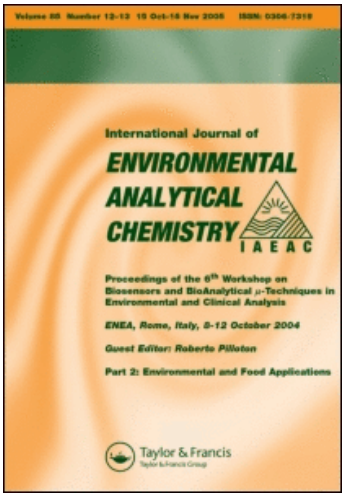

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Improving analytical confidence in the determination of PCBs in complex matrices by a sequential GC-MS/MS approach

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A method is described for achieving increased confidence in the selective determination of PCBs using capillary gas-liquid chromatography with tandem mass spectrometry (GC-MS/MS). It is well known that quantitation of PCBs by MS is susceptible to a false positive interference that arises from the co-elution of a higher PCB homolog with a lower PCB homolog (i.e., M-Cl^+ from a higher homolog is M^+ for a lower homolog). Because the elution order of the PCB congeners is not exactly proportional to increasing Cl content, frequent switching from MS/MS windows for specific homologs must take place. This approach can yield significant errors when matrix-induced retention time shifts occur. We therefore explored an alternative approach that involves repetitive analyses of a single extract. We developed and optimized a method that requires three injections, with homolog classes sequentially monitored as: 1-4-7-10, 2-5-8, and 3-6-9, respectively. The sequential design of the method entails the use of separate, broad MS/MS windows for each homolog class, thereby minimizing adverse matrix effects on retention variability. However, a consequent tripling of overall analysis time is incurred for each sample. The homolog classes are determined with high confidence (99%) that overlapping higher homolog fragments do not interfere with the quantitation of lower homologs. The method was demonstrated for extracts in small samples (~750–1000 mg) from seven different freshwater biota species (n = 20) to illustrate a wide range of matrix-induced shifts. Application of the method resulted in more accurate quantitation, correcting an average 5.3% relative error (false positive bias) in observed concentration.

Keywords: polychlorinated biphenyls; quantitation; gas chromatography; mass spectrometry; congener coelution; biological tissue

1. Introduction

Polychlorinated biphenyls (PCBs) continue to be a well-studied class of semi-volatile organic contaminants (SVOCs) because of their widespread distribution, environmental persistence, tendency to bioaccumulate, and potential health effects [1–3]. Analytical methods that can accurately identify and quantify all 209 congeners are of interest, particularly for certain types of toxicological studies [4–7]. Of the wide range of analytical techniques that have been studied for PCBs, gas-liquid chromatography (GC) has been shown to provide the highest level of separation efficiency [1,8–14]. However,

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a comprehensive review of GC methods for PCBs has shown that resolving all 209 congeners continues to remain an elusive goal [15–17]. Co-elution is a problem that has plagued PCB and similar multi-congener semi-volatile organic (SVOC) compound analyses since their measurement began in the mid 1960s [18,19].

We recently reported the observation of matrix-induced retention time shifts in several unusual biota sample types during the determination of PCB congeners by GC-MS/MS [20]. Unlike standards and "predatory" species (e.g., yellow perch, lake whitefish, and lake trout) that we studied, we found an increased (random) variability in the precision (as per cent relative standard deviation) of the retention times (up to four-fold) during the elution of "co-planar" PCBs (as defined by the World Health Organization [1]) in species that were "exotic" to Lake Michigan (including alewife, bloater chub, and slimy sculpin). In our previous work [20], we measured the imprecision present in the retention times of 533 chromatographic peaks and found that while predatory fish (perch, whitefish, and lake trout) had a low average variation (0.24% average RSD), the variation for organisms in lower trophic levels (zooplankton, alewife, chub, deepwater sculpin, and slimy sculpin) was significantly greater (1.1% average RSD). We surmised that the standard method we applied was not "robust" for the invasive species, and we speculated that this arose because – unlike the filets used from predatory fish – the "lower" organism samples were completely homogenized prior to extraction. Thus we suspected that the extraction method which had been optimized for predatory fish filets (i.e., skeletal muscle and adipose tissue) was not optimal for internal organ tissues. Interestingly, the imprecision in retention was highest for alewife, which also has the distinction of being the only species that we sampled that has adapted to freshwater from a marine origin. Matrix-induced retention time shifts coupled to the variability intrinsic to any measurement (e.g., as a result of instrument drift, routine maintenance, seasonal changes, analyst bias, etc.) serve only to further complicate the accurate interpretation of these chromatograms [21].

In the absence of ideal chromatographic efficiency, computational approaches have been studied to try to provide congener-specific information for SVOCs such as PCBs. Chemometric techniques that have been explored for distinguishing among the constituents of a complex mixture include those based upon the use of mathematical methods such as Partial Least Squares Analysis and Fourier Spectral Deconvolution [22,26]. A recent example described by Zeng and co-workers used "two product ion fragments generated from a parent ion associated with the (co-eluting) isomers for quantitation" [27]. In their approach, congener-specific identification of co-eluting isomers focused on the use of an internal standard and relative response factors for each homolog class. The result was the development of a model that approximated the relative contributions from each co-eluting isomer, despite the specific nature of the analyte's composition and/or conformation. Given that fragmentation during EI ionization is a function of congener conformation and gas-phase kinetics, the application of chemometric techniques that treat all congeners within the same homolog class as identical will be less than ideal.

Several empirical approaches have been reported to address shortcomings in chromatographic efficiency and thereby provide congener-specific information. Experimentally, both multi-dimensional separation and detection approaches have been reported. The most successful separation methods are those based upon comprehensive two-dimensional GC ($GC \times GC$) [15,28–31]. However, quantitation models based on specific retention time windows in $GC \times GC$ can be difficult to apply because of the marked increase in the overall variation of analyte retention that is often encountered [32].

To address this issue, Pierce and co-workers have recently demonstrated that a ''retention time alignment algorithm'' can be effectively applied for data preservation in each chromatographic dimension [33]. Nevertheless, the GC × GC approach has proven difficult to implement in laboratories that concentrate on samples from many different sources because of the aforementioned problem with matrix-induced variation in retention [27,34]. A more popular approach has been the use of multi-dimensional detection approaches in which a selective detector is applied to gain an additional dimension of information [35]. Detectors for GC that are most commonly used for PCB quantitation are the electron capture detector (ECD) and the mass spectrometer (MS). The hallmark of the ECD is its remarkable sensitivity, although a limited dynamic range, interferences from other ECD-sensitive analytes, and the limited qualitative information provided by retention time continue to be drawbacks [36]. Although MS is at a disadvantage relative to the ECD in terms of sensitivity, much better qualitative information is provided in the mass spectrum – parent ion and daughter ion fragmentation patterns as well as characteristic chlorine isotope ratio patterns. Further selectivity is provided in consecutive fragmentation experiments or MS^n experiments, though the higher homolog interference can still be problematic in MS/MS [1].

In MS/MS, frequent switching among mass-to-charge (m/z) windows for specific homologs must take place because the elution order of the 209 PCB congeners is not exactly proportional to increasing chlorine content. For example, given the elution order of the congeners on the highest efficiency GC columns that are commercially available, one would have to switch between homolog m/z segments >75 times during a single chromatographic run. Increased analytical confidence may be gained by repetitive analyses of a single extract as a means to minimize the monitoring of m/z windows for the parent ions of each homolog class, albeit at the expense of increased analysis time. In addition, programming frequent changes in the m/z windows is further complicated by random changes in retention time as demonstrated by our previous work. Such shifts would mean it is not possible to ensure the desired homolog would appear in the programmed segment and may contribute to misidentification in congener-specific analysis. As pointed out by Cochran and Frame [16], extensive calibration curves, non-linear calibration functions, and relative response factors are not reliable methods for assessing retention time variability in a chromatogram. The most rigorous strategy would be to use the Method of Standard Addition for 209 deuterated PCB standards, but this would be impractical because of the high cost of these standards as well as the labour-intensive calibration and data reduction that would be required. If just 10 deuterated PCBs were used for each homolog class, one would find that the retention shifts within a given homolog class are not always uniform [20]. Therefore, in light of these concerns, in the work presented herein we describe the development and optimization of a method in which a series of consecutive injections of a given sample extract are made to achieve high confidence that co-eluting PCBs are quantified selectively. The method is designed to be comprehensive in its ability to quantify each of the 209 PCB congeners with high confidence.

2. Method

2.1 *Standards and reagents*

All reagents were analytical reagent (AR) grade or better. Organic solvents were HPLC grade (Acros Organics, Morris Plains, NJ, USA). PCB standards and surrogates were

obtained from AccuStandard (New Haven, CT, USA) and internal standards were purchased from Supelco (Bellefonte, PA, USA). All glassware was acid-washed for at least 48 hours in 5% (v/v) AR-grade nitric acid to remove background contamination. Surrogates (tetrachloro-*m*-xylene and decaCB) and internal standards (2-fluorobiphenyl and acenaphthylene) were prepared in HPLC-grade n-hexane and stored in amber glass vials with Al-lined caps at 0°C. Standard reference material (SRM) 1946, ''Lake Superior Fish Tissue'', was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) and lyophilized prior to use. Solid-phase extraction (SPE) tubes were purchased from Supelco, including LC-Silica (5 g) and ENVI^{TM} Florisil (0.5 g).

2.2 *Biota sample preparation*

Tissue was homogenized in a blender, spread in a thin layer on a Pyrex Petri dish (9 cm diam.), and lyophilized under vacuum at −34°C. Drying time averaged between 2 and 4 days. Dried samples were immediately covered by pre-baked aluminum foil and stored in amber vials at <0°C.

2.3 *PCB extraction*

Lyophilized biological tissue samples (~1 g) were finely ground and transferred to a Greenchem^{TM} extraction vessel (CEM Corporation, Mathews, NC, USA). Latent water content was determined gravimetrically ($n=3$) in ~100 mg sub-samples; the target water content for microwave assisted extraction (MAE) was 15% (w/w). Samples containing less than the target water content were supplemented with hexane-washed reagent water. Following addition of 20 mL of 20:80 (v/v) acetone:hexane, the vessel was fortified with 2 µg of each surrogate compound. Prepared samples and a control vessel were placed in a MarsX^{TM} analytical microwave system (CEM Corp.) equipped with an ESP-1500 Plus Pressure Control System, Fiber Optic Temperature Control System, and Solvent Sensor. Extraction was performed with a 10 min temperature ramp to 115°C, a 10 min hold at the target temperature, and a ~45 min cool-down period. The extract was transferred to a 250 mL round bottom flask and evaporated to ~1 mL using a microscale rotary evaporator (VWR International, Batavia, IL, USA) at 22°C. The extract was then transferred to a conditioned LC-Silica SPE tube and eluted with n-pentane under gentle vacuum. The eluate was concentrated to ~0.5 mL using the rotary evaporator and transferred to a conditioned ENVI^{TM} Florisil SPE tube and eluted with n-pentane under gravity flow. The eluate was then transferred to a 25 mL round bottom flask and rotary evaporated to ~200 µL. The final extract was transferred to a glass vial and stored at 0°C.

2.4 *PCB determination*

Extracts from representative species of the various trophic levels ($n=20$) with relatively high concentrations of co-planar PCBs were chosen. Total PCB concentration in the samples was determined by gas chromatography-tandem mass spectrometry (GC-MS/MS) using a Saturn 2000 system (Varian, Walnut Creek, CA, USA), which consisted of the following components: Model 3800 gas chromatograph with Model 1079 split/splitless programmed temperature vaporization (PTV) injector, electron impact (EI) ionization

Table 1. Optimized method conditions for the GC-MS/MS method.

Chromatograph	
Sample Volume	7 μL
Injection Rate	0.5 μL/s
Split Ratio	100 : 1
Split Vent Closed	0.30–2.00 min
Split Vent Flow	10.0 mL/min
Column	HT8 (8% v/v phenyl); 0.22 mm × 50 m, 0.25 μm film thickness
Oven Program – A	Hold at 90°C for 2 min
Oven Program – B	Gradient at 4°C/min to 170°C
Oven Program – C	Gradient at 2°C/min to 300°C
Oven Program – D	Hold at 300°C for 8 min
Total run-time	95 min
Mass Spectrometer	
Mass Range	10–650 m/z
Filament Delay	17 min
Filament emission	10 μA
Ion Trap Temperature	220 °C
Axial Modulation	4.5 μA
Peak Threshold	0
Background Mass	125 μ
Mass Defect	(−)50 mmμ/100 μ
Target Ion Count	2000
MS/MS	
Mass Window	1 μ
Low/High Offset	4/4 DAC steps
Ejection RF	48 m/z
Ejection Amplitude	40 V
Isolation Time	5 ms
Broadband Amplitude	30 V
CID Time	5 ms
CID Bandwidth	0 kHz
Modulation Range	0DAC
Modulation Rate	5600 μs/DAC

source (+70 eV); quadrupole ion trap mass filter (10 to 650 m/z range, unit resolution). Separation was performed using a HT8 capillary column (50 m × 0.22 mm with 0.25 μm film; SGE, Austin, TX, USA). The mobile phase was ultrahigh-purity (99.999%) helium (Praxair, Milwaukee, WI, USA) at a constant flow rate (1.2 mL min^{-1}) maintained by electronic flow control.

The GC-MS/MS method is summarized in Table 1. Sample extracts were fortified with internal standard and brought to a volume of 1.0 mL using *n*-hexane and introduced to the GC by large-volume injection. The following sequence was optimal: a 1 μL air plug, 1 μL solvent plug, 7 μL sample plug, and a final 1 μL solvent plug. For programmed temperature vaporization (PTV), the initial temperature (90°C) was held for 18 s with a split ratio of 100:1. The split vent was closed for 1.7 min, the injector temperature was ramped to 310°C at a rate of 180°C min^{-1}, and at 2.0 min the split vent was re-opened (100:1). For the oven program, the temperature was held at 90°C

for 2.0 min and then increased at 4°C min^{-1} to 170°C followed by 2°C min^{-1} to 300°C, and finished with an 8 min hold at 300°C. Between each standard and unknown, *n*-hexane blanks were injected to determine the extent of carry-over.

Automated library searching was performed using the NIST Mass Spectral Database (vers. 3.0). Data acquisition and reduction were accomplished using Varian's Saturn software (vers. 5.5.2). Separate calibration models were built for each of the 10 homolog classes using a Dry Color Manufacturer's Assoc. (DCMA) set of standards; separate models for each homolog class were constructed at five levels (0, 0.1, 0.5, 1.0 and 2.0 $\mu g\,g^{-1}$) in triplicate. The final PCB concentrations determined in the biota samples (chromatographic peak area) were corrected by using the average recovery of surrogate standards normalized by the internal standards. The MAE GC-MS/MS method was validated by using NIST SRM 1946 "Lake Superior Lake Trout" ($n = 3$). The recovery for two of the quantifiable co-planar PCBs certified in this SRM (i.e., that were above instrumental detection limits) (PCB 105 and PCB 118) averaged $92.1 \pm 11.9\%$, which is within the certified range [20].

3. Results and discussion

3.1 *Method development*

The creation of false-positive (Type I) errors by the formation of lower PCB homologs during EI ionization is well-known [1]. This occurs because EI ionization produces molecular ions ("parent ions" denoted as M^+ in the mass spectrum) as well as lower m/z fragments derived from M^+ ("daughter ions"). All of the parent and daughter ions are trapped in the mass analyser, whether they are from the homolog of interest at that retention time or from fragments of a higher homolog that has lost one or more chlorine atoms that is eluting in that same retention window. The parent ion is then subjected to collision-induced dissociation (CID) and produces the daughter ions that are used for quantitation. Without correcting for the contribution from higher homologs that may have formed daughter ions during EI ionization, inaccurate quantitation of the congener of interest may occur for the lower homolog. In contrast, if a lower order congener concentration is significantly higher than a co-eluting higher homolog, the contribution made by the higher homolog is negligible. For example, a triCB can form diCB and monoCB daughters during EI ionization. During conventional GC-MS/MS of a sample containing a triCB, lower homologs are indeed observed (Figure 1). We investigated the extent of this type of EI-induced artifact formation on all 10 homolog classes (Figure 2). Even when applying the most efficient GC method for congener-specific separation [15], we observed that the co-elution of different homologs could result in ~22 instances of false-positive quantitation that involve ~49 congeners (Table 2). The random and increased variation in retention time that we observed for lower trophic species (i.e., up to four times that observed for predatory fishes) further increases the likelihood of this type of error [20].

To address this problem, tandem MS (MS/MS) methods have been developed for PCBs, albeit in simple matrices (e.g., water) [37]. Tandem MS can be successfully applied by the careful selection of only those daughter ions that do not overlap with fragments in the mass spectra of lower homologs [37]. However, the sensitivity of MS/MS markedly decreases because the judicious selection of non-overlapping daughter ions leads one to choose fragments of relatively low intensity. For low-mass samples from complex matrices, such as the whole-organism biological samples that we studied, both sensitivity

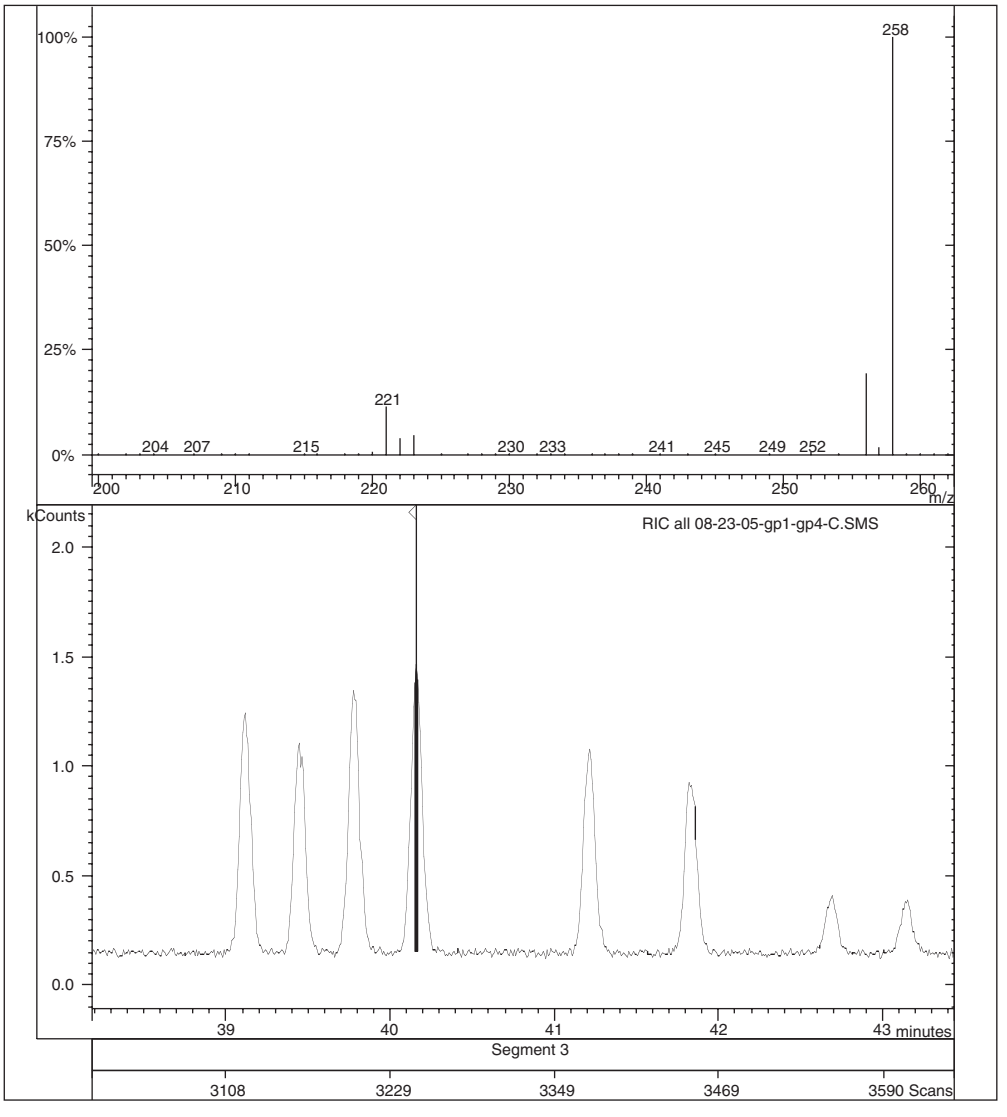

Figure 1. Example mass spectrum (top) and chromatogram (bottom) for a triCB (m/z 258) and the formation of a diCB daughter (m/z 221), creating a false positive error of ~15%.

and selectivity are of paramount importance. Chemometric methods have also been reported to address the higher homolog error, albeit using "simulated" data sets [23,24,38]. These methods assume that the congeners in each homolog class will fragment identically under all conditions. However, not only are there significant differences in EI mass spectra for congeners within a given homolog class, but also the unpredictable variability in the chromatographic retention process as a result of matrix effects can invalidate this approach.

The method we have developed consists of a *series* of injections of the *same* sample extract. While the increase in analysis time is certainly burdensome, the higher

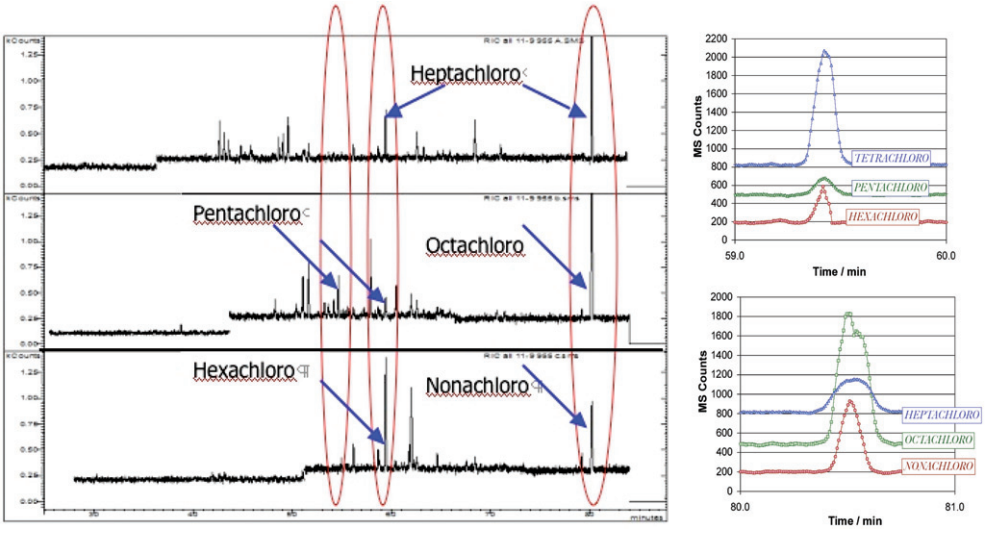

Figure 2. Comparison of overlap among several homolog classes. The formation of pentaCB and tetraCB artefacts from a hexaCB (top right) and octaCB and heptaCB artifacts from a nonaCB (bottom right) is shown.

confidence in the quality of the data obtained is the paramount consideration. The method we developed is similar, at least in principle, to ''multiple chromatographic fingerprinting'' that has been reported for other analytes [39]. Of course, a single injection of the extract to resolve the homolog classes sufficiently for accurate quantitation would be ideal – and given a repeatable and precise elution order for a given matrix, the homolog classes could be identified and quantified. However, overlap among homolog classes is extensive so frequent switching between homolog classes would be necessary. On the other hand, 10 separate injections would be the most conservative approach to provide the highest confidence (i.e., use of MS/MS for a single homolog class throughout each chromatogram). Clearly this is unacceptable from a time standpoint, and therefore we endeavored to create a method for which 99% confidence in avoiding homolog class overlap was realized – albeit with an overall increase in analysis time. We found that by using three injections, we could achieve this goal. The method is designed as follows (see Table 3):

(1) The first injection (''A'') isolates parent ions of the monoCBs, tetraCBs, heptaCBs, and decaCBs, thereby accounting for four consecutive MS/MS segments.
(2) For the second injection (''B''), three MS/MS segments are used – for diCBs, pentaCBs, octaCBs.
(3) The third injection (''C'') quantifies the remaining triCB, hexaCB, and nonaCBs.

The method is therefore referred to as the ''A-B-C'' sequential method. (Several other segments are included in each run, i.e., to delay electron-impact ionization until the solvent peak has eluted and to quantify the internal and surrogate standards.) It is important to note that the method developed based upon the ''higher homolog correction'' focuses only on the correction for the loss of a single chlorine atom and does not take into account losses of two or more chlorine atoms from co-eluting higher homologs. The error arising

Table 2. Comparison of homolog class elution order for development of the A-B-C method.

Elution order	IUPAC#	# of Cl	Elution order	IUPAC#	# of Cl
1	1	1	69	83	5
2	2	1	70	136	6
3	3	1	71	86	5
4	10	2	72	97	5
5	4	2	73	89	5
6	9	2	74	115	5
7	7	2	75	87	5
8	6	2	76	154	6
9	8 and 5	2 and 2	77	85	5
10	19	3	78	10 and 81	2,4
11	18	3	79	151	6
12	11	2	80	144	6
13	17	3	81	147	6
14	13	2	82	135	6
15	24	3	83	77/82	4,5
16	27	3	84	149	6
17	15	2	85	124	5
18	32	3	86	143	6
19	16	3	87	134/107/131	6,5,6
20	54	4	88	123	5
21	23	3	89	133	6
22	34	3	90	118	5
23	29	3	91	165	6
24	26	3	92	146/114	6,5
25	25	3	93	132/179	6,7
26	31	3	94	122	5
27	28/53	3 and 4	95	153	6
28	51	4	96	176	7
29	21	3	97	141	6
30	33/45/20	3/4/and 3	98	105	5
31	22	3	99	137	6
32	46	4	100	130	6
33	36	3	101	178	7
34	52/69	4 and 4	102	163	6
35	43	4	103	138	6
36	49	4	104	160	6
37	47/48/75	4, 4, and 4	105	158/175	6,7
38	44	4	106	187/182	7,7
39	59	4	107	183/129	7,6
40	42	4	108	126	5
41	35	3	109	185	7
42	64	4	110	159/202	6,8
43	71/103	4 and 5	111	174/128	7,6
44	41	4	112	177/201	7,8
45	37	3	113	167	6
46	68	4	114	171	7
47	100	5	115	197	8
48	40	4	116	173	7
49	57	4	117	200	8
50	67	4	118	156/172	6,7
51	63	4	119	157	6

(*Continued*)

Table 2. Continued.

Elution order	IUPAC#	# of Cl	Elution order	IUPAC#	# of Cl
52	102	5	120	180	7
53	95	5	121	193	7
54	74	4	122	191	7
55	121/155/91	5,6,5	123	198	8
56	70	4	124	199	8
57	80	4	125	170	7
58	66	4	126	190/196/203	7,8,8
59	96/55	5,4	127	169	6
60	84/92	5,5	128	208	9
61	125	5	129	207	9
62	90	5	130	195	8
63	101	5	131	189	7
64	60	4	132	194	8
65	56	4	133	205	8
66	152	6	134	206	9
67	99	5	135	209	10
68	119	5			

Table 3. Design of the MS/MS segments in the A-B-C method.

Method	Segment no.	Homolog	Begin (min)	End (min)
A	1	Mono	25.2	27.9
A	2	Tetra	39.1	54.3
A	3	Hepta	61.2	74.5
A	4	Deca	79.7	End of run
B	1	Di	28.8	35.9
B	2	Penta	46.2	63.0
B	3	Octa	67.5	77.7
C	1	Tri	33.4	44.7
C	2	Hexa	53.4	71.0
C	3	Nona	73.9	78.8

from the interference of higher homologs that have lost two (or more) chlorines would be of lesser magnitude given the general distribution of daughter ions formed during fragmentation.

To build the A-B-C method, we adjusted our method to include all 209 congeners based on data supplied by SGE, Inc. for the HT8 column. To verify the elution order, we compared the retention times for 55 standards to those reported by SGE (*results not shown*). The linear model describing the retention ($y = 0.9994x + 0.0292$; $R = 0.994$) was then used to establish retention time windows for all 209 congeners. This allowed us to identify specific segments that we could use for efficient division of the homolog classes (Figure 3). It is apparent when looking at two consecutive homolog classes in Figure 3,

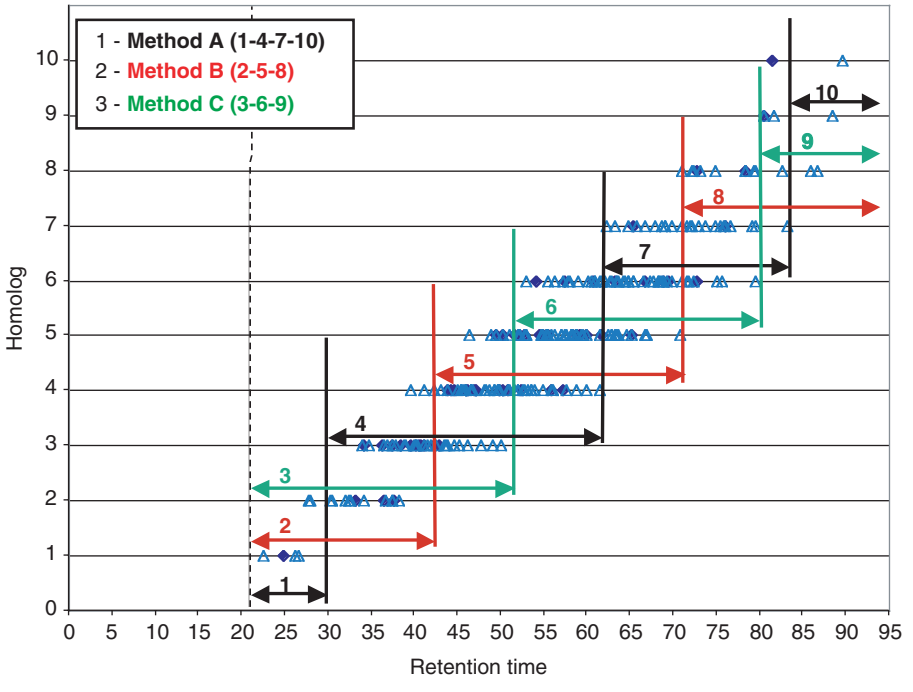

Figure 3. Design of the A-B-C method showing the three injections and the corresponding MS/MS segments used for homolog discrimination.

for instance the tetraCB and pentaCB classes, that there is significant overlap in the retention times of congeners in these classes. Further examination, particularly in the tetraCB to heptaCB regions of the chromatogram, indicated severe homolog overlap. Based on these observations, we found that at least three injections of the same extract (separated by at least two homolog classes) must be used to maximize the efficient separation (at the 99% confidence level, i.e., $3 \times \sigma$) of these classes by GC-MS/MS (Figure 3). Thus our approach does not eliminate the problem of the co-elution of homologs. Rather, it uses a straightforward correction when co-elution and variation in ionization efficiency might otherwise produce inaccurate results.

Another aspect of the A-B-C method that is important is the placing of more separation emphasis on the GC-MS/MS aspect of the method, rather than upon the extraction procedure. Consider that in addition to the silica gel and Florisil SPE steps that we used in our conventional "clean up" procedure, we explored several other "clean up" approaches for the lower trophic level species that employed a wide variety of stationary phase functionalities – but to no avail. That notwithstanding, the A-B-C method could compensate for matrix-induced retention variability, thereby making matrix-specific procedural modifications less necessary. It may be more efficient in the long run to repetitively analyse problematic ("dirtier") samples by using the conservative A-B-C approach rather than to devote time to the development and implementation of matrix-dependent alternative "clean up" procedures. To address the burdensome total analysis time, "Fast GC" methods show promise [40] – albeit implementation of the A-B-C approach using Fast GC methods would result in reduced confidence in correcting for

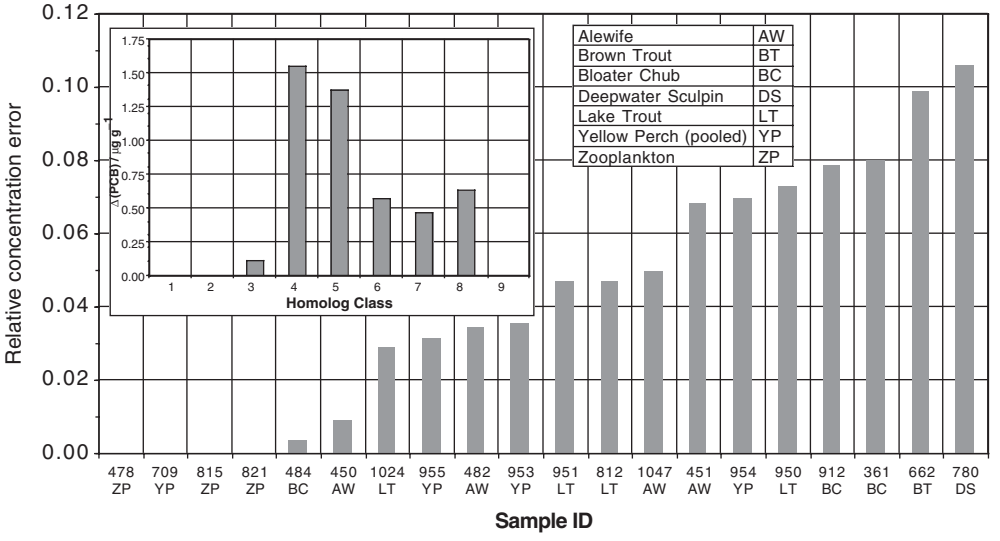

Figure 4. Application of the method to samples of Lake Michigan biota ($n = 20$).

Table 4. Average relative errors found in comparing the A-B-C method (“Corr”) with uncorrected data (“Raw”).

Homolog class	Raw (µg/g)	Corr (µg/g)	Change (µg/g)	%
Monochloro	0.015	0.015	0.000	0.0
Dichloro	0.101	0.101	0.000	0.0
Trichloro	0.017	0.011	−0.005	−2.2
Tetrachloro	0.596	0.519	−0.078	−7.7
Pentachloro	1.101	1.033	−0.069	−6.1
Hexachloro	0.704	0.675	−0.028	−2.1
Heptachloro	0.556	0.532	−0.023	−6.7
Octachloro	1.294	1.262	−0.031	−5.9
Nonachloro	0.254	0.254	0.000	0.0
Averages	*0.515*	*0.489*	*−0.026*	*−5.3*

higher homolog interferences because of the larger number of co-eluting congeners that are present.

3.2 *Method application*

Twenty extracts were selected from the archive ($n = 175$) of our recent study [20] based on the high co-planar PCB concentrations that were measured and their representativeness of the trophic structure in Lake Michigan. Sample extracts were brought to a

volume of 1.0 mL with reagent n-hexane immediately prior to GC-MS/MS. In Figure 4 (inset), we found that the predominant classes were tetraCBs and pentaCBs, with the hexa- through octaCBs present at moderate levels; the other homolog classes were low or negligible in concentration. These overall trends are not surprising given the typical distribution of homologs in Aroclor mixtures [1]. We suspect that the biased distribution in the tetraCB to octaCB classes that we observed in the uncorrected data may have been partially the result of the false-positive influence of "second" daughter ions, i.e., $(M\text{-}Cl_2)^+$ on the lower homologs. That is, the correction that we applied is only for the "first" daughter ion $(M\text{-}Cl)^+$ that is created by the ionization of a higher homolog. The A-B-C method could be expanded to include $(M\text{-}Cl_2)^+$ ions from higher homologs as a further refinement. For the 20 samples that we studied, the average error (false positive bias for the lower homolog) was 5.3% and the total error observed for the 20 samples was 31% (Table 4). It is important to note that, not surprisingly, the decaCB surrogate that we used interfered by forming artifactual nonaCBs, octaCBs, etc. – these data were ignored. The distribution relative to the different species that we studied is also presented in Figure 4. To understand the observed inter- and intra-species differences, we are presently studying correlations between the PCB data and factors that include habitat, food source, and lipid content.

4. Conclusions

The three-injection GC-MS/MS technique described herein is based on achieving 99% confidence ($3 \times \sigma$) that homolog overlap does not occur. Without this correction it is inevitable that false positive errors will be reported in the concentration of PCBs in complex environmental samples. Although the automated method throughput is low, inaccurate results obtained more quickly are of little value. In addition, the method does not require the application of a complicated chemometric scheme which assumes identical ionization reactivity throughout each homolog class. Furthermore, although chemical ionization (CI) would seem to be a logical alternative to EI to lessen the higher-homolog interference, fragmentation of M^+ to lower homologs can still be significant in CI and could thereby result in the same type of false positive interferences as observed in our study [38]. Modifications to the method that would permit higher throughput include: (a) the use of narrower retention windows for the homolog classes (i.e., at lower than 99% confidence); and (b) applying the method only to the most common homolog classes (e.g., tetraCB – octaCB congeners).

Further investigations are underway to refine the higher-homolog correction method by expanding it to account for interferences beyond adjacent homologs. Extension of the method to other SVOCs for improved confidence in preventing homolog overlap errors (e.g., dioxins, furans, toxaphenes, etc.) may also be worthy of examination.

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