna)[18]	Daily intake in the Europe[19]	RfD[20]
1	MHC-1	110	2	unavailable		unavailable
2	Q1	110	210	150		unavailable
3	*p,p'*-DDE	6	<LOD	120		500*
4	Σ PCBs	5	18	360	10-45 (adults)/ 27-50 (<6 years old)	20*
5	Σ PBDEs	2	4	unavailable		100*
6	HCB	0.8	1	unavailable		800

*Since RfD values of *p,p'*-DDE, total PCBs and total PBDEs are not available, RfD values of *p,p'*-DDT, Aroclor 1254 and BDE 47 were used instead of them, respectively.

F)

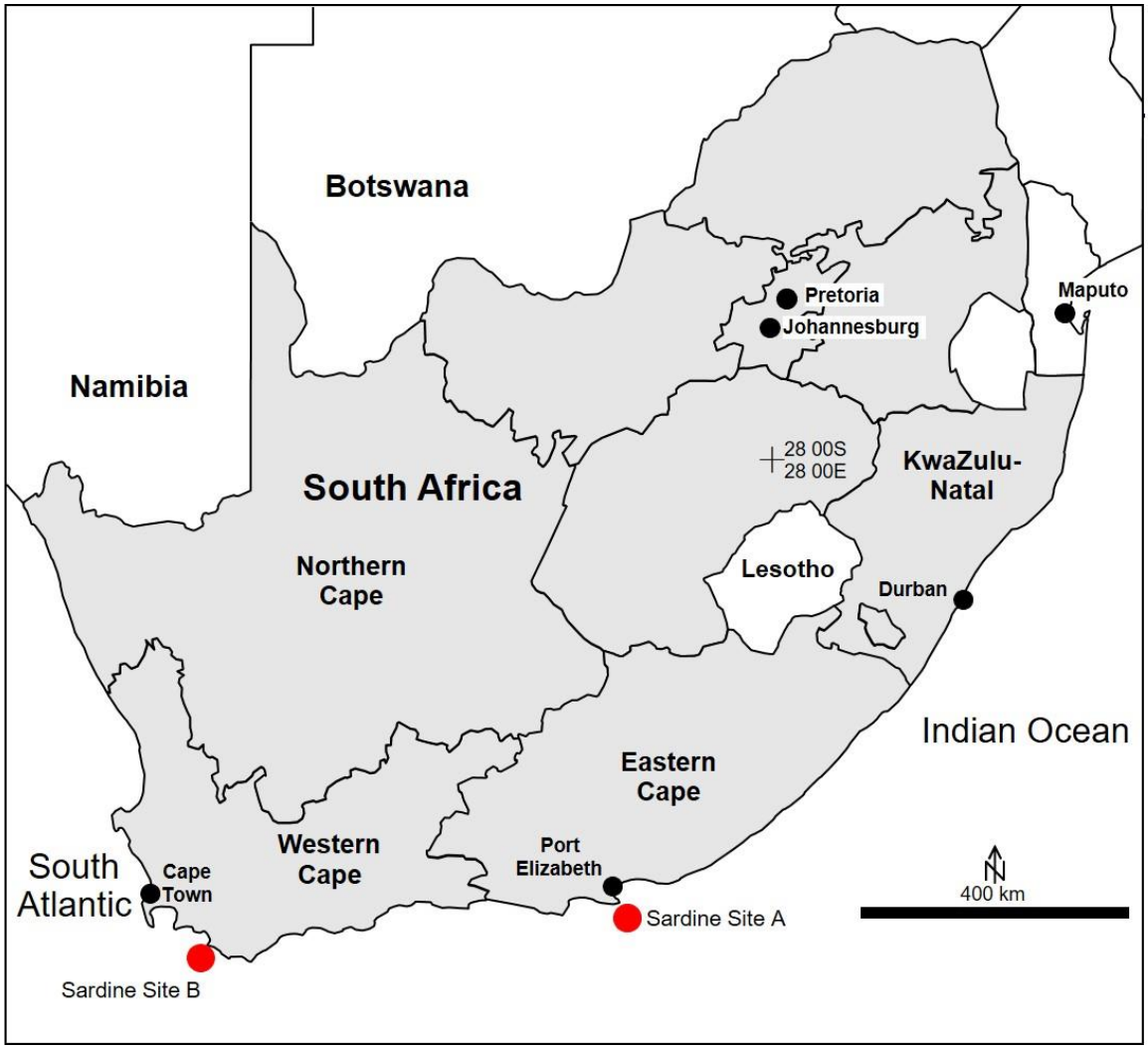

Figure S1. Map of the sampling locations along the coast of South Africa.

G)

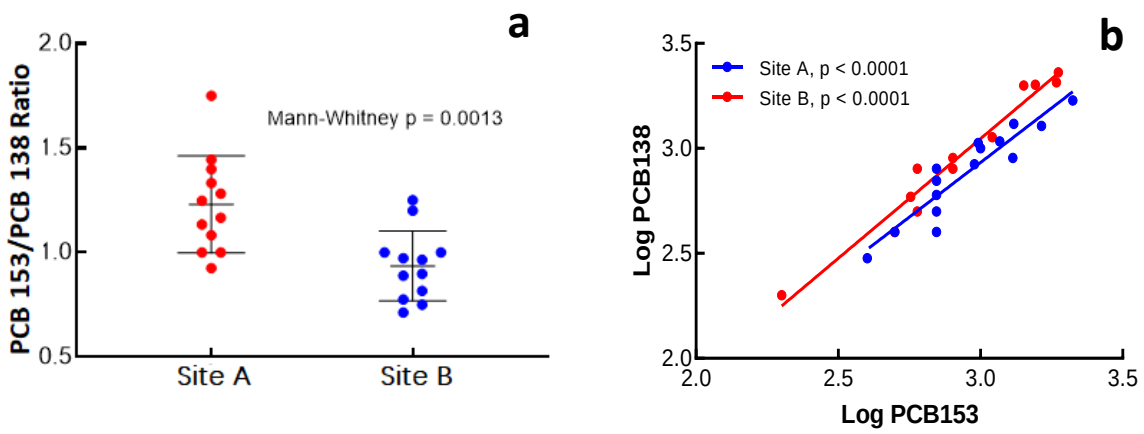

Figure S2. (a) The ratio of PCB 153/PCB 138 and **(b)** regression of concentrations of PCB 153 against PCB 138 in sardines (*Sardinops sagax*) from the Indian Ocean (site A) and the South Atlantic (site B).

H)

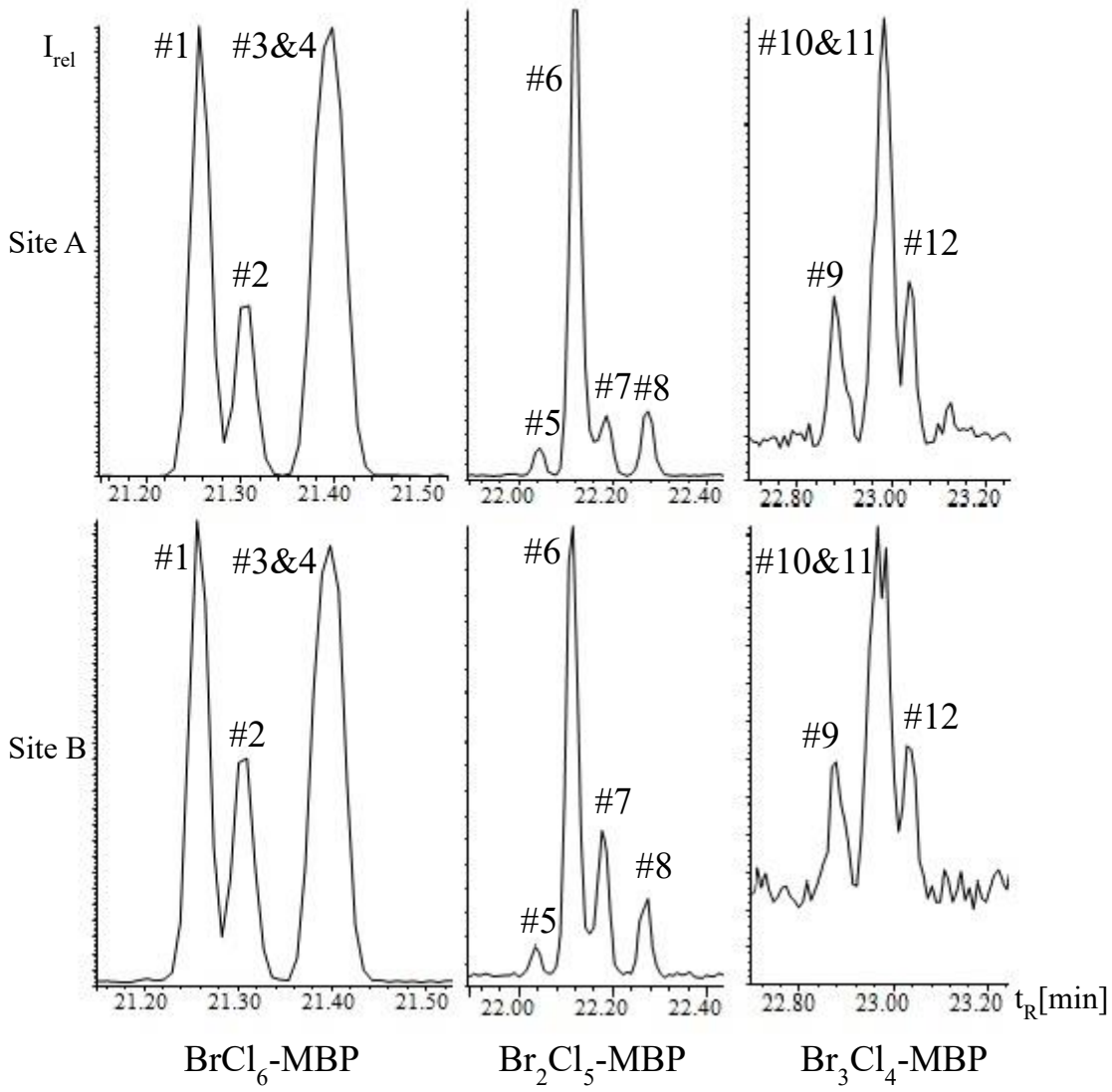

Figure S3. GC/ECNI-MS ion chromatograms of mixed brominated-chlorinated 1´-methyl-1,2´-bipyrroles (PMBPs) of sardines (*Sardinops sagax*) from the Indian Ocean (site A) and the South Atlantic (site B) with four $BrCl_6$-MBPs monitored by *m/z* 432 (#1-4, #3 and # 4 co-eluted), four Br_2Cl_5-MBPs monitored by *m/z* 476 (#5-8) and four Br_3Cl_4-MBPs monitored by *m/z* 520 (#9-12, #10 and #11 co-eluted).

I)

REFERENCES

(1) Eibler, D.; Krüger, S.; Skírnisson, K.; Vetter, W. Combined thin layer chromatography and gas chromatography with mass spectrometric analysis of lipid classes and fatty acids in malnourished polar bears (*Ursus maritimus*) which swam to Iceland. *J. Chromatogr. B.* **2017**, *1046,* 138–146.

(2) Matissek, R.; Steiner, G.; Fischer, M. *Lebensmittelanalytik;* Springer Spektrum: Berlin, Heidelberg, **2014.**

(3) Hale, M. B.; Brown, T. Fatty acids and lipid classes of three underutilized species and changes due to canning. *Mar. Fish. Rev.* **1983**, *45*, 45–48.

(4) Vetter, W.; Weichbrodt, M.; Hummert, K.; Glotz, D.; Luckas, B. Combined microwave-assisted extraction and gel permeation chromatography for the determination of chlorinated hydrocarbons in seal blubber and cod livers. *Chemosphere.* **1998**, *37*, 2439–2449.

(5) Specht, W.; Tilkes, M. Gas-chromatographische Bestimmung von Rückständen an Pflanzenbehandlungsmitteln nach Clean-up über Gel-Chromatographie und Mini-Kieselgel-Säulen-Chromatographie. *Fres. Z. Anal. Chem.* **1980**, *301*, 300-307.

(6) Bendig, P.; Vetter, W. Improved GC/ECNI-MS sensitivity of decabromodiphenyl ether determination on 30 m columns by increasing the carrier gas flow after a modified gel permeation chromatographic cleanup protocol employing cyclohexane/ethyl acetate as eluent. *Int. J. Environ. An. Ch.* **2013**, *93*, 1019–1029.

(7) van Leeuwen, S P J; Boer, J. de Advances in the gas chromatographic determination of persistent organic pollutants in the aquatic environment. *J. Chromatogr. A.* **2008**, *1186,* 161–182.

(8) Boer, J. de; Allchin, C.; Law, R.; Zegers, B.; Boon, J. P. Method for the analysis of polybrominated diphenylethers in sediments and biota. *Trac-Trend Anal. Chem.* **2001**, *20,* 591–599.

(9) Steinwandter, H.; Schlüter, H. Beiträge zur Verwendung von Kieselgel in der Pesticidanalytik. *Z. Anal. Chem. (Fresenius' Zeitschrift für Analytische Chemie).* **1977**, *286,* 90–94.

(10) Rashid, U.; Anwar, F.; Moser, B. R.; Ashraf, S. Production of sunflower oil methyl esters by optimized alkali-catalyzed methanolysis. *Biomass Bioenerg.* **2008**, *32,* 1202–1205.

(11) Indarti, E.; Majid, M. I. A.; Hashim, R.; Chong, A. Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. *J. Food Compos. Anal.* **2005**, *18,* 161–170.

(12) Otto, M. *Analytische Chemie*, 4th ed.; Wiley-VCH: Weinheim, **2014.**

(13) Hardy R.; Keay J. N. Seasonal variations in the chemical composition of Cornish mackerel, Scomber scombrus (L), with detailed reference to the lipids. *Int. J. Food Sci. Tech.* **1972**, *7,* 125–137.

(14) Gámez-Meza, N.; Higuera-Ciapara, I.; La Calderon de Barca, A. M.; Vázquez-Moreno, L.; Noriega-Rodríguez, J.; Angulo-Guerrero, O. Seasonal variation in the fatty acid composition and quality of sardine oil from *sardinops sagax caeruleus* of the gulf of California. *Lipids.* **1999**, *34*, 639–642.

(15) Bandarra N. M.; Batista I.; Nunes M. L.; Empis J. M.; Christie W. W. Seasonal Changes in Lipid Composition of Sardine (Sardina pilchardus). *J. Food Sci.* **1997**, *62*, 40–42.

(16) FAO Fisheries & Aquaculture - Species Fact Sheets - Sardinops ocellatus (Pappe, 1854).

(17) Wu, Q.; Bouwman, H.; Uren, R. C.; van der Lingen, C. d.; Vetter, W. Halogenated natural products and anthropogenic persistent organic pollutants in chokka squid (*Loligo reynaudii*) from three sites along the South Atlantic and Indian Ocean coasts of South Africa. *Environ. Pollut.* **2019**, *255*, 113282.
(18) Hisamichi, Y.; Haraguchi, K.; Endo, T. Levels of mercury and organochlorine compounds and stable isotope ratios in three tuna species taken from different regions of Japan. *Environ. Sci. Technol.* **2010**, *44*, 5971–5978.
(19) Update of the monitoring of levels of dioxins and PCBs in food and feed. *EFSA.* **2012**, *10*, 1-82.
(20) United States Environmental Protection Agency (U.S.EPA). IRIS (the Integrated Risk Information System). https://www.epa.gov/iris.