CAPE Technologies

High Performance PCB-TEQ Immunoassay Kit (PCB1)
High Performance Dioxin/Furan Immunoassay Kit (DF1)

Technical Note TN-005

Carbon Column Fractionation and Analysis of Samples for PCB-TEQ

Existing Carbon Column Method: The preparation of samples for dioxin/furan analysis by US EPA Method 4025m is described in CAPE Technologies Application Note AN-008. This method uses a two stage coupled column system for cleanup of an extract in an aliphatic solvent (such as hexane or hexane/tetradecane). The second stage of this cleanup is an activated carbon mini-column which is used to capture the dioxin/furan portion of the sample for analysis with the DF1 Immunoassay Kit. The protocol described in Application Note AN-008 calls for loading the sample onto the carbon mini-column, washing with 6 mL of 1:1 hexane:toluene in the forward direction, then reversing the column to elute the dioxin/furan sample with 12 mL of toluene. It is very simple to modify this protocol to allow capture of the dioxin-like PCB fraction from the same sample.

Fractionation Protocol: The protocol modification noted above is as follows:

1) after removing the carbon column from its acid silica column during the sample loading (step F7/8), the column is placed on a clean empty reservoir for washing of the carbon column alone (as in the first portion of AN-008 step F9)
2) the column is washed in the forward direction with 5 mL hexane (new step)
3) the dioxin-like PCB fraction is eluted in the forward direction with 6 mL of 1:1 hexane:toluene and captured for analysis (exactly as in AN-008 step F9, except that the eluate is captured here)
4) if analysis of the dioxin/furan fraction is required, continue as normal in AN-008 (step 10); reverse elute with 12 mL toluene to obtain the dioxin/furan fraction

Analysis of Eluted PCB's: The captured dioxin-like PCB fraction is exchanged for immunoassay analysis using the same protocol as described for dioxin/furan analysis. An aliqout of immunoassay keeper is added and the sample is evaporated under a nitrogen stream with gentle heating. The residue is centrifuged and methanol is added to dilute the sample prior to addition to the immunoassay tube. The complete PCB immunoassay analysis procedure is described in detail in the PCB-TEQ Kit Insert (IN-PCB1).

Supporting Data: The original design of the carbon column method in AN-008 was intended to remove as many potentially interfering compounds as possible from the dioxin/furan sample. The protocol as outlined in AN-008 captures in the dioxin/furan fraction all the tetra- and higher chlorinated PCDD's and PCDF's which contribute to the TEQ and are detected by the DF1 immunoassay. The preceding hexane:toluene fraction described above contains the major crossreacting PCDD/F, 237-triCDD, as well as the 12 WHO dioxin-like PCBs. Other PCBs are flushed through the carbon column during the hexane washes before and after the carbon column is removed from the acid silica column, before the hexane:toluene fraction. This carbon column elution behavior has been verified using stable isotope labeled dioxin/furan and PCB congeners, analyzed by GC-HRMS (plot on page 2).

The CAPE Technologies PCB-TEQ Immunoassay and the fractionation protocol described above were evaluated in a 2004 demonstration project as part of the Superfund Innovative Technology Evaluation (SITE) Program. The full report (110 pages) and a brief abstract are available on the CAPE Technologies web site. The EPA concluded that the PCB-TEQ kit, with the cleanup method described above, could be an effective screeing procedure for PCB TEQ.

Parallel Analysis of TEQ from PCDD/Fs and PCB's: The carbon column fractionation described here allows a single sample to be extracted and prepared for immunoassay analysis using both the DF1 Dioxin/Furan Kit and the PCB1 PCB-TEQ Kit. The resulting data can be combined to give a total TEQ value from PCDD/F's and PCB's, as well as defining the relative contributions of the two components. The amount of time required for this combined analysis is only marginally greater than for either analysis alone. In addition to the "piggybacked" sample preparation by carbon column fractionation, the immunoassays can be run concurrently, with slightly staggered incubation times. The potential economic, logistic, and scientific benefit of this approach for assessment of either unknown sites or known PCB/dioxin sites is huge.

Figure 1. Verification of carbon column fractionation by HRGC-HRMS. A mixture of individual congener standards was applied to the carbon column and processed as described above. The first step was washing in the forward direction with 30 mL hexane. The second step was washing in the forward direction with 6 mL of 1:1 toluene:hexane. The third step was elution in the reverse direction with 12 mL toluene. Each fraction was analyzed by high resolution gas chromatography coupled to high resolution mass spectrometry.