

AN EFFICIENT AND GREEN CLEANUP SYSTEM FOR ANALYSIS OF DIOXIN/FURANS, DIOXIN-LIKE PCBS AND PBDES

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Abstract

We have used a fast and relatively cost effective method, the CAPE Technologies coupled carbon-acid silica column, to improve the efficiency for the analysis of PCDD/Fs, dioxin-like PCBs and PBDEs in environmental samples. Blank concentrations for total 24 PBDEs (3-Br to 10-Br) ranged from 0.483 to 1.39 ng/column and the main congeners detected were BDE-47, BDE-209 and BDE-99. The MDL for total PBDEs using the new cleanup process was determined to be 4.47 ng/g. Since 2008 we have tested 64 real samples of different environmental matrices using this method. The recovery range was 65.7%~103% for ¹³C₁₂-PCDD/Fs, 52.2~81.8% for ¹³C₁₂-PCBs, and 36.7%~106% for ¹³C₁₂-PBDEs. These results meet the QA/QC criteria of US EPA Methods 1613B, 1668A and 1614. We have successfully applied this technique to biological samples, sediments, and dustfall samples, and are now extending its application to other sample matrices.

Introduction

The toxicity of the anthropogenic environmental pollutants PCBs and PCDD/Fs requires that these groups be analysed to protect human health¹. A similar threat from PBDEs has increased public concern, so this group has also been included in the Taiwan EPA monitoring program. However, simultaneous analysis of these three groups of toxic chemicals in a single sample has been seldom reported.^{2,3} These compounds are easy to extract from samples together, but no unified cleanup has been described. Our goal in this work has been to develop a single sample cleanup procedure which is fast, easy, cheap, environmental friendly, and efficient. The ideal method will allow all three groups to be extracted and cleaned up together, followed by separation for analysis.

The traditional time-consuming cleanup procedures such as silica, alumina and carbon column have been widely adopted for analysis of PCDD/Fs and DLPCBs. Much research at the Taiwan EPA has been devoted to shortening the time required for sample cleanup. Automated systems, such as Automatic Cleanup Robot and the Power-Prep System⁴ have been evaluated. Though these systems perform well, their cost for both equipment and disposable columns is too high for most commercial labs. Therefore, we have developed an alternative method, the CAPE coupled carbon-acid silica column. This system was designed for cleanup of samples for immunoassay analysis, so it is fast, easy, and cost-effective.⁵ We have used a modification of the original CAPE Technologies cleanup procedure for this study. Also, the original CAPE carbon columns were found to have a high PBDE background, so a new ultra-clean carbon column has been used here. We demonstrate that this combination of modified protocol and new ultra-clean carbon column can be used in a single process to prepare samples for analysis of 17 PCDD/Fs, 12 DLPCBs, and 24 PBDEs (3-Br to 10-Br, including BDE-209).

Materials and Methods

All solvents were pesticide residue grade and were purchased from Merck, Tedia, and Sigma–Aldrich. Silica gel (100–200 mesh) was obtained from Fisher. Cellulose and glass filter thimbles were obtained from Sartorius. Standard solutions were obtained from Wellington Laboratories and included the following: **PCDD/Fs**- 1613-LCS (Labeled Compound Stock Solution), 1613-ISS (¹³C-1,2,3,4-TCDD and ¹³C-1,2,3,7,8,9-HxCDD Internal Standard Spiking Solution), 1613-CSS (Cleanup Standard Spiking Solution), 1613-PAR (Native PCDD/Fs, Precision and Recovery Stock Solution), and 1613CVS (EPA Method 1613, Calibration and Verification Solutions CS1-CS5); **PCBs**- WP-LCS (WHO ¹³C -PCBs Surrogate Spiking Solution), WP-ISS (WHO ¹³C-PCBs, Internal Standard Solution), WP-STK (Native PCB Solution), WP-CVS (“Dioxin-Like” PCBs, Calibration and Verification Solutions CS1-CS7); and **PBDEs**- MBDE-MXE (Mass-Labelled PBDE Solution/Mixture), BDE-CVS-EISS (Mass-Labelled PBDE Internal Standard Solution), BDE-MXE (Native PBDE Solution/Mixture), BDE-CVS-E (BDE-CVS-E, Calibration Solutions CS1-CS5).

All samples required the addition of 3 different internal standards before extraction. Extracted samples were evaporated to near dryness and transferred for cleanup. Mass labeled cleanup standards for PCDD/F analysis were added before cleanup.

CAPE Technologies Cleanup System

The coupled carbon-acid silica column system was developed by CAPE Technologies for use with their Dioxin/Furan Immunoassay Kit. This immunoassay, using a simple oxidation-only cleanup, was originally validated by the USEPA as Method 4025 for screening soil and sediment samples. The application of the coupled column cleanup system expanded the ability to analyze real environmental samples by increasing the efficacy of the cleanup. At the same time the sensitivity of the immunoassay was extended into the low pg/g range. The resulting method is referred to as modified Method 4025 or Method 4025m. This cleanup method provides simple and effective fractionation of PCDD/Fs and DL-PCBs⁷, thus allowing immunoassay screening of environmental and biological samples at low pg/g levels for both PCDD/Fs and DL-PCBs^{9,10}.

We developed this method in our lab as a backup method for the screening method DR-CALUX[®] that we established in 2004. During this process we found that the CAPE Technologies coupled column system can be applied to preparation of samples for GC-MS analysis also. In order to get better recovery of isotope standards and meet the QA/QC criteria of US EPA Methods 1613B, 1668A, and 1614, a new carbon column was developed (product code CCXC-60; Ultra Clean Carbon Mini-Columns for Cleanup of PBDE Sample Extracts). The original cleanup procedures^{6, 7} were modified to the following: Pretreat the extract with bulk acid-silica if necessary (add copper to remove sulfur for sediment samples). Apply extract to CAPE coupled carbon-acid silica column, set up as shown in Figure 1. Use stopper/stopcock assembly and syringe to pressurize the column and maintain a drop-wise flow rate of 0.5-1.0 mL per minute when eluted by solvent. Add 40 mL n-hexane to prewash the acid-silica column, then use 10 mL n-hexane to wash the coupled carbon-acid silica column set. Add 2 mL n-hexane to sample extract for transfer to column, then repeat 2 times. Add 10 mL n-hexane to elute the sample from the acid silica column and load the target analytes onto the carbon column. Add 2 x 10 mL n-hexane to continue eluting the column, this time collecting all solvent passed through column to recover one fraction containing both dioxin-like PCBs and PBDEs. Remove the carbon mini-column from the acid silica column and transfer to a clean empty column. Add 5-5.5 mL of 1:1 toluene:n-hexane to elute column. Combine this eluate with all solvent passed through column to complete the dioxin-like PCB/PBDE fraction. Reverse the carbon mini-column on the empty column and add 20-30 mL of toluene to elute. Collect all solvent passed through column for the PCDD/F fraction. Evaporate both fractions to near dryness and transfer to autosampler vials. Add recovery standards for analysis by HRGC/HRMS. DLPCBs and PBDEs are separated during analysis.

HRGC/HRMS

The analysis of prepared sample extracts was performed on a HRGC (HP 6890)/ HRMS (JEOL JMS-700). PCDD/Fs and DLPCBs were separated using a DB-5MS 60 m column and PBDEs were separated using a DB5-HT 15 m column. The HRMS was operated at >10000 resolution for PCDD/Fs and DLPCBs and at >5000 resolution for PBDEs, using EI ionization at 35 eV. All measurements were made in selective ion recording (SIR) mode, monitoring the two most abundant ions in the cluster.

Results and Discussion

The unified cleanup method using CAPE Technologies coupled carbon-acid silica column was compared to other cleanup methods. As summarized in Table 1, the CAPE process used fewer columns and less solvent. The method also costs less and requires less time to operate. The CAPE coupled carbon-acid silica column cleanup process only needs n-hexane and toluene to elute. Uniformly manufactured preppacked columns allow easy quality control and can be used immediately with no preparation. Columns are sealed after manufacture, so storage requires only a dry space and no energy. Shorter process times mean less cost and easier operation. These advantages, plus the reduced solvent volumes make this a relatively 'green' cleanup process.

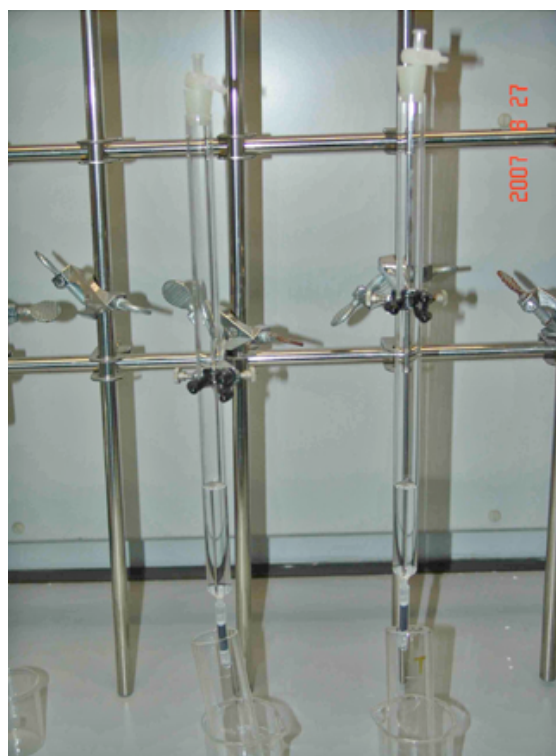


Fig. 1: CAPE coupled carbon-acid silica column

Blank Testing and Real Sample Testing

All solvents and other materials were tested for PBDEs contamination. Congener data were collected for PBDEs in 12 blank carbon columns. In 2008, the unified sample cleanup described above was applied to 47 biological samples (including QA/QC samples), which were analyzed for 17 PCDD/Fs, 12 DLPCB and 24 PBDEs (3-Br to 10-Br, include BDE-209). In 2009, the unified sample cleanup was applied to 18 sediment and dustfall samples (including QA/QC samples). Experimental results are presented below.

Improvement of CAPE Carbon Columns for PBDE Analysis

We had previously analyzed more than 1000 samples of various environmental matrices using the CAPE coupled carbon-acid silica column system for PCDD/Fs and DLPCBs. But circumstances now require that we analyze PBDEs in addition to PCDD/Fs and DLPCBs. Initial testing of the CAPE coupled carbon-acid silica column cleanup process to analyze PBDEs showed high background PBDE levels in the carbon mini-column. The blank carbon columns contained PBDE concentrations from 0.9 to 56.3 ng/column. Since the highest of these levels are unacceptable for analysis of environmental samples, we cooperated with CAPE Technologies to determine the source of the contamination. Analysis by GC-MS showed that the largest contribution was from a polyurethane foam pad used in the packaging to protect the columns during shipping and storage. A new clean production process was introduced and different packaging was used to produce a new product for cleanup of PBDE sample extracts. Testing of 12 of the new columns gave blank concentrations for total PBDEs from 0.483 to 1.39 ng/column, with the main congeners BDE-47, BDE-209 and BDE-99 (Fig 2). Because these congeners are the same as in most environmental samples, additional effort is under way to reduce these blank levels further. Testing of the new columns in the established cleanup process gave an MDL for total PBDEs of 4.47 ng/g. Although the PBDEs standards included 1-Br and 2-Br, these congeners were easily lost during cleanup, either during the coupled column cleanup or in the N₂ evaporation. Since these congeners are not of major importance in the sample, they were excluded from the total PBDE calculation, which included 24 congeners from 3-Br to 10-Br (including BDE-209).

Analysis of Field Samples

Table 2 and Figures 3 to 5 present recovery results from testing of field samples. Table 2 shows the range of recovery values for different matrices, from 65.7% to 103% for ¹³C₁₂-PCDD/Fs, from 52.2% to 81.8% for ¹³C₁₂-DLPCBs, and from 36.7% to 106% for ¹³C₁₂-PBDEs. Figure 3 shows that recovery of TCDD/F and OCDD mass labeled standards was lower than for other congeners. Figure 4 shows that average recovery for DLPCB was nearly equal. Figure 5 shows that recovery of BDE-209 was lower than other BDE congeners, but this is because the HRMS program is not suitable for it. Performance properties of the activated carbon used in the CAPE Technologies mini-carbon column are very similar to AX-21, so capture of PCDD/Fs, DLPCBs, and PBDEs is quite effective. Data from 6 different analysts show that the recovery values do not vary by analyst.

While the recovery values observed were lower than those obtained with the Power-Prep System⁴, they are within the QA/QC criteria of US EPA Methods 1613B, 1668A and 1614. The cost of the CAPE coupled carbon-acid silica column system is much less than the Power-Prep System, both for materials and equipment at start-up and for the disposable columns required for each sample. The cleanup procedure is simple, user-friendly and needs much less solvent. The total operation time for 8-10 samples is about 3 hours. Batch size is flexible and each coupled carbon-acid silica column set can be operated independently, allowing several analysts to perform the clean-up procedure at the same time.

Previously we had demonstrated that the CAPE Technologies coupled carbon-acid silica column system can be used for cleanup of a wide variety of environmental matrices, such as stack gas, ambient air, water, waste, plant samples, etc.⁵ The present study expands that capability to include PBDEs, demonstrating a new unified system that can enhance productivity substantially in the analysis of PCDD/Fs, DLPCBs and PBDEs. We hope this simple, inexpensive, efficient, and 'green' cleanup system can be widely applied in the future.

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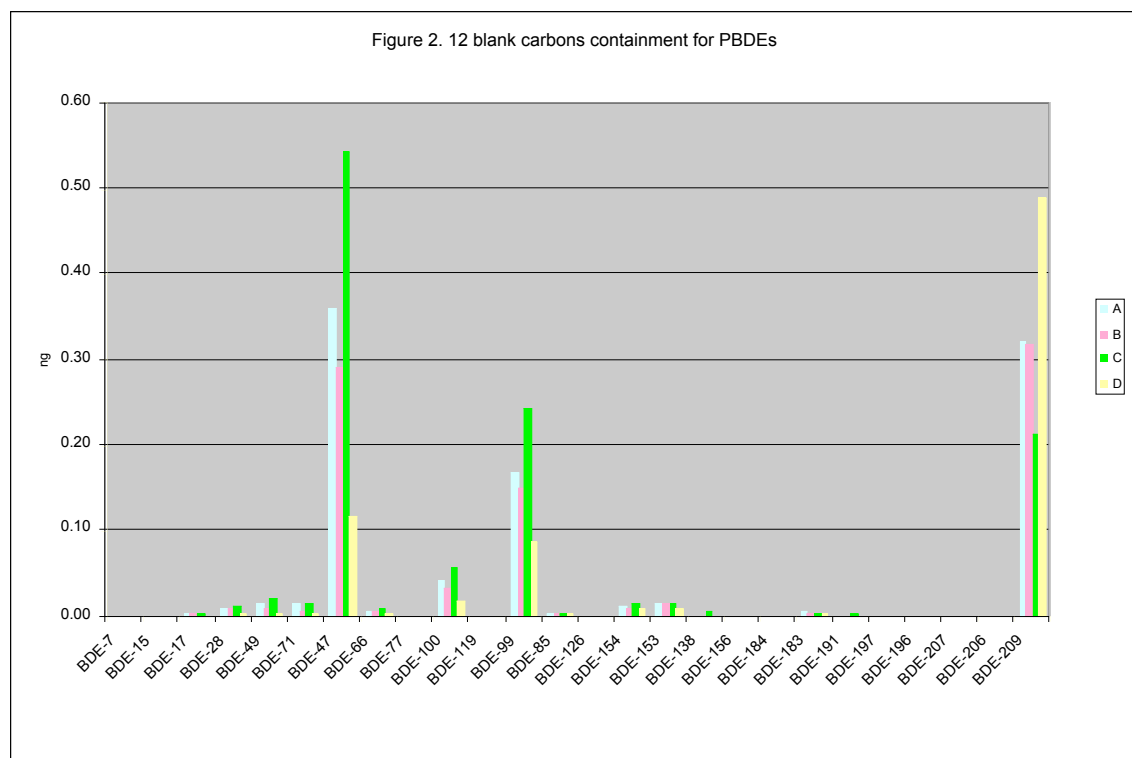
Table 1. Comparison of CAPE coupled carbon-acid silica column cleanup process with other cleanup processes

Cleanup process	Columns Used	Solvents Used	Total Solvent Used Per Sample	Apparatus cost	Column Cost Per Sample	Operation Time Per Batch	Electrical Power Required
Traditional Cleanup ⁸	Silica Alumina Carbon	n-hexane DCM Toluene	~350 mL	low	~\$25	~24 hr	Power for Oven
Power-Prep System	Silica Alumina Carbon	n-hexane DCM Toluene Benzene	~720 mL	More than \$180000	~\$60	~5 hr	Computer Pump Control Unit
CAPE column	Silica Carbon	n-hexane Toluene	~140 mL	Less Than \$1000	~\$25	~3 hr	none

Note 1. Above data do not include pretreatment processes for special matrices.

2. Total solvent amounts include all solvents for column pre-cleaning and elution.

3. Batch operation time includes column setup, pre-cleaning, loading, and elution for 8-10 samples.



Biotic / TW EPA PBDE test columns and solvent sample

A-D = sets of 3 columns in 40 mL vial

A = stored tightly wrapped in green PUF in a clean 40 mL vial (no LDPE bag)

B = stored in small LDPE bag in a clean 40 mL vial (no green PUF)

C = stored in a clean 40 mL vial with recycled desiccants (no PUF or LDPE bag)

D = stored in a clean 40 mL vial with new desiccants (no PUF or LDPE bag)

Table 2. The real samples IS recovery used CAPE columns cleanup processes to analysis PCDD/Fs, DLPCBs, and PBDEs

congeners	biological samples analysis in 2008					sediment and dustfall samples analysis in 2009				
	average(µM)	min(µM)	max(µM)	numbers	SD(%)	average(µM)	min(µM)	max(µM)	numbers	SD(%)
13C-2,3,7,8-TeCDF	65.7	30.2	94.1	47	17.5	79.3	64.9	101.7	17	9.84
13C-1,2,3,7,8-PeCDF	85.7	57.0	109	47	14.6	92.7	70.0	111.4	17	11.8
13C-2,3,4,7,8-PeCDF	87.5	60.5	116	47	15.0	94.4	70.9	116	17	13.1
13C-1,2,3,4,7,8-HxCDF	91.7	68.5	111	47	11.1	101	80.6	121.1	17	11.7
13C-1,2,3,6,7,8-HxCDF	96.0	70.5	113	47	9.69	99.7	84.0	115	17	10.0
13C-2,3,4,6,7,8-HxCDF	92.7	70.3	110	47	9.59	98.5	81.8	113.9	17	10.0
13C-1,2,3,7,8,9-HxCDF	84.7	58.4	111	47	14.0	103	86.3	120.4	17	8.70
13C-1,2,3,4,6,7,8-HpCDF	89.4	69.8	103	47	10.9	95.3	66.2	113.4	17	12.2
13C-1,2,3,4,7,8,9-HpCDF	76.4	47.4	96.2	47	13.9	86.6	68.4	109.7	17	12.0
13C-2,3,7,8-TeCDD	68.9	33.8	95.9	47	14.5	89.4	68.4	105	17	10.1
13C-1,2,3,7,8-PeCDD	85.0	62.6	107	47	13.9	93.1	73.3	109.2	17	11.0
13C-1,2,3,4,7,8-HxCDD	83.1	53.0	102	47	11.5	91.9	72.6	110.1	17	11.7
13C-1,2,3,6,7,8-HxCDD	92.5	67.6	115	47	10.8	98.8	71.8	117.9	17	12.1
13C-1,2,3,4,6,7,8-HpCDD	81.0	53.7	103	47	12.4	90.5	65.9	109.3	17	11.2
13C-OCDD	69.5	41.4	96.0	47	13.6	77.2	46.7	96.1	17	11.7
37Cl-2,3,7,8-TCDD	67.9	34.8	87.7	47	12.9	93.5	74.4	116.2	17	11.5
Total average for PCDD/Fs	82.4				12.9	92.8				11.2
13C-PCB #81	52.8	29.7	88.7	47	15.7	67.5	25.5	104	17	17.8
13C-PCB #77	56.1	29.7	96.9	47	16.3	69.8	27.4	105	17	17.7
13C-PCB #123	52.2	26.6	80.6	47	12.8	62.3	25.2	86.8	17	18.0
13C-PCB #118	54.2	27.1	81.3	47	12.3	65.4	25.3	88.2	17	18.2
13C-PCB #114	52.2	26.8	77.9	47	12.2	59.6	25.5	82.8	17	18.2
13C-PCB #105	55.2	27.0	85.4	47	12.6	68.4	29.2	88.3	17	15.2
13C-PCB #126	57.7	28.7	88.4	47	13.1	74.4	33.1	93.4	17	15.3
13C-PCB #167	66.7	30.1	83.2	47	10.2	70.8	39.0	92.5	17	17.8
13C-PCB #156	66.4	30.4	89.0	47	10.0	74.0	45.0	99.7	17	16.4
13C-PCB #157	66.6	30.5	89.8	47	9.8	77.3	46.9	104	17	15.2
13C-PCB #169	64.7	31.6	103	47	11.9	81.8	54.5	102	17	12.4
13C-PCB #189	74.1	30.7	95.8	47	11.6	79.4	48.3	121	17	17.8
Total average for DLPCBs	59.9				12.4	70.9				16.7
BDE-28L	106	26.4	147	47	35.7	79.0	27.2	148	17	34.7
BDE-47L	119	60.6	150	47	26.1	87.7	38.8	150	17	32.0
BDE-99L	119	72.4	146	47	20.0	91.8	53.8	126	17	21.0
BDE-154L	102	58.3	148	47	18.8	76.9	54.5	93.5	17	9.94
BDE-153L	99.0	65.6	146	47	14.1	85.3	66.0	101	17	9.14
BDE-183L	90.9	64.3	145	47	16.2	75.0	60.4	88.9	17	7.57
BDE-197L	86.4	55.6	140	47	19.8	94.2	56.3	130	17	22.2
BDE-207L	56.7	36.0	101	47	13.1	73.2	38.6	113	17	22.9
BDE-209L	36.7	21.0	61.7	47	10.6	67.9	20.1	144	17	30.0
Total average for PBDEs	88.3				19.9	77.7				22.5

