AOAC Official Method 990.07
Polychlorinated Biphenyls (as Aroclor 1254) in Serum
Gas Chromatographic Method
First Action 1990
Final Action 1992

(Certain chlorinated pesticides, e.g., p,p'-DDE, o,p'-DDT, and p,p'-DDT, when present at concentrations of at least 1, 2, and 2 ng/mL, respectively, interfere in determination.)

Results of Interlaboratory Study:

Aroclor 1254 (in vitro spike)

Average recovery at 8.58 ppb (ng/mL) = 89%
s_r = 1.44; s_R = 1.64; RSD_r = 18.8%; RSD_R = 21.5%
Average recovery at 16.8 ppb (ng/mL) = 83%
s_r = 2.87; s_R = 2.95; RSD_r = 20.5%; RSD_R = 21.1%
Average recovery at 41.8 ppb (ng/mL) = 77%
s_r = 3.38; s_R = 4.84; RSD_r = 10.2%; RSD_R = 14.6%
Average recovery at 84.3 ppb (ng/mL) = 77%
s_r = 9.14; s_R = 13.5; RSD_r = 14.1%; RSD_R = 20.8%

Aroclor 1254 (in vivo spike)

Average recovery at 10 ppb (ng/mL) = 100%
s_r = 0.67; s_R = 2.06; RSD_r = 6.7%; RSD_R = 20.6%
Average recovery at 25 ppb (ng/mL) = 88%
s_r = 2.13; s_R = 3.52; RSD_r = 9.2%; RSD_R = 16.0%
Average recovery at 50 ppb (ng/mL) = 78%
s_r = 2.50; s_R = 4.25; RSD_r = 6.4%; RSD_R = 10.9%
Average recovery at 100 ppb (ng/mL) = 79%
s_r = 4.58; s_R = 8.14; RSD_r = 5.8%; RSD_R = 10.3%

A. Principle

Serum is denatured with methanol and extracted with hexane–ethyl ether (1 + 1). Extracts are eluted with hexane through deactivated silica gel in adsorption chromatographic column. PCBs are determined as Aroclor 1254 (AR 1254) by electron-capture gas chromatography using characterized AR 1254 standard.

Table 990.07 Calculation of relative response factors

<table>
<thead>
<tr>
<th>AR 1254 concentration in GC standard, ng/mL</th>
<th>PCB peak 125 concentration in GC standard, ng/mL</th>
<th>Response, area count</th>
<th>RRF (a) for peak 125</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.16</td>
<td>4.3929</td>
<td>49433</td>
<td>1188850</td>
</tr>
<tr>
<td>56.13</td>
<td>8.7828</td>
<td>94923</td>
<td>1154970</td>
</tr>
<tr>
<td>135.15</td>
<td>21.0834</td>
<td>221989</td>
<td>1123250</td>
</tr>
<tr>
<td>270.3</td>
<td>42.1668</td>
<td>485107</td>
<td>1122570</td>
</tr>
</tbody>
</table>

\(a\) Weight percent factor for PCB peak 125 in AR 1254 = 15.6. DCB concentration in GC standard solutions = 47 ng/mL. Volume injected = 3 μL (injection volume not used in calculation; for information only).

B. Apparatus

(a) Gas chromatograph.—Electron-capture detector interfaced to 1.8 m x 2 mm id glass column packed with 3% GC GE-SE-30 on 80–100 mesh Gas-Chrom Q (Alltech-Applied Science Laboratories), or equivalent nonpolar liquid phase [i.e., one that provides same elution order for PCB peaks as shown in Table 970.52B (see 10.1.01)] and support. Operating conditions: injector 250°C, column 205°C, detector 330°C \(^{63}\)Ni. Nitrogen flow 20 mL/min. Condition column 72 h at 250°C before use. [Note: These conditions may have to be altered to achieve reasonable elution time (≤30 min) for internal standard, and GC system must be sufficiently sensitive to detect AR 1254 at 5 ng/mL in 4 mL (assumed to be 4 g) serum.]

(b) Adsorption chromatographic column.—Glass, 18 cm x 9 mm, with or without stopcock, but with 50 mL reservoir.

c) Wide-diameter funnel.—Powder funnel: top, 100 mm id; stem, 20 mm od x 30 mm.

C. Reagents

(a) Solvents.—Distilled-in-glass or nanograde quality: n-hexane, methanol, ethyl ether, isooctane, and benzene. (Caution: Exercise extreme caution to preclude inhalation of benzene, a known carcinogen. Use only in well-ventilated environment.)

(b) Sodium sulfate.—Anhydrous, granular.

c) Silica gel.—For adsorption chromatography. Woelm, active, 70–150 mesh.

d) Keeper solution.—1% (w/v) paraffin oil (NF [white, light, domestic—viscosity 125/135]) in hexane.

(e) Aroclor 1254 standard solution.—Aroclor 1254. Dissolve 5–10 mg standard in 100 mL nanograde isooctane.

(f) Decachlorobiphenyl (DCB) internal standard solution.—Dissolve 1.0 mg DCB (Analabs, Inc.) in 2–3 mL nanograde benzene and dilute to 100 mL in nanograde isooctane.

(g) Glass wool.—Dimethyldichlorosilane-treated.

D. Preparation of Sodium Sulfate

Treat Na$_2$SO$_4$, C(b), as follows: Pre-rinse all glassware with acetone, then with hexane, and allow to air dry before use. Add to 1 L beaker about half its volume of Na$_2$SO$_4$. Add adequate amount of nanograde hexane to create slurry. Stir mixture with glass rod and decant hexane. Repeat these steps for total of 6 washes. Use wide-diameter funnel to pour about equal amounts of washed Na$_2$SO$_4$ into two 500 mL glass reagent bottles. Stopper bottles with dimethyldichlorosilane (DMDSCS)-treated glass wool and invert over glass beaker until all excess hexane is drained. Remove remain-
ing hexane by placing bottles in 30°C vacuum oven overnight. Store hexane-washed Na₂SO₄ in 130°C oven. Remove Na₂SO₄ as needed and allow sufficient time to cool in vacuum desiccator before use.

E. Preparation of Silica Gel

(a) Water for deactivation.—Prerinse all glassware with acetone, then with hexane, and allow to air dry before use. Add 100 mL water and 25 mL nanograde hexane to 200 mL separatory funnel with glass stopper and Teflon stopcock. Extract water by gentle (to avoid emulsion) inversion several times. Let phases separate and drain water into another separatory funnel. Discard hexane extract. Repeat extraction 2 more times, discarding hexane phase after each extraction. After third extraction, drain water into clean container for storage. Monitor water for signs of bacterial growth; discard water when growth is observed.

(b) Deactivation.—Weigh 20 g active silica gel, C(e), into 250 mL beaker, cover beaker with aluminum foil (shiny side out), punch several holes in foil, and let beaker stand ≥24 h in 130°C oven. Cool silica gel in vacuum desiccator. Weigh dried silica gel into flask with Teflon-lined screw cap. Add water down sides of flask to constitute 3% of total weight (e.g., add 0.62 g H₂O to 20 g silica gel).

Wrap Teflon-lined cap with tape to seal flask. Shake wetted gel until no clumps are evident. Gently rotate flask for 3 h on mechanical rotator and let stand tightly capped overnight before use. Silica gel prepared in this manner maintains its elution characteristics ≥7 days stored on bench top.

(c) Silica gel performance.—Evaluate performance of silica gel by eluting (as in Adsorption Chromatography) and extracting (as in Extraction of Serum) serum (equivalent to 4 mL [ca 4 g]) that was in vitro spiked with AR 1254 at 25 ppb (ng/g) just before elution. Recovery of AR 1254 (corrected for AR 1254 in unspiked serum) should be ≥70% and should elute in second hexane fraction.

F. Relative Response Factors and Calculations

(a) GC standard solutions.—Use AR 1254 standard solution, C(e), to prepare solutions in hexane at 4 concentration levels of AR 1254 (20–200 ng/mL). Add DCB internal standard solution, C(f), at concentration to produce ca 50% full scale deflection.

(b) Relative response factors (RRFs).—Inject 2–3 μL aliquot of each GC standard solution and obtain PCB and DCB area counts. Use mean weight percent data in Table 990.07 to determine concentration for each PCB peak at each concentration level and compute RRF values as follows:

\[ RRF = \frac{PA}{PA'} \times \frac{Q}{Q'} \]  

(1)

where \( PA \) and \( PA' \) = area of PCB and DCB (IS) peaks, respectively; and \( Q \) and \( Q' \) = amount PCB and DCB injected, respectively. After RRFs are computed, determine mean, standard deviation, and relative standard deviation for the concentration levels for each PCB peak. These data indicate linearity of system. RSD ≤10% should be attainable for most PCB peaks except 58, 174, and 203.

(c) Calculations.—Once RRF values are generated for the 11 PCB peaks shown in Table 990.07, (see 10.1.01), amount for any PCB peak can be calculated as follows:

\[ Q = \frac{PA \times Q' \times RRF}{PA'} \]  

(2)

Divide result obtained for equation 2 by test portion weight to find concentration for that PCB.

(d) Example calculation.—The following uses data for PCB peak 125 as example of calculation by IS technique.

Calculation of RRF for PCB peak 125⁴: Use equation 1 to calculate RRF at each concentration level, and then calculate mean, SD, and CV.

Quantitation of PCB peak 125 in unknown serum: Serum volume extracted = 4.0 mL (4.0 g); final serum concentration = 4000 mg/1000 μL (4.0 g/mL); DCB concentration = 47 ng/mL; response (area count) for PCB peak 125 = 94,407; for DCB = 1,209,120.

Response of PCB peak 125 in area counts is nearest to response in AR 1254 standard at 56.3 ng/mL so that RRF is used in equation 2 to calculate concentration:

\[ \text{PCB 125, ng/g} = \frac{94,407 \times 2.2737 \times 47}{1,209,120 \times 4} = 2.0859 \text{ ng/g} \]

See Table 990.07.

G. Extraction of Serum

Pipet 4 mL serum into clean 16×125 mm culture tube that has Teflon-lined screw cap. Add 2 mL methanol, cap, and swirl tube briefly on vortex mixer. Add 5 mL hexane–ethyl ether (1 + 1) to tube, cap, vortex-mix briefly, and then mix 15 min at 50–55 rpm on rotary mixer. Centrifuge 6 min at 1800 rpm. Use Pasteur pipet (disposable glass, previously rinsed with acetone and hexane) to transfer supernate to 20×150 mm culture tube that has Teflon-lined screw cap. Repeat extraction twice. Combine extracts, add 5 drops of keeper solution, C(d), and reduce solvent volume, at room temperature, to ca 0.5 mL under gentle stream of prepurified nitrogen.

H. Adsorption Chromatography

Prepare adsorption chromatographic column, B(b), as follows: Plug column with small pledget of silanised glass wool. Add (1) anhydrous Na₂SO₄ to height of 10 mm, (2) 3.0 g of 3% deactivated silica gel, and (3) anhydrous Na₂SO₄ to height of 10 mm.

Pre-wash column with 20 mL hexane and add concentrated extract in 0.5 mL. Rinse sample tube with three 0.5 mL portions of hexane and transfer each wash to head of column. Elute column with 5 mL hexane and discard first 7 mL eluate (0.5 mL concentrated sample + three 0.5 mL washes + 5 mL eluant). Add sufficient hexane to collect 15 mL eluate in 15 mL conical centrifuge tube. Add 5 drops of keeper solution C(d) and concentrate eluate just to dryness in 40°C water bath under gentle stream of prepurified nitrogen. Add 1.0 mL DCB standard solution (at same concentration used in GC standards). Proceed with analysis by electron-capture gas chromatography.

I. Gas Chromatography

Match PCB peaks detected in extract to PCB peaks in AR 1254 standard by comparison of retention times. Allow variation of ±5% in retention times. To decide which calibration factor to use for quantitation, match peak areas to standard with nearest response. If response is between 2 standards, average calibration factors from 2 standards. If response exceeds highest standard by 10%, dilute sample with DCB internal standard solution and reanalyze. If response is less than lowest standard, use calibration factor from lowest standard.