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NOVEL GC SEPARATION CHARACTERISTICS FOR 209 PCB CONGENERS – THE HT8-PCB COLUMN REVISITED

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Introduction

Polychlorinated Biphenyls (PCBs) are known for long as being toxic with different toxicological action modes of PCBs, comprising dioxin-like (dl-) and non-dioxin-like (ndl-) effects, depending on the individual chlorine substitution scheme. This has recently been addressed e.g. by Californian legislative Proposition 65[1], and by generally being recognised as human carcinogens by the IARC[2] leading to the demand of precisely analysing the total PCB content in terms of determining all 209 possible congeners with sufficient quality.
Since the 1990s, GC separation of PCB congeners has been improved, especially with respect to different chromatographic phases used for separation. The recognition that there are major issues with overall performance of "single run methods" (e.g. separation problems on DB5-type columns for PCBs #28/ #31 and #138/#16x) led to further developments in this field of work. One rather new type of structural backbones have been GC columns on carborane base, replacing the arylen moiety of e.g. a 5ms-type column by m-carborane cages. In the time between 2010 and 2014, we made observations regarding different separation characteristics for individuals of this column type whilst analysing the total of all possible PCBs. This has been identified to be a problem with synthesis of the stationary phase at that time in use due to cessation of chemical production of the original m-carborane phase and its chemical re-development. Our aim to maintain a high-class one-column-separation of PCBs brought us to accompany this process and to investigate the HT8-PCB properties as it is present today. The actual state of the applicability of this GC phase for analysis of all 209 PCBs is presented in this work.

Materials and methods

The work was carried out using an Agilent 6890N GC (coupled with an Waters Micromass Autospec Ultima sector field HRMS. Standards were provided by AccuStandard, New Haven, USA. Tests have been performed on GC columns of the type HT8-PCB (Trajan Scientific and Medical, Australia, former SGE) with the dimensions 60m * 0.25 mm i.d (no information about film thickness available) in the form as it has been available since begin of 2015. Injection was performed in splitless mode with 2 µL volume. Carrier gas was Helium at constant flow with a flow rate of 1,2 mL min-1. The temperature programme was as follows: 120°C @1.5 min; 20°C/min to 180°C; 2°C/min to 260°C; 5°C/min to 300 °C / 8 min. The general method and approach for analysing marine biota has been described elsewhere[3].

Results and discussion:

The focus for the present work has been put upon GC separation. The HT8-PCB column in its present form shows similar good separation properties as the formerly used versions. The physicochemical properties and polarity appear to be similar in comparison, even if the total number of separations decreased slightly. Some separations are unique, notably the possibility to completely separate the important marker PCBs #138 and #180 from other PCBs of the same chlorination degrees.

The column has been examined for its separation characteristics especially in order to have the best possible separation for a broad variety of samples, e.g. biota samples in a single-run analysis. The most important PCBs to quantify are the 12 dioxin-like PCBs (#77, #81, #105, #114, #118, #123, #126, #156, #157, #167, #169, #189) as well as the 6 indicator-PCBs (#28, #52, #101, #138, #153, #180). Most of these are separable from all other congeners as well as sufficiently separated from PCBs of a lower chlorination degree, therefore analysable under standard MS conditions and resolution. Critical points for performance may be PCB #52, which is affected by co-elution of #69, #105 with close elution of #127 and #118 with close elution of #106. The importance of these critical separations will depend on the congener patterns of analysed samples. Other critical points might arise from possible proximity of fragment ions of close eluting PCB congeners of higher chlorination degrees. Care has to be taken especially regarding analysis of TetraCB #81 in presence of PentaCB #120 and analysis of TetraCB #77 in presence of HexaCB #144.

With the chosen setup, a maximum separation/reporting of 178 congeners (as distinguishable peak tops) is possible with 33 congeners completely in co-elution and not being separable at all and further 13 congeners in almost complete co-elution, thus for practical purposes not usable. 136 congeners were separated as individual congeners at minimum 50% valley and 43 congeners partially overlapping to 50-90% with other congeners, thus being amenable to approximative quantification under favourable circumstances. For reporting purposes, quantification was narrowed down to 166 separations for reasons of method robustness and constant data quality. It is proposed to use a criterion of 50% valley for robust separations on long term use of the column, in order to maintain a consistent reporting of co-elutions.

The column performance has been examined using 3 individual columns in its present form reflecting the latest "production version" of the as it has been delivered since around begin 2015. The results mark the begin of the commercial availability of the HT8-PCB carborane phase in its present form. For the given data, virtually no differences in separation between the 3 individual columns could be observed proving stability of the stationary phase.
For purpose of a stable analysis of biota samples under long-term conditions, a couple of separations were considered as co-elutions even if a separation of down to 50 % would have been feasible on an unused column.
Investigation of column robustness is still ongoing. Up to now, the presented method has been applied over a time range of 9 months to over 650 samples – mainly biota being fish and fish oil which have passed a single column without the proposed GC separation criteria (166 separations, peaks >= 50 % valley not considered separated) being compromised in spite of a certain typical general performance deterioration observed under such heavy workload, where we observed that, depending on different structures, the single co-elutions suffered to a different extent.

Regarding quality, a specific pair of GC separation criteria has been established in order to asses chromatographic quality of the column. For this reason, two closely eluting but not too far overlapping pairs of peaks have been selected as GC performance markers, based on the procedures of US EPA method 1668C[4] which also make use 2 peak pairs for this purpose. As first pair PentaCBs #110/#120 were used for mid-early eluting compounds and the second pair being HeptaCBs #174/#181 as relatively late eluting compounds. The choice for congeners of the Penta- and HeptaCBs has been influenced by the fact that the typical distribution of PCB congeners encountered in biota samples would maximize between Penta- and HeptaCBs. For the last eluting PCB (#209, DecaCB), it has been decided to let DecaCB elute after > 50 minutes of runtime. Having the unique separation characteristics of the column as it performs in our method, it seemed useless to adapt to the 55 minutes of minimal runtime which US EPA1668C method requests for use of a SPB-octyl column. Anyway, it has to be doubted whether the use of a fixed retention time without any further guidelines on getting to it can be considered as a "quality criterion" in itself.
The main results are shown in **figure 1** where separation for all PCB congener groups are shown for a standard solution of approx. 5 pg μL^{-1} as well as in **table 1** where separations, separation degrees as well as example retention times are listed.
As a conclusion, the present version of the HT8-PCB column proves to be as suitable as the previous version regarding separation efficiency as well as stability. It has anyway to be remarked that some single separations are not as good as on the original column. But the advantages of separating e.g. PCBs #138 and #180 completely whilst maintaing most of the other separations of dl- and ndl-PCBs prevail.

Figure 1: PCB congeners as separated on a HT8-PCB column (2015 version
Table 1: PCB separation on a HT8-PCB column (2015 version) Remarks-column: Percentage = overlap with closest eluting neighbour, (-) = no separation, (!)=almost no separation

References:
1. The Safe Drinking Water and Toxic Enforcement Act of 1986 = California Proposition 65 (1986)
2. B. Lauby-Secretan et.al.: The Lancet Oncology, Volume 14, Issue 4, Pages 287 - 288, April 2013;
3. Neugebauer F., Ast C., Paepke O.: Organohalogen Compounds 74, 28-31 (2012)
4. US EPA Method 1668C Chlorinated Biphenyl Congeners in Water, Soil,Sediment, Biosolids, and Tissue by HRGC/HRMS, April 2010

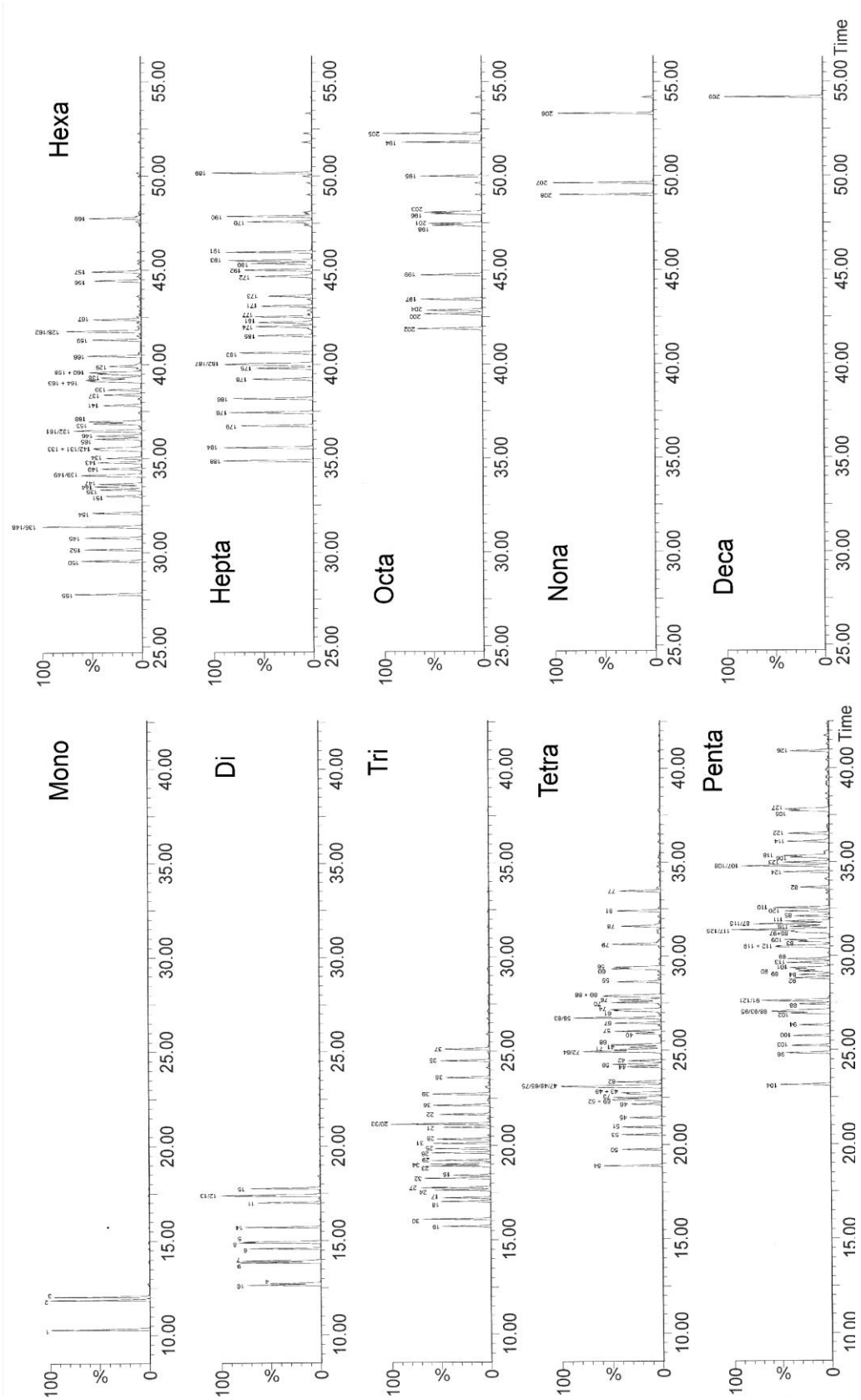

Figure 1: PCB congeners as separated on a HT8-PCB column (2015 version

Cl	PCB #	RT	Remarks	Cl	PCB #	RT	Remarks	Cl	PCB #	RT	Remarks	Cl	PCB #	RT	Remarks
Mo	1	10,30		Tr	38	23,57		Pe	97 (+#86) (+#117/#125)	31,24	(!); 90%; (-)	Hx	163 (+#164)	39,08	90%
Mo	2	11,86		Te	44	24,09		Hx	136/148	31,29	(---)	Hp	178	39,12	
Mo	3	12,03		Te	59	24,22		Pe	117/125 (+#86/#97)	31,33	(---); 90%	Hx	138	39,21	
Di	10	12,62		Te	42	24,41		Pe	116	31,49		Hx	160 (+#158)	39,41	90%
Di	4	12,71		Tr	35	24,46		Te	78	31,54		Hx	158 (+#160)	39,49	90%
Di	9	13,82		Pe	96	24,80		Pe	87/115	31,63	(---)	Hp	175	39,71	
Di	7	13,91		Te	72/64 (+#71)	24,90	(---); 80%	Pe	111	31,78		Hx	129	39,82	
Di	6	14,56		Te	71 (+#72/#64)	24,99	80%; (---)	Hx	154	32,02		Hp	182/187	39,92	(---)
Di	8 (+#5)	14,86	90%	Tr	37	25,07		Pe	85	32,05		Hx	166	40,37	
Di	5 (+#8)	14,91	90%	Te	41	25,12		Pe	120	32,31		Hp	183	40,52	
Di	14	15,68		Pe	103	25,18		Te	81	32,35		Pe	126	40,85	
Tr	19	15,70		Te	68	25,26		Pe	110	32,50		Hx	159	41,22	
Tr	30	16,08		Pe	100	25,71		Hx	151	32,93		Hp	185	41,43	
Di	11	16,98		Te	40	25,86		Hx	135	33,26		Hx	128/162	41,67	(---)
Tr	18	17,01		Te	57	25,98		Te	77	33,41		Oc	202	41,80	
Tr	17	17,21		Pe	94	26,28		Hx	144	33,42		Hp	174	41,92	
Di	Dez 13	17,36	(---)	Te	67	26,41		Hx	147	33,56		Hp	181	42,13	
Tr	24	17,61		Te	58/63	26,69	(---)	Pe	82	33,58		Hx	167	42,30	
Di	15	17,73		Pe	102 (+#98/#93#95)	26,89	70%	Hx	139/149	34,02	(---)	Hp	177	42,44	
Tr	27	17,74		Pe	98/93/95 (+#102)	27,00	70%	Hx	140	34,38		Oc	200	42,58	
Tr	32	18,24		Te	61 (+#74)	27,07	90%	Pe	124	34,41		Oc	204	42,77	
Tr	16	18,39		Te	74 (+#61)	27,12	90%	Hx	143	34,71		Hp	171	43,00	
Te	54	18,85		Pe	88	27,39		Pe	107/108	34,72	(---)	Oc	197	43,36	
Tr	23	18,89		Te	70	27,50		Hp	188	34,79		Hp	173	43,53	
Tr	34	18,99		Pe	91/121	27,57	(---)	Pe	123	34,92		Hx	156	44,35	
Tr	29	19,18		Te	76	27,64		Hx	134	34,95		Hp	172	44,58	
Tr	26	19,58		Hx	155	27,72		Pe	106 (+#118)	35,19	80%	Oc	199	44,65	
Te	50	19,72		Te	80 (+#66)	27,82	(!)	Pe	118 (+#106)	35,27	80%	Hx	157	44,83	
Tr	25	19,80		Te	66 (+#80)	27,86	(!)	Hx	(!) 142 (+#131)(+#133)	35,37	(!); 70%	Hp	192	44,92	
Tr	31	20,09		Te	55	28,59		Hx	(!) 131 (+#142)(+#133)	35,42	(!); 70%	Hp	180	45,27	
Tr	28	20,32		Pe	92	28,77		Hx	(!) 133 (+#142)(+#131)	35,48	90%	Hp	193	45,42	
Te	53	20,50		Pe	84	28,94		Hp	184	35,49		Hp	191	45,87	
Te	51	20,90		Pe	89 (+#90+#101)	29,13	50%	Hx	165	35,97		Oc	198 (+#201)	47,29	50%
Tr	21	20,94		Pe	90 (+#89+#101)	29,22	50%/70%	Pe	114	36,03		Oc	201 (+#198)	47,39	50%
Tr	20/33	21,12	(---)	Te	60 (+#56)	29,28	70%	Hx	146	36,13		Hp	170	47,48	
Te	45	21,40		Pe	101 (+#89+#90)	29,31	70%	Hx	132/161	36,40	(---)	Hx	169	47,67	
Tr	22	21,62		Te	56 (+#60)	29,36	70%	Pe	122	36,46		Hp	190	47,77	
Tr	36	22,11		Hx	150	29,48		Hp	179	36,65		Oc	196 (+#203)	47,91	70%
Te	46	22,12		Pe	113	29,59		Hx	153 (+#168)	36,78	50%	Oc	203 (+#196)	47,99	70%
Te	69 (+#52)	22,33	(!)	Pe	99	29,78		Hx	168 (+#153)	36,89	50%	No	208	48,92	
Te	52 (+#69)	22,37	(!)	Hx	152	30,09		Hp	176	37,36		No	207	49,54	
Te	73	22,47		Pe	112 (+#119)	30,42	(!)	Pe	105 (+#127)	37,67	80%	Oc	195	49,91	
Te	43 (+#49)	22,66	(!)	Pe	119 (+#112)	30,47	(!)	Hx	141	37,75		Hp	189	50,09	
Tr	39	22,70		Te	79	30,59		Pe	127 (+#105)	37,76	80%	Oc	194	51,72	
Te	49 (+#43)	22,71	(!)	Hx	145	30,70		Hp	186	38,10		Oc	205	52,18	
Te	47/48/65/75	23,07	(---)	Pe	83 (+#109)	30,70	80%	Hx	137	38,30		No	206	53,24	
Pe	104	23,12		Pe	109 (+#83)	30,79	80%	Hx	130	38,58		Dc	209	54,09	
Te	62	23,29		Pe	86 (+#97) (+#117/#125)	31,17	(!); 90%; (-)	Hx	164 (+#163)	39,02	90%				

Table 1: PCB separation on a HT8-PCB column (2015 version) Remarks-column: Percentage = overlap with closest eluting neighbour, (-) = no separation, (!)=almost no separation