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Advances in the Application of the Perchlorination Technique for the Quantitation and Confirmation of Polychlorinated Biphenyls (PCBs)

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During the course of development of a method for the analysis of ambient airborne PCB, it became apparent that it would be necessary to apply the perchlorination method of ARMOUR (1973) to quantitate the airborne PCB collected. Ambient airborne PCB at several of the locations sampled could not be closely matched to an Aroclor® pattern; hence, quantitation could not be accomplished without an exhaustive peak by peak identification of each species. The perchlorination of all PCB present to decachlorobiphenyl (DCB) greatly simplified quantitation and improved method detectability. Several problems in application of the Armour method had to be overcome, however, to achieve satisfactory results for airborne PCB. The method did not yield quantitative results when attempts were made to perchlorinate PCB species of less than tri-chloro substitution; and even for Aroclors, quantitative recovery was not achieved for less than 1 ug quantities of PCB. After rather extensive experimentation, and several modifications to the original Armour method, quantitative recovery was achieved for even the most volatile PCB species in sub-microgram quantities.

METHOD

The sample is extracted with hexane or other appropriate solvent, concentrated, and cleaned up using silica gel or Florisil as specified by routine PCB analytical procedures. Since the perchlorination reaction cannot be carried out in hexane, all residual hexane must be removed from the extract prior to perchlorination. Even small amounts of residual hexane will result in the formation of a black, solid residue upon the addition of the antimony pentachloride ($SbCl_5$). This severely reduces PCB recovery. The hexane is removed by azeotropic evaporation from a hexane/chloroform mixture. To the 1.0 ml extract in a Kuderna-Danish (K-D) apparatus, add 10 ml of chloroform. Concentrate by slow boiling to 4 ml. Repeat

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this azeotropic evaporation three additional times by adding 10 ml increments of fresh chloroform in order to remove all traces of hexane. Attempts to remove the hexane by direct evaporation without the use of the chloroform inevitably resulted in the volatile loss of dichlorobiphenyl and monochlorobiphenyl from test solutions.

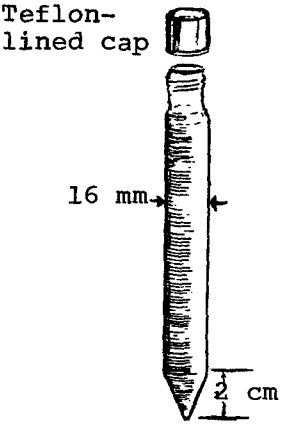

Figure 1. Perchlorination Reaction Vial

Quantitatively transfer the extract to a reaction vial, prepared as shown in Figure 1, using three chloroform rinses. The reaction vial is a 16 x 100 mm glass screw top culture tube with teflon-lined screw cap. The culture tube has been modified by drawing the bottom into a cone shape. By use of a reaction vial of this design, the analyst may achieve concentration of the solvent to 0.1 ml without going to dryness. Add two boiling chips and immerse the reaction vial upright in a 70°C water bath to a depth of 6 ± 2 cm. Increase the water bath temperature slowly until the solvent begins to boil at 72-76°C. Concentrate slowly to a volume of approximately 0.1 ml. Under no circumstances should the water bath temperature be permitted to exceed 76°C or the solvent will be evaporated to dryness. If either of these occurs, PCB will be lost by volatilization and consequent recoveries will be low. The final volume (0.1 ml) may be determined with sufficient accuracy by comparison of the solvent remaining with another reaction vial containing 0.1 ml of chloroform. Cap the reaction vial immediately and allow to cool.

To the concentrated sample extract in the reaction vial, add 0.2 ml of $SbCl_5$ and immediately re-seal the vial tightly with the teflon-lined screw cap. Reagent grade $SbCl_5$ from several commercial sources has been found to contain DCB and bromononachlorobiphenyl (BNCB), as reported by TROTTER and YOUNG (1975). The DCB contaminant results in a positive blank value, and the BNCB interferes with the perchlorination of the lower-substituted PCB species. The $SbCl_5$ selected for use must first be analyzed for these contaminants.

Place the reaction vial containing the sample into a preheated (160 ± 3°C) aluminum block heater for a period of 15 hours. It was found that the use of higher reaction temperatures resulted in poor recovery of

di- and mono-substituted PCB species. Apparently these species were not stable at the higher temperatures. Lower reaction temperatures also resulted in poor recovery due to incomplete conversion to DCB.

After the reaction period, remove the reaction vial from the heater and allow to cool to room temperature. Then cool in an ice bath. Cautiously vent pressure from the reaction vial in a fume hood, directed away from the analyst. Add 1 ml of 6 N HCl. Replace the cap tightly and shake for 30 seconds. The HCl inactivates the excess perchlorination reagent.

To extract the DCB from the reaction mixture, add 1 ml of hexane to the reaction vial, shake vigorously for 30 seconds, and carefully draw off the hexane layer with a disposable pipet. Place this hexane extract on the top of a 6 mm x 12 cm disposable pipet packed with 2 g of anhydrous Na_2SO_4. This column is prewashed with hexane. Repeat hexane extraction of the reactants in this manner five times to assure quantitative recovery of DCB. After passing all six increments of the hexane extract through the Na_2SO_4 column, pass two additional 1 ml portions of fresh hexane through the column. Collect all fractions in a 10 ml graduated K-D apparatus. Connect a modified micro-Snyder column to the K-D apparatus, add a single boiling chip, and evaporate in a water bath (70°C) to less than 0.5 ml. Add fresh hexane to bring the volume back to exactly 1.0 ml on the graduated K-D receiver. Cap the receiver tightly or transfer the contents to a sealed container to avoid evaporation of solvent prior to gas chromatographic analysis. Store in a cold, dark location.

Analyze and quantitate the DCB present in the perchlorinated extract by gas chromatography using an electron capture detector (GC/EC).

At PCB concentrations below 50 ng, correction should be made for a reagent blank value. Reagent blanks are generally less than 10 ng.

TEST RESULTS

Recovery studies of the modified perchlorination method described above yielded the results reported in Table 1 for a PCB test mixture and Aroclor 1016. This table shows quantitative recovery is achieved for these most volatile PCB species over the range of at least 100 ng to 10 ug.

TABLE 1

PERCHLORINATION RECOVERY VS. CONCENTRATION TEST RESULTS

PCB	PCB Concentration (ug)	Number of Replicates	Percent Recovery	Standard Deviation
Aroclor 1016	10.0	3	103	3.7
Aroclor 1016	5.0	3	105	2.1
Aroclor 1254	3.0	1	113	--
Test Mixture	1.18	3	98	6.5
Aroclor 1016	0.750	4	93	5.6
Test Mixture	0.412	2	101	1.1
Test Mixture	0.103	3	101	4.7

A ruggedness test (YOUDEN and STEINER 1975) was performed on the modified perchlorination method to assess the likelihood of achieving comparable interlaboratory results for PCB at low concentrations. The ruggedness test incorporated the seven factors listed in Table 2. These factors were varied from nominal (procedure specified) conditions to the degree that might be expected of a competent analyst following the written procedure. Each determination number consisted of triplicate analysis of a PCB test mixture containing 25 percent monochlorobiphenyl, 55 percent dichlorobiphenyl (three isomers) and 20 percent trichlorobiphenyl at a total concentration of 0.824 ug PCB. The test involved 24 separate analyses and, hence, has statistical reliability.

The ruggedness test results are tabulated in Table 3. All factors showed a difference of less than that of the blank and, hence, the challenging conditions that were applied in no way affected the outcome of the determination. The difference observed for the blank (no variation) determination was +3.25 percent, which is satisfactorily low. An estimate may be made of the analytical standard deviation as follows:

$$\text{standard deviation} = \sqrt{2/7 \; D_i^2}$$

where D_i is the difference (Table 3) measured for each factor. When the data from Table 3 are entered into this formula, an estimated analytical standard deviation of 3.0 percent results.

TABLE 2

PERCHLORINATION RUGGEDNESS TEST FORMAT AND DATA

Factor Value	Determination Number 1	2	3	4	5	6	7	8
Extract Transfer Rinse Volume A = 4.0 ml a = 1.0 ml	4.0	4.0	4.0	4.0	1.0	1.0	1.0	1.0
Reaction Temperature B = 160°C b = 165°C	160	160	165	165	160	160	165	165
$SbCl_5$ Reagent Volume C = 0.2 ml c = 0.1 ml	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1
Final Extract Volume D = 0.1 ml d = 0.3 ml	0.1	0.1	0.3	0.3	0.3	0.3	0.1	0.1
Blank E = e no variation	--	--	--	--	--	--	--	--
Solvent Evaporation Temp. F = 72°C f = 76°C	72	76	76	72	72	76	76	72
Water Bath Immersion Depth G = 4 cm g = 9 cm	4	9	9	4	9	4	4	9
Result	S	T	U	V	W	X	Y	Z
Percent Recovery (%)	98.7	99.7	95.8	93.9	94.6	99.5	91.9	99.1
Standard Deviation (%)	8.9	4.1	3.2	4.2	--	4.4	0.6	3.5

TABLE 3

PERCHLORINATION RUGGEDNESS TEST RESULTS

Rank	Factor	Difference (%)
1	Blank	+3.25
2	Reaction Temperature	+2.95
3	$SbCl_5$ Volume	-2.80
4	Final Extract Volume	1.40
5	Water Bath Immersion Depth	-1.30
6	Extract Transfer Method	0.75
7	Solvent Evaporation Temperature	0.15

The results of ruggedness testing of the modified perchlorination method indicate the method is "rugged" and therefore immune to modest departures from habitual

routine on the part of the analyst. Furthermore, all method variables are in control. Variation within the specified limits of the procedure should not affect the outcome of the analysis. Competent trace analytical chemists following the above procedure in different laboratories should therefore achieve comparable analytical results.

A detailed step-by-step analytical procedure may be obtained from the authors upon request.

ENVIRONMENTAL APPLICATION

Figure 2A is a GC/EC chromatogram of an ambient airborne PCB sample collected on polyurethane foam in a high-volume sampling system. Those peaks indicated by a "•" match peaks in various Aroclor patterns. However, there is not a pattern match for any single Aroclor. Accurate quantitation of the PCB present is not possible by conventional pattern matching techniques. Figure 2B is the same sample after perchlorination. This sample contains 660 ng DCB, representing an airborne concentration of 8 ng/m^3 as DCB.

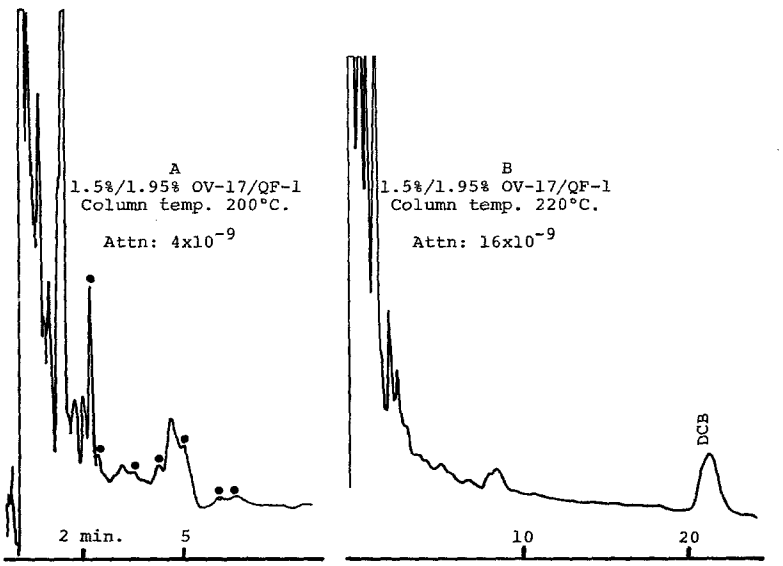

Figure 2. (A) GC/EC Chromatogram of an Ambient Air Sample
(B) GC/EC Chromatogram After Perchlorination of the Same Sample

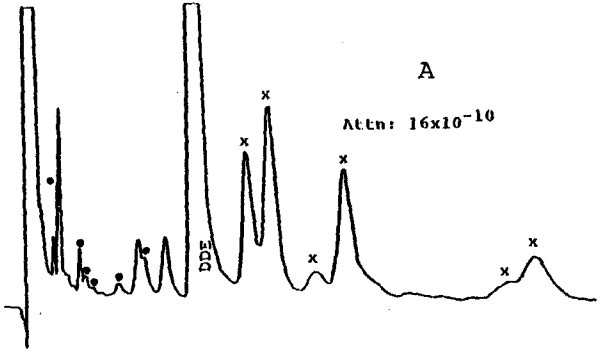

Figure 3. (A) GC/EC Chromatogram of a Human Blood Serum Extract

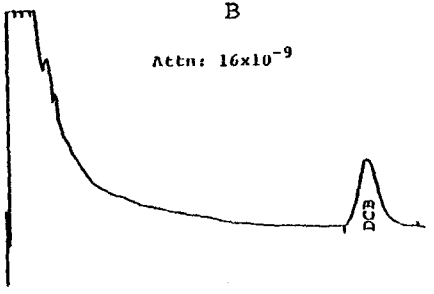

Figure 3. (B) GC/EC Chromatogram of the Same Blood After Perchlorination

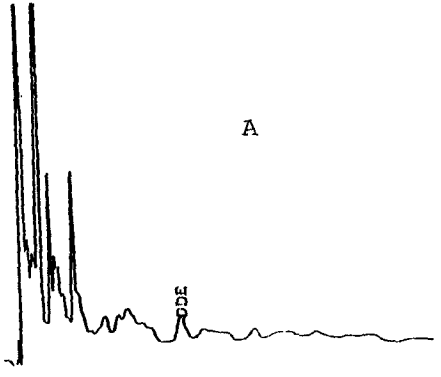

Figure 4. (A) GC/EC Chromatogram of a Groundwater Sample Extract

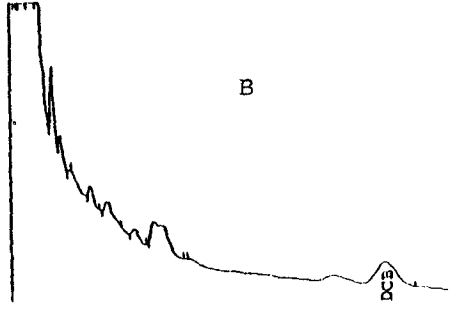

Figure 4. (B) GC/EC Chromatogram of the Same Groundwater Sample After Perchlorination

Figure 3A is a chromatogram of an extract of PCB in human blood serum. A number of peaks, indicated by a "•", match peaks in Aroclor 1242. Others, indicated by an "X", match peaks in Aroclor 1254. Using conventional pattern matching techniques, this sample was determined to contain 58 ug/ml Aroclor 1242 (based on 6 peaks) and 40 ug/ml Aroclor 1254 (based on 6 peaks). Perchlorination of this sample (Figure 3B) yielded 127 ng/ml DCB. Applying the DCB to Aroclor conversion factors of ARMOUR (1973), it appears that the pattern quantitation technique yields values approximately 30 percent high. This positive bias of the pattern quantitation technique was noted on a substantial number of blood serum samples.

Figure 4A is a chromatogram of an 800 ml groundwater sample extract showing possible traces of PCB; but no Aroclor pattern can be matched. Figure 4B shows the same sample extract after perchlorination. PCB is easily quantified at 0.6 ug/l as DCB. This chromatogram demonstrates the increased detectability that can be achieved by use of the perchlorination technique for environmental samples.

ACKNOWLEDGEMENTS

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