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Fingerprinting polychlorinated biphenyls (PCBs) in environmental samples using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry

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Abstract

A GCxGC-TOFMS installed with a Rtx-PCB (60 m x 0.18 mm x 0.18 µm) in the first dimension and Rxi-17 (1.5 m x 0.1 mm x 0.1 µm) column in the second dimension was used to separate 188 out of 209 congeners. A further 12 congeners were identified through additional data processing resulting in the identification of a total of 200 congeners. However, caution is advised if these 12 congeners were to be used in quantitative assessments. The remaining 9 co-eluting congeners were three doublets (CB65 + CB62, CB160 + CB163 and CB201 + CB204) and one triplet (CB20 + CB21 + CB33). This method was tested on five Aroclors and resulted in the separation of all congeners present in the heavier Aroclor mixtures A1254 and A1260. The suitability of this method for applications in biological matrices was demonstrated on extracted whiting and guillemot liver samples which resulted in the identification of 137 individual PCBs in the whiting liver sample and 120 in the guillemot sample. Fingerprinting was able to show clear differences in the PCB signature of the two animals. This highlights the potential of this method for PCB fingerprinting in environmental forensics studies and other assessments that require congener specific analysis.

Key words
Polychlorinated biphenyls, Comprehensive two-dimensional gas chromatography, Time of flight mass spectrometry, Aroclor

Highlights

188 out of 209 PCBs separated for quantitation in one analytical run

200 out of 209 PCBs identified in one analytical run

All PCBs separated in Aroclors A1254 and A1260

137 individual congeners identified in a whiting liver sample

The results highlight the potential of this method for PCB fingerprinting

1. Introduction

Polychlorinated biphenyls (PCBs) were first discovered in environmental samples in 1966 [1,2]. However, due to their structural similarity, the separation of all 209 PCBs still presents a significant analytical challenge. In order to achieve positive identification and accurate quantification, all target compounds must be sufficiently resolved. In toxicological studies it is important to separate the World Health Organisation 12 dioxin like PCBs (WHO12) [3]. However, in environmental forensics studies a greater number of congeners often need to be separated to identify processes such as microbial degradation [4], volatilisation [5] and biotransformation in humans [6]. Having the ability to separate and identify specific PCB congeners is essential in environmental studies that require PCB fingerprinting.

Early analytical techniques such as EPA methods 8082 and 608 focused on calculating total PCB concentrations as spills to the environment occurred from technical mixtures such as Aroclors rather than from individual congeners. As more was understood about PCBs it became ever more important to identify and quantify individual PCB congeners. In 1997 Frame [7] documented the retention times and elution orders of all 209 congeners on 20 GC columns which significantly improved the ability of analytical chemists to confidently identify and quantify PCBs in an unknown sample. However, no single GC column is currently able to separate all 209 congeners and so it was necessary to manage datasets with co-elutions or analyse samples on multiple GC columns. The development of comprehensive two-dimensional gas chromatography can significantly increase peak capacity and therefore potentially remove the need to undertake repeat sample injections on multiple GC columns [8]. The demand for congener specific analysis lead to the development of EPA method 1668c which states that over 180 individual congeners can be separated using a dual column system.

Recently PCB analysis using GCxGC-TOFMS has been used to separate more than 190 individual PCB congeners along with simultaneous identification of other organohalogenated contaminants [9,10]. Separation of 198 PCBs was undertaken by Harju et al. [11] in 2003 using GC x GC-μECD equipped with a 60 m DB-XLB in the first dimension (^{1}D) and 2.25 m

BPX-70 in the second dimension (^{2}D). This resulted in co-elutions of one triplet and four doublets. No co-elutions were recorded for either the 12 'dioxin like' congeners (WHO12) or European indicator PCBs (EC7), in addition the run time was up to 4 hours long. Separation of 194 congeners was reported by Focant et al. [12] in 2004 using GCxGC-TOFMS equipped with a 60 m DB-XLB in ^{1}D and 2.5 m BPX-50 in ^{2}D; However this resulted in co-elutions of one triplet and six doublets. One co-elution was recorded for a WHO12 congener (CB123) and two co-elutions were recorded for the EC7 congeners (CB101 and CB153). Separation of 196 congeners was reported by Zapadlo et al. [13] in 2011 using GC x GC-TOFMS equipped with a 30 m SPB-Octyl in ^{1}D and 1.8 m SLB-IL59 in ^{2}D. However this resulted in co-elutions of one triplet and five doublets. No co-elutions were recorded for the WHO12 congeners and two co-elutions were recorded for the EC7 congeners (CB101 and CB153).

GCxGC-TOFMS has therefore been shown to be a powerful technique for resolving PCB congeners. Several papers have been published on PCB separation using a variety of column combinations [9-13]; however none of these studies investigated the use of the recently developed Rtx-PCB column (commercially available since 2005). This paper reports the separation achieved using a PCB specific low polarity column in ^{1}D with a stationary phase which is predominantly dimethylpolysiloxane (Rtx-PCB), coupled with a mid-polarity column in ^{2}D with a stationary phase which is 50% diphenyl, 50% dimethyl polysiloxane (Rxi-17). This method was tested on the five most common Aroclor solutions [14] and its application to biological matrices was demonstrated through analysis of extracted liver samples taken from a whiting obtained from the English Channel and a guillemot recovered from the coast of Great Britain.

2. Materials and methods

2.1. Chemicals, standards and samples

Individual PCB congeners were identified using nine PCB congener standard calibration mixtures (CS1 to CS9; AccuStandard) containing 10 µg mL^{-1} of each PCB in 1 mL of isooctane. Approximately 50 µL of CS1 – CS9 were combined to produce a solution containing all 209 congeners (209 PCB solution). Five certified PCB technical mixtures (99% purity) were obtained at a concentration of 1000 µg mL^{-1} including; Aroclor 1016, Aroclor 1254 and Aroclor 1260 (Fisher Chemicals), Aroclor 1242 and Aroclor 1248 (Greyhound Chromatography). Prior to analysis, all Aroclors were made up to concentrations of 10 mg L^{-1} through serial dilution with hexane (for dioxins, furans and PCBs; Sigma-Aldrich). One whiting and one guillemot liver sample were prepared using established techniques for extraction of organic biomarkers in marine tissue reported by Brown et al. [15]. Briefly this involved; freeze drying and grinding samples into a powder before sonication and extraction with dichloromethane/methanol. Extracts were filtered, dried and re-suspended in hexane and the non-polar fraction separated by column chromatography (SiO_2). Samples were evaporated to dryness and reconstituted with 10 µL of hexane prior to analysis.

2.2 GCxGC-TOFMS analysis and data processing

Samples were analysed on a time-of-flight mass spectrometer, (Leco, St. Joseph, MI Pegasus 4D) coupled to a two dimensional gas chromatograph (Agilent Technologies 7890A) equipped with a thermal modulator (Leco, St. Joseph, MI). The gas chromatograph was installed with a Rtx-PCB (60 m x 0.18 mm x 0.18 µm) ^{1}D column and a Rxi-17 (1.5 m x 0.1 mm x 0.1 µm) ^{2}D column. One µL of sample was injected in splitless mode, analytical blanks were run with each batch of up to 10 samples and the same 209 PCB mix solution was analysed in triplicate.

The GC inlet temperature was set at 280 ºC with a helium carrier gas flow rate of 1 mL min^{-1} and a purge time of 120 s at a flow rate of 20 mL min^{-1}. The primary oven temperature was initially set at 90 ºC for 1 min, then increased to 150 ºC at 30 ºC min^{-1}, then to 300 ˚C at 1 ˚C min^{-1}. The secondary oven temperature was set at 20 ˚C higher than the primary oven temperature. The modulator temperature was also set at 20 ˚C higher than the primary oven temperature, the modulation period was 3 s with a hot-pulse duration of 700 ms and the cooling time was 800 ms. The transfer line and detector temperature was set at 300 ˚C. The mass spectrometer was operated with a source ionisation energy of 70 eV, detector voltage of 1800 V and the data acquisition rate of 100 spectra s^{-1} for 100 – 550 Daltons.

The run time for each sample was 153 min. All data files were processed using ChromaTOF software. The processing method was set to identify 10,000 peaks with a signal-to-noise ratio of greater than 10:1. Throughout this paper PCBs are referred to using the Guitart et al. [16]numbering system.

2.3 Quality control procedures

Analytical blanks were run with each batch of approximately 10 samples. All samples were spiked with a $^{13}C_{12}$ internal standard. Standard mixtures were analysed in triplicate to check that separation was consistently achieved. Standard solutions in decreasing concentrations were analysed to establish the limit of detection for individual congeners; the lowest concentration of an individual congener that could be consistently detected was 1 pg µL^{-1}.

3. Results and discussion

3.1. Identification of all 209 congeners

Calibration solutions CS1 to CS9 were run individually and retention times were compared with literature values on elution order produced by Leco Corporation [17] to identify each congener. Figure 1 shows the two-dimensional retention times for all 209 PCB congeners, grouped by their degree of chlorination.

<<Figure 1>> Plotted peak apexes representing the two-dimensional retention times for all 209 PCB congeners

Analysis of the 209 PCB solution resulted in the separation of 188 individual PCBs with nine doublets and one triplet that could not be resolved by comprehensive two dimensional chromatography with mass spectrometric detection. Co-eluting PCBs were investigated to see

if they could be identified by further data interpretation. This included comparing slices of the one dimensional chromatogram as well as spectral deconvolution which was undertaken using the ChromaTOF software. The results of which are presented in the supplementary material. Using further data interpretation, six of the co-eluting doublets were identified in all three triplicate samples (CB4 + CB10, CB67 + CB58, CB95 + CB88, CB84 + CB89, CB101 + CB90 and CB175 + CB182). This resulted in the identification of a total of 200 of the 209 PCBs in the mixture (Table 1). Although the separation was sufficient to allow for each congener to be visually identified, quantification of these congeners would need to be undertaken using further data analysis. There were no isobaric overlaps between PCBs and fragments of higher homologues -2Cl (e.g. hexa-CB fragment -2Cl and tetra-CB). However, there were several co-elutions within higher homologous series -1Cl (e.g. hexa-CB fragment -1Cl and penta-CB). These should also be considered when undertaking quantitative analysis.

<<Table 1>> Elution order and retention times of all 209 congeners

When including the congeners that were resolved by further data interpretation, no co-elutions were recorded that involved either the WHO12 or EC7 congeners. Figure 2 presents the nine co-eluting doublets and one triplet that could not be separated by comprehensive two-dimensional chromatography, along with their percentage abundances in the five major Aroclors [18].

<<Figure 2>> Summary of congeners that could not be resolved by chromatography are presented as contour plots along with their documented retention times and percentage abundance in the five main Aroclors.

The co-elutions from this study are presented in Table 2 along with co-elutions for various column combinations reported by other authors. This is intended to help future researchers decide on the column combination that would best suit their needs.

<<Table 2>>

3.2. Identification of PCBs in 5 Aroclors

PCBs were produced as commercial mixtures such as Aroclors. During the manufacturing process there are several congeners that do not have favourable chlorine substitution patterns, such as PCBs with one heavily chlorinated biphenyl and one un-chlorinated biphenyl [18]. These PCBs are therefore unlikely to be present in detectable concentrations in environmental and animal tissue samples. Analysis of the five most common Aroclors (A1016, A1242, A1248, A1254 and A1260) was undertaken to identify co-elutions in these commercial mixtures. Results were compared with the database of PCBs in Aroclors compiled by Frame [18] which was based on data from Frame et al. [19]. All PCBs present in the heavier Aroclor mixtures A1254 and A1260 were identified, along with 113 of the 115 congeners in A1248 (co-elutions of CB88 + CB95), 96 of the 99 congeners in A1242 (co-elutions of CB20 + CB21 + CB33) and 63 of the 66 congeners in A1016 (co-elutions of CB20 + CB21 + CB33).

As with the 209 PCB solution, this method was unable to separate CB33 + CB20 + CB21 in any Aroclor. However, the sample of A1248 did not contain detectable concentrations of CB20 which allowed CB33 to be resolved from CB21.

3.3. Identification of PCBs in animal tissue sample

The application of this method to biological matrices was demonstrated through analysis of extracted liver samples taken from a whiting and guillemot. These samples were used as an example of the complex matrices that are often experienced in environmental forensics investigations. This method identified 137 individual PCBs in the whiting liver, with a further 18 tentatively identified with a signal-to-noise ratio <10. This method also identified 120 PCBs in the guillemot liver with a further 11 tentatively identified with a signal-to-noise ratio <10. The same 112 PCBs were positively identified in both samples. The relative proportions of the peak areas of these 112 congeners clearly showed a different PCB signature in each animal (Figure 3). This highlights the potential of this method for PCB fingerprinting in environmental forensics studies.

<<Figure 3>> PCB signature in whiting and Guillemot liver. One hundred and twelve PCBs were resolved by GCxGC-TOFMS in both samples. PCBs are arranged by elution order on the first dimension column (Rtx-PCB 60 m x 0.18 mm x 0.18 µm)

Several other persistent organic pollutants (POPs) were also identified in the same tissue samples by library matching against the NIST database, including DDE, chlordane, hexachlorobenzene, Mirex®, nonachlor and heptaclor isomers, indicating that this method could also be used as a broader screening tool. p'p'-DDE co-eluted with CB154, but these compounds could be distinguished by their different mass spectra. In both samples, a peak was detected for the co-elutions of CB20 + CB21 + CB33, CB88 + CB95, CB89 + CB84, CB90 + CB101, CB182 + CB175 and CB201 + CB204. Spectral deconvolution was able to separate CB20 + CB33, CB89 + CB84, CB90 + CB101, and CB182 + CB175 in the whiting liver and CB89 + CB84, CB90 + CB101 and CB182 + CB175 in the guillemot liver. CB163 could not be consistently resolved from CB160 in the 209 PCB mix analysed in triplicate. However, CB160 did not appear to be present at detectable concentrations (1 pg μL^{-1}) in the biological samples and therefore the peak was recorded as CB163.

The ability of spectral deconvolution to separate co-eluting congeners varied between the different samples and therefore caution is advised when undertaking quantitative analysis of these congeners as it may be better to report them as co-eluting congeners. The spectra for congeners from the same level of chlorination are very similar; however it has been shown that by using a combination of retention time and ion ratio information it is possible to confidently identify individual congeners [17].

4. Conclusions

One hundred and eighty eight of the 209 PCB congeners were separated using a Leco Pegasus 4D GCxGC-TOFMS installed with a Rtx-PCB (60 m x 0.18 mm x 0.18 µm) in the first dimension and Rxi-17 (1.5 m x 0.1 mm x 0.1 µm) in the second dimension A further 12 congeners were identified through additional data processing, resulting in the identification of

a total of 200 congeners. However, caution is advised if these 12 congener are used for quantitative assessments. The only congeners that could not be resolved were CB30 + CB20 + CB21, CB65 + CB62, CB160 + CB163, CB201 + CB204. This method was tested on five Aroclors and its suitability for application to biological matrices was demonstrated on extracted whiting and guillemot liver samples. All PCBs present in the heavier Aroclor mixtures A1254 and A1260 were separated. One hundred and thirty seven individual PCBs were positively identified in a whiting liver sample which had a distinctly different signature when compared with a guillemot liver sample. This highlights the potential of this method for PCB fingerprinting in environmental forensics studies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere. 2014.04.061.

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Tables

Table 1. Elution order and retention times of all 209 congeners recorded using a Leco Pegasus 4D GCxGC-TOFMS equipped with a 60 m Rtx-PCB and 1.5 m Rxi-MS. Congeners in bold are present at >1% in the five main Aroclors, congeners in italics are present at <0.01% in the five main Aroclors. WHO12 congeners are marked with *, EC7 congeners are marked with $^{+}$

Represents co-eluting congeners

Represents congeners separated by further processing

PCB	Chlorine position	^{1}D Retention Time (min)	^{2}D Retention Time (s)	PCB	Chlorine position	^{1}D Retention Time (min)	^{2}D Retention Time (s)
	Mono-chlorobiphenyls				Penta-chlorobiphenyls cont.		
1	2	28.35	1.64	*120*	*245-3'5'*	*87.1*	*2.12*
2	3	34.75	1.67	**97**	**245-2'3'**	**87.45**	**2.22**
3	4	36.2	1.67	*116*	*23456*	*88.5*	*2.22*
	Di-chlorobiphenyls			**87**	**234-2'5'**	**89.3**	**2.21**
4	**2-2'**	**37.45**	**1.91**	*111*	*235-3'5'*	*89.8*	*1.89*
10	26	37.55	1.87	117	2356-4'	89.85	2.14
9	25	42.35	1.81	115	2346-4'	90.05	2.16
7	24	42.65	1.79	85	234-2'4'	90.25	2.23
6	2-3'	43.9	1.88	109	235-3'4'	90.95	1.92
5	23	45.2	1.95	**110**	**236-3'4'**	**91.15**	**2.22**
8	**2-4'**	**45.7**	**1.88**	82	234-2'3'	92.9	2.38
14	*35*	*48.55*	*1.75*	124	345-2'5'	95.25	2.05
11	*3-3'*	*52.55*	*1.84*	*108*	*2346-3'*	*95.95*	*2.08*
12	34	53.65	1.89	123*	345-2'4'	96.15	2.11
13	3-4'	54.6	1.85	*107*	*234-3'5'*	*96.3*	*2.07*
15	4-4'	56.75	1.85	*106*	*2345-3'*	*96.55*	*2.12*
	Tri-chlorobiphenyls			**118*$^{+}$**	**245-3'4'**	**97.3**	**2.08**
19	26-2'	48	2.12	122	345-2'3'	98.45	2.24
30	*246*	*49.75*	*1.88*	114*	2345-4'	99.25	2.17
18	**25-2'**	**52.2**	**2.01**	**105***	**234-3'4'**	**102.3**	**2.25**
17	**24-2'**	**52.8**	**1.99**	*127*	*345-3'5'*	*103.45*	*1.92*
27	26-3'	53.9	2.05	*126**	*345-3'4'*	*109.65*	*2.1*
24	236	54.6	2.04		Hexa-chlorobiphenyls		
16	**23-2'**	**55.6**	**2.16**	*155*	*246-2'4'6'*	*80.35*	*2.11*
32	26-4'	56.25	2.05	*150*	*236-2'4'6'*	*84.95*	*2.25*
34	35-2'	57.55	1.91	*152*	*2356-2'6'*	*85.9*	*2.35*
23	*235*	*58*	*1.92*	*145*	*2346-2'6'*	*87.15*	*2.36*
29	245	58.65	1.94	*148*	*235-2'4'6'*	*88*	*2.09*
26	25-3'	60.3	1.92	154	245-2'4'6'	89.5	2.11
25	24-3'	60.85	1.94	136	236-2'3'6'	89.6	2.38
31	**25-4'**	**62.55**	**1.93**	151	2356-2'5'	92.35	2.17
28^{+}	**24-4'**	**63.1**	**1.94**	135	235-2'3'6'	92.7	2.22
33	**34-2'**	**63.3**	**2.06**	144	2346-2'5'	93.25	2.18
21	*234*	*63.35*	*2.09*	147	2356-2'4'	94.05	2.21
20	23-3'	63.5	2.08	149	236-2'4'5'	94.15	2.25
22	**23-4'**	**65.75**	**2.09**	*143*	*2345-2'6'*	*94.6*	*2.37*
36	*35-3'*	*67.5*	*1.82*	139	2346-2'4'	94.75	2.22
39	*35-4'*	*69.95*	*1.86*	*140*	*234-2'4'6'*	*95.2*	*2.25*
38	*345*	*70.15*	*1.98*	134	2356-2'3'	96.4	2.32
35	34-3'	73.25	1.98	*142*	*23456-2'*	*96.7*	*2.36*
37	34-4'	75.75	1.99	133	235-2'3'5'	97	2.06

PCB	Chlorine position	^{1}D Retention Time (min)	^{2}D Retention Time (s)
	Tetra-chlorobiphenyls		
54	*26-2'6'*	*57.85*	*2.3*
50	*246-2'*	*60.1*	*2.06*
53	25-2'6'	62.3	2.15
51	24-2'6'	63.45	2.15
45	236-2'	65.05	2.22
46	23-2'6'	66.1	2.3
73	*26-3'5'*	*66.35*	*2.03*
69	*246-3'*	*67.15*	*1.98*
43	235-2'	67.35	2.09
52$^+$	**25-2'5'**	**67.9**	**2**
48	245-2'	68.25	2.1
49	**24-2'5'**	**68.75**	**2.02**
47	24-2'4'	69.55	2.02
65	*2356*	*69.55*	*2.08*
62	*2346*	*69.65*	*2.08*
75	246-4'	69.85	1.99
44	**23-2'5'**	**71.4**	**2.16**
59	236-3'	72.05	2.12
42	23-2'4'	72.25	2.17
71	26-3'4'	72.95	2.19
41	234-2'	73.5	2.25
*72**	*25-3'5'*	*74.2*	*1.87*
64	236-4'	74.85	2.14
68	*24-3'5'*	*75*	*1.91*
40	23-2'3'	75.05	2.32
57	235-3'	76.5	1.97
58	*23-3'5'*	*77.4*	*2.04*
67	245-3'	77.4	1.99
61	*2345*	*78.2*	*2.12*
76	345-2'	78.75	2.13
63	235-4'	79.1	2.01
74	245-4'	79.9	2.01
70	**25-3'4'**	**80.4**	**2.03**
66	**24-3'4'**	**81.15**	**2.06**
80	*35-3'5'*	*82.15*	*1.77*
55	234-3'	82.35	2.13
56	**23-3'4'**	**83.65**	**2.2**
60	234-4'	84.85	2.16
79	*34-3'5'*	*88.2*	*1.92*
78	*345-3'*	*89.4*	*2.01*
*81**	*345-4'*	*92.1*	*2.05*
77	34-3'4'	94.2	2.08
	Penta-chlorobiphenyls		
104	*246-2'6'*	*69.2*	*2.21*
96	236-2'6'	73.9	2.36
103	246-2'5'	74.55	2.06
100	*246-2'4'*	*75.9*	*2.06*
94	235-2'6'	76.15	2.2
102	245-2'6'	77.5	2.22
98	*246-2'3'*	*78.4*	*2.2*
93	2356-2'	78.7	2.23
88	2346-2'	79.3	2.23
95	236-2'5'	79.35	2.2
121	*246-3'5'*	*79.45*	*1.93*
91	236-2'4'	80.8	2.21
92	235-2'5'	82.8	2.04
89	234-2'6'	83.25	2.36
131	2346-2'3'	97.15	2.35
165	*2356-3'5'*	*98*	*2.02*
146	235-2'4'5'	98.3	2.08
161	*2346-3'5'*	*98.75*	*2.04*
168	*246-3'4'5'*	*99.35*	*2.12*
153$^+$	**245-2'4'5'**	**99.55**	**2.1**
132	**234-2'3'6'**	**100**	**2.4**
141	2345-2'5'	101.8	2.18
137	2345-2'4'	102.9	2.24
130	234-2'3'5'	103.8	2.24
164	236-3'4'5'	103.95	2.25
138$^+$	**234-2'4'5'**	**104.9**	**2.26**
160	*23456-3'*	*105.15*	*2.22*
163	2356-3'4'	105.2	2.2
129	2345-2'3'	105.35	2.37
158	2346-3'4'	105.75	2.22
166	23456-4'	108.25	2.28
159	*2345-3'5'*	*109.25*	*2.02*
162	*235-3'4'5'*	*110.2*	*2.06*
128	234-2'3'4'	110.3	2.43
167*	245-3'4'5'	111.45	2.08
156*	2345-3'4'	115.75	2.22
157*	234-3'4'5'	116.4	2.27
*169**	*345-3'4'5'*	*124*	*2.09*
	Hepta-chlorobiphenyls		
188	*2356-2'4'6'*	*96.3*	*2.24*
184	*2346-2'4'6'*	*97.65*	*2.25*
179	2356-2'3'6'	100.8	2.36
176	2346-2'3'6'	102.2	2.38
186	*23456-2'6'*	*102.95*	*2.47*
178	2356-2'3'5'	104.85	2.18
182	*2345-2'4'6'*	*105.9*	*2.25*
175	2346-2'3'5'	105.95	2.21
187	2356-2'4'5'	106.45	2.22
183	2346-2'4'5'	107.5	2.24
185	23456-2'5'	109.8	2.27
174	2345-2'3'6'	110.45	2.38
181	*23456-2'4'*	*111.45*	*2.34*
177	2356-2'3'4'	112.4	2.38
171	2346-2'3'4'	113.25	2.4
173	23456-2'3'	113.7	2.46
172	2345-2'3'5'	115.3	2.22
192	*23456-3'5'*	*115.85*	*2.12*
180	**2345-2'4'5'**	**116.7**	**2.22**
193	2356-3'4'5'	117	2.21
191	2346-3'4'5'	117.85	2.24
170	2345-2'3'4'	122.05	2.42
190	23456-3'4'	122.95	2.33
189*	2345-3'4'5'	128.9	2.21
	Octa-chlorobiphenyls		
202	2356-2'3'5'6'	111.2	2.32
201	2346-2'3'5'6'	112.7	2.36
204	*23456-2'4'6'*	*112.7*	*2.36*
197	2346-2'3'4'6'	114.15	2.39
200	23456-2'3'6'	117.1	2.49
198	23456-2'3'5'	121.6	2.29
199	2345-2'3'5'6'	121.85	2.34
196	2345-2'3'4'6'	123.05	2.37

PCB	Chlorine position	^{1}D Retention Time (min)	^{2}D Retention Time (s)	PCB	Chlorine position	^{1}D Retention Time (min)	^{2}D Retention Time (s)
84	236-2'3'	83.35	2.34	203	23456-2'4'5'	123.3	2.33
90	*235-2'4'*	*83.9*	*2.08*	195	23456-2'3'4'	129.05	2.52
101^{+}	**245-2'5'**	**83.95**	**2.05**	194	2345-2'3'4'5'	132.9	2.36
113	*236-3'5'*	*84.2*	*2.05*	205	23456-3'4'5'	134.15	2.33
99	**245-2'4'**	**84.9**	**2.1**		Nona-chlorobiphenyls		
119	246-3'4'	86.3	2.09	208	23456-2'3'5'6'	126.8	2.46
83	235-2'3'	86.45	2.2	207	23456-2'3'4'6'	128.4	2.49
125	345-2'6'	86.6	2.24	206	23456-2'3'4'5'	137.95	2.47
112	*2356-3'*	*86.7*	*2.09*		Deca-chlorobiphenyls		
86	2345-2'	86.8	2.27	209	23456-2'3'4'5'6'	141.85	2.6

Table 2. Comparison of co-eluting congeners in different column combinations

	Megson et al. 2013 (this study)	**Harju et al. 2003**	**Focant et al. 2004**	**Zapadlo et al. 2011**
Column set up	^{1}D - 60m Rtx-PCB ^{2}D - 1.5m Rxi-17	^{1}D - 60m DB-XLB ^{2}D - 2.25m BPX-70	^{1}D - 60m DB-XLB ^{2}D - 2.5m BPX-50	^{1}D - 30m SPB-Octyl ^{2}D - SLB-IL59
Co-elutions	CB 4/10[a] CB 20/21/30 CB 65/62 CB 58/67[a] CB 88/95[a] CB 84/89[a] CB 90/101[a] CB160/163 CB 175/182[a] CB 201/204	CB 47/62/65 CB 42/59 CB 86/112 CB 106/109 CB 175/182	CB 20/21/33 CB 66/155[b] CB 77/144[b] CB 84/89 CB 90/101 CB 107/123 CB 153/168	CB 12/13 CB 62/75 CB 70/76 CB 90/101/113 CB 97/125 CB 153/168
Total number of PCBs resolved	188/209	198/209	194/209	196/209

[a] These congeners could be identified manually . However caution is advised when undertaking quantitative analysis.

[b] Co-elution between tetra-CB and Hexa-CB -2Cl fragment.

Figures

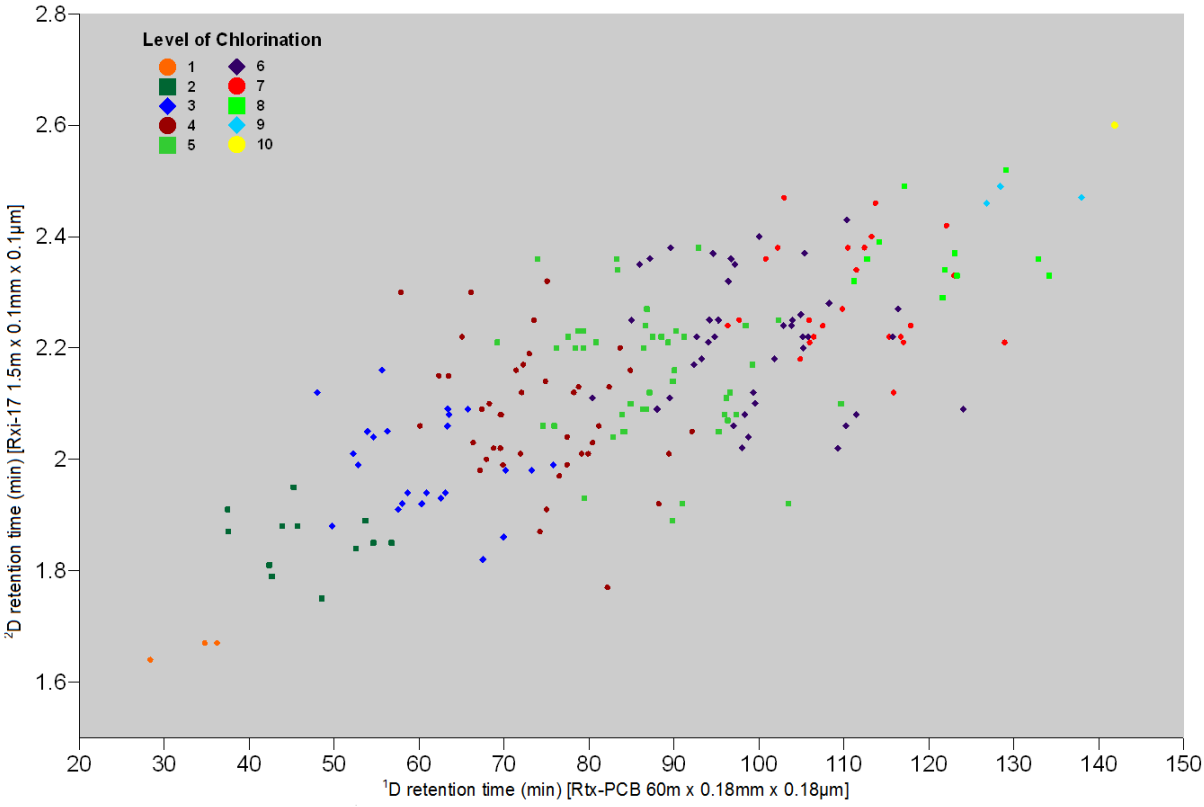
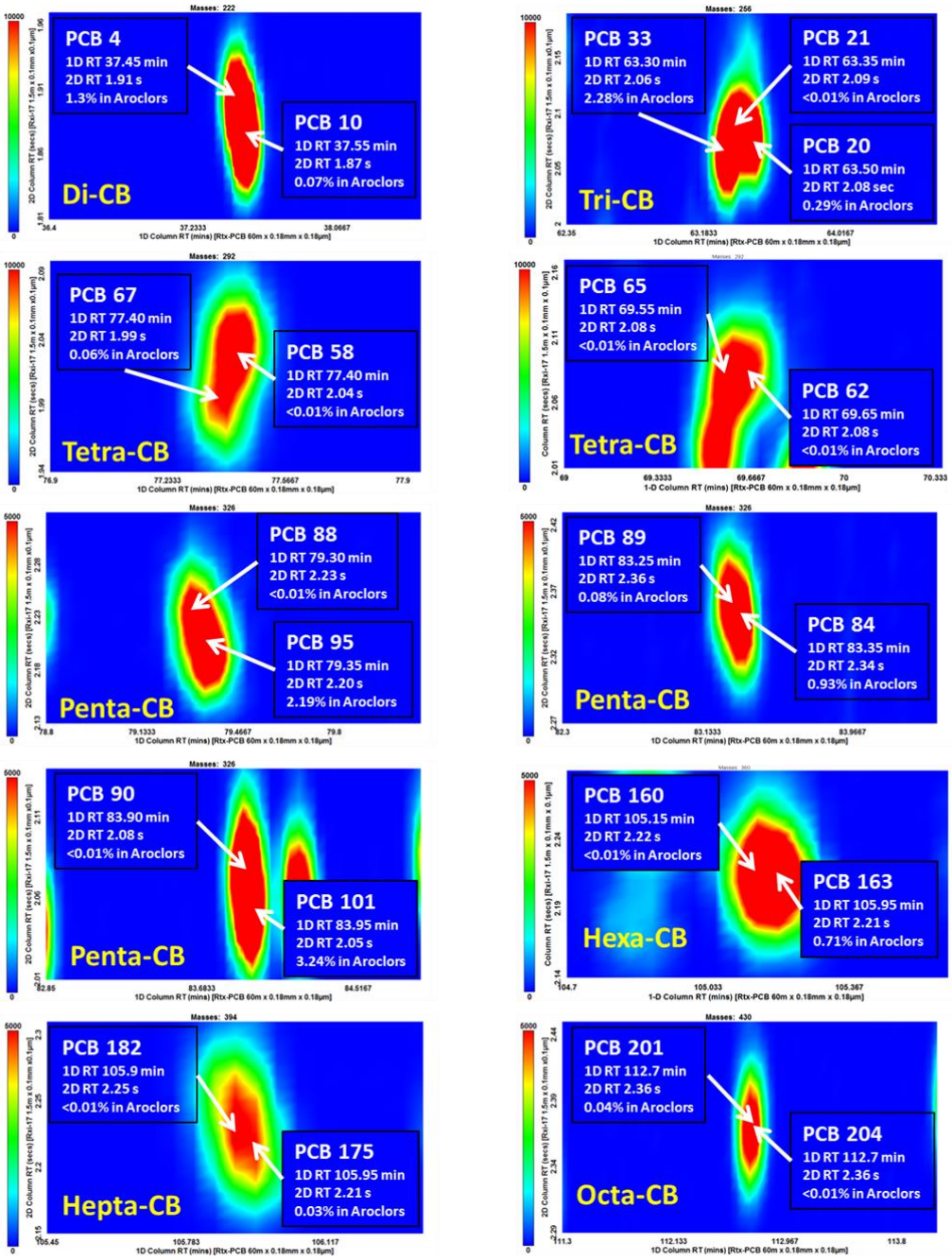


Figure 1. Plotted peak apexes representing the two-dimensional retention times for all 209 PCB congeners



	Mono-CB	Di-CB	Tri-CB	Tetra-CB	Penta-CB	Hexa-CB	Hepta-CB	Octa-CB	Nona-CB	Deca-CB	Total
Number of Congeners	3	12	24	42	46	42	24	12	3	1	209
Peaks identified by GCxGC-TOFMS	3	11	22	40	43	41	23	11	3	1	198
Co-elutions of PCB (B/Z number) on GCxGC-TOFMS	none	4[5]/10[5]	20/21/33	65/62 67[5]/58[5]	88[5]/95[5] 89[5]/84[5] 90[5]/101[5]	160/163	182[5]/175[5]	201/204	none	none	10

[5] Congeners separated by further data interpretation

Figure 2. Summary of congeners that could not be resolved by chromatography are presented as contour plots along with their documented retention times and percentage abundance in the five main Aroclors.

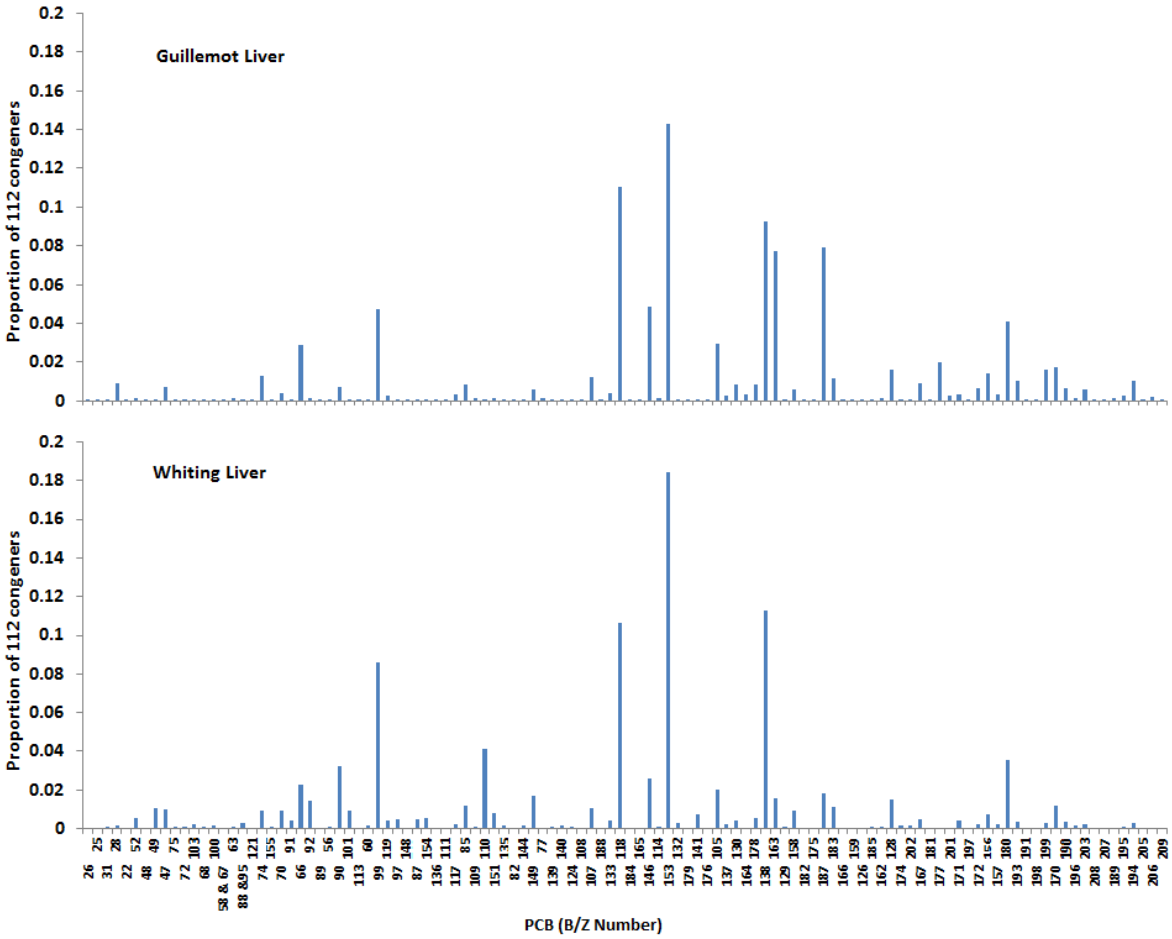


Figure 3. PCB signature in whiting and guillemot liver. One hundred and twelve PCBs were resolved by GCxGC-TOFMS in both samples. PCBs are arranged by elution order on the first dimension column (Rtx-PCB 60 m x 0.18 mm x 0.18 µm).