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SAMPLE PREPARATION FOR THE MEASUREMENT OF POLYCHLORINATED DIOXINS AND FURANS BY THE PROCEPT RAPID DIOXIN ASSAY

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Introduction

The Procept Rapid Dioxin Assay (Eichrom Technologies, Inc.) is an Aryl hydrocarbon-Receptor (AhR) based assay which utilizes Polymerase Chain Reaction (PCR) to quantify levels of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F) in samples.[1] Under appropriate conditions, when exposed to PCDD/F or similar compounds, the AhR forms an adduct including a DNA response element (DRE). A small DNA molecule mimicking this DRE can be tagged with a florescent probe and amplified using PCR to allow the measurement of very low levels of the DNA molecule and indirectly the amount of PCDD/F. However, compounds similar to PCDD/F, including polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH) and certain brominated flame retardants (BFR) can also interact with the AhR leading to significant cross-reactivity for these classes of compounds.[2] Since, it is often desirable to measure PCDD/F at very low concentrations, and these dioxin-like compounds can be present in samples at levels much higher than PCDD/F, the PCDD/F must be separated from the compounds with significant cross-reactivity prior to measurement of PCDD/F by the Procept Rapid Dioxin Assay. This paper will outline several methods for isolating PCDD/F from other AhR cross-reactive compounds, focusing primarily on PAH.

Materials and Methods

The Procept Rapid Dioxin Assay was obtained from Eichrom Technologies, Inc. and Hybrizyme Corporation. Dioxin, Furan and PCB standards were obtained from Cambridge Isotope Laboratories. PAH standards were obtained from Accustandard, Inc.. Solvents were obtained from Sigma Aldrich and were of HPLC grade. Deionized water was obtained from a Milli-Q2 water purification system. Florisil, alumina and silicas were obtained from Sigma Aldrich. Trace metal grade sulfuric acid was obtained from Fisher Scientific. PCR reagents were obtained from Stratagene, Inc.. Diatomaceous earth was obtained from Dionex Corporation.

Results and Discussion

In Table 1, the response factors on the Procept Rapid Dioxin Assay for selected PCDD/F, PCB and PAH are listed. In general, these response factors are similar to the WHO 98 TEF values. Although no TEF values have been assigned for PAH compounds, several of these compounds have significant response on the Procept Rapid Dioxin Assay. Therefore, it is important to remove PAH compounds from samples prior to analysis by this method.

The sample preparation method recommended for the determination of PCDD/F by high resolution gas chromatography-mass spectrometry in US EPA method 8290 offers many options for removing PAH compounds. Method 8290 includes: contact with concentrated sulfuric acid, a multi-layer silica column, an alumina column and finally an activated carbon column.

Contact of sample extracts dissolved in organic solvents with concentrated sulfuric acid can significantly reduce the amount of many PAH compounds. For example, in Figure 1, over 99% of benzo(g,h,i)perylene is removed by a single one minute contact with concentrated sulfuric acid, as shown by the decrease in the UV-VIS absorbance observed following contact. Following treatment with concentrated sulfuric acid, additional removal of PAH compounds can be achieved by passing sample extracts through a column of silica gel coated with sulfuric acid. The presence of PAH compounds in the sample is normally revealed by the discoloration of the silica column (yellow, red, brown, black, depending on the type and amount of PAH present).

Table 1.

Congener	WHO 98 TEF	Procept Assay response factor
2,3,7,8 TCDD	1	1
1,2,3,7,8 PCDD	1	0.55
1,2,3,4,7,8 HxCDD	0.1	0.35
1,2,3,6,7,8 HxCDD	0.1	0.1
1,2,3,7,8,9 HxCDD	0.1	0.49
1,2,3,4,6,7,8 HpCDD	0.001	0.013
1,2,3,4,6,7,8,9 OCDD	0.0001	0.0000028
2,3,7,8 TCDF	0.1	0.06
1,2,3,7,8 PCDF	0.05	0.14
2,3,4,7,8 PCDF	0.5	0.32
1,2,3,4,7,8 HxCDF	0.1	0.39
1,2,3,6,7,8 HxCDF	0.1	0.17
1,2,3,7,8,9 HxCDF	0.1	0.28
2,3,4,6,7,8 HxCDF	0.1	0.1
1,2,3,4,6,7,8 HpCDF	0.01	0.053
1,2,3,4,7,8,9 HpCDF	0.01	0.016
1,2,3,4,6,7,8,9 OCDF	0.0001	0.00046
PCB-81 (3,4,4',5)	0.0001	0.000045
PCB-77 (3,3',4,4')	0.0001	0.000034
PCB-126 (3,3',4,4',5)	0.1	0.014
PCB-169 (3,3',4,4',5,5')	0.01	0.001
PCB-123 (2',3,4,4',5)	0.0001	0.0000089
PCB-118 (2,3',4,4',5)	0.0001	$<3 \times 10^{-7}$
PCB-114 (2,3,4,4',5)	0.0005	0.00001
PCB-105 (2,3,3',4,4')	0.0001	$<3 \times 10^{-7}$
PCB-167 (2,3',4,4',5,5')	0.00001	0.000001
PCB-156 (2,3,3',4,4',5)	0.0005	0.000029
PCB-157 (2,3,3',4,4',5')	0.0005	0.000043
PCB-189 (2,3,3',4,4',5,5')	0.0002	$<3 \times 10^{-7}$
Indeno(1,2,3-cd)pyrene	N/A	0.8
Benzo(k)fluoranthene	N/A	0.54
Benzo(b)fluoranthene	N/A	0.59
Dibenzo(a,h)anthracene	N/A	0.29
Benzo(a)pyrene	N/A	0.13
Benzo(a)anthracene	N/A	0.054
Chrysene	N/A	0.036
Benzo(g,h,i)perylene	N/A	0.0038

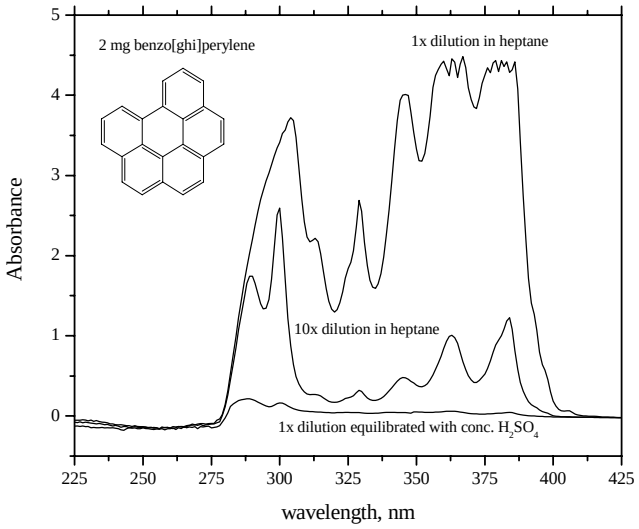
acenaphthylene, anthracene, fluorene, naphthalene, fluoranthene, phenanthrene, pyrene, acenaphthene, 2-methylnaphthalene, 2-chloronaphthalene, biphenyl, 2,4-dichlorophenol, 3,4-dichlorophenol and toluene showed no measurable response at 10 ppm.

Figure 2 shows the dry weight distribution ratio (Dw) for several PAH compounds on alumina and Florisil. Dw is a measure of how well a compound is retained and is proportional to the number of free column volumes to the elution peak maximum under a given set of conditions.[3] As can be seen in Figure 2, the retention of the PAH compounds decreases as the concentration of methylene chloride increases. However, the authors have been unable to identify conditions under which significant decontamination from PAH compounds can be achieved while PCDD/F are still retained by either alumina or Florisil. The PCDD/F and PAH compounds with significant cross-reactivity on the Procept Rapid Dioxin Assay tend to co-elute from these column materials. The main benefit of using the alumina or Florisil column appears to be decontamination of the PCDD/F fraction from many PCB congeners.

Based on these preliminary results, reagent blanks and spiked samples were determined by extracting diatomaceous earth (certified PCDD/F, PCB, PAH free) using an accelerated solvent extraction (ASE) apparatus and then processing the extract using the sulfuric acid pretreatment, silica column and Florisil column. The purified extract was then dissolved in 200 µL of heptane and processed using the Procept Rapid Dioxin Assay. Table 2 shows the results for four replicates of reagent blanks, starting with toluene or heptane:acetone (3:1) as the ASE solvent. If the resulting extract is

added to the Procept Assay without any further treatment a significant background response is observed, corresponding to 9 ppt TCDD for heptane:acetone (3:1) and 15 ppt TCDD for Toluene. However, if the extract (in heptane) is contacted briefly with a small aliquot of concentrated sulfuric acid, the response observed by the Procept Assay is reduced to about 1 ppt TCDD in both cases and is essentially the same as the response observed for blank solvent. This suggests that some PAH is accumulated during the sample preparation from solvents, glassware or the Florisil column.

Figure 1.



The final contact with sulfuric acid is an easy way to remove any small amounts of PAH accumulated during the sample preparation and is made simple by using heptane to dissolve the purified sample extract. Furthermore, solutions of PCDD/F standards in heptane have been observed to be stable to contact with concentrated sulfuric acid for over 1 month at room temperature.

Typically, aqueous soluble solvents such as methanol are used to introduce samples in to the Procept Assay. However, volatile water-immiscible solvents can also be used. Figure 3 shows response curves for 2,3,7,8-TCDD in methanol, hexane and heptane. The response curves, shown as threshold cycle (Ct, PCR parameter equal to the number of temperature cycles at which a certain level of fluorescence is reached) vs ppt TCDD, are essentially identical.

Figure 2.

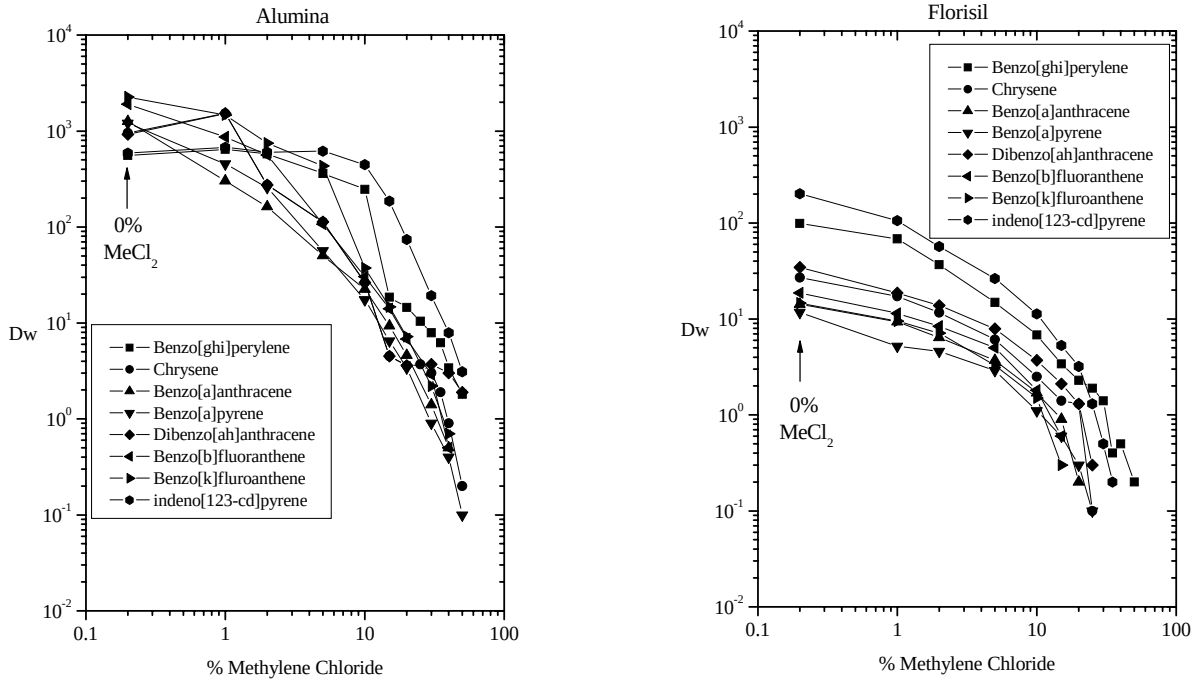


Figure 4 shows the results for diatomaceous earth spiked with various levels of a mixed standard containing tetra-, penta-, hexa- and octa-chloro dibenzo-*p*-dioxins and furans. Following the same sample preparation method as the reagent blanks, with the final sulfuric acid contact immediately prior to introduction into the Procept Assay, a linear, nearly 1:1 response is observed for the TEQ measured by the Procept Assay plotted versus the TEQ measured by GC-HRMS for samples ranging from 4-500 ppt. These results are very promising, and future work will extend to the study of samples of several different matrices (soils, food, feed, fly ash) and the simplification of the sample preparation method. Also, the benefits of using an activated carbon column during the sample preparation will be explored.

Table2.

	Reagent Blank[a]	
	No Sulfuric Acid	**Sulfuric Acid**
ASE Solvent	**Post-Treatment**	**Post-Treatment**
Toluene	9 + 5 ppt	1.1 + 0.5 ppt
Heptane:Acetone (3:1)	15 + 8 ppt	1.6 + 0.9 ppt

[a]For 5 gram sample size, 200 μL final extract volume

Figure 3.

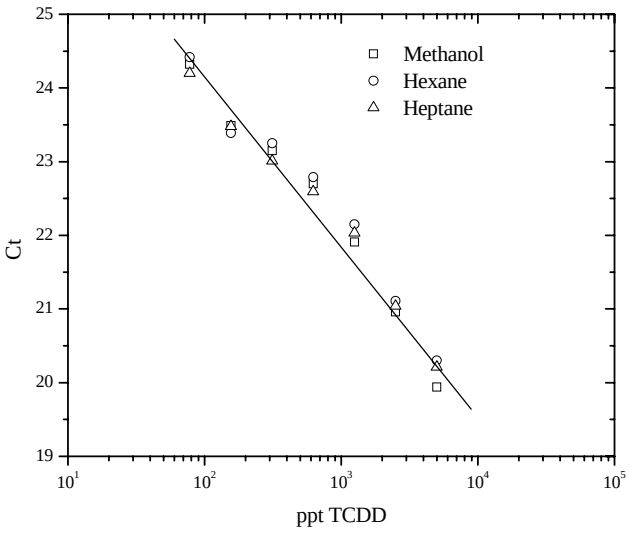
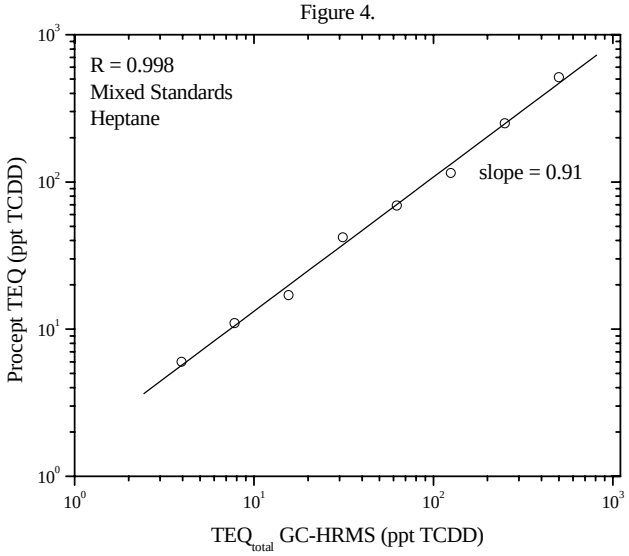


Figure 4.



References

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