A MONOCLONAL ANTIBODY BASED IMMUNOAFFINITY COLUMN FOR ISOLATION OF PCDD/PCDF FROM SERUM

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Introduction

Polychlorinated aromatic compounds such as dioxins, dibenzofurans, and PCBs are ubiquitous environmental contaminants. These compounds have long half-lives, are slow to degrade, resist metabolism, and hence can bioaccumulate in the food chain. Adding to the complexity of analysis, these compounds have numerous congeners that have similar physical properties and are present in trace amounts. Because of these properties, current analysis for dioxins/dibenzofurans requires laborious cleanup procedures that involve multiple column procedures and consume large quantities of potentially hazardous solvents. To detect trace amounts of dioxins/dibenzofurans, instrumentation such as high resolution GC-high resolution MS is needed. Consequently, current analysis for dioxins/dibenzofurans is very costly (ca. US $1000/sample).

For regulatory or risk assessment purposes, a high sample throughput is needed in order to deliver timely data to avoid the spread of contamination and subsequent economic consequences. The use of an antibody-based affinity column has been explored as a means to shorten the length of time needed for the dioxin analysis and decrease the amount of solvent consumption[1]. In this report, we update the use of serum sample cleanup by a monoclonal antibody based immunoaffinity column (IAC) for the dioxins/ dibenzofurans/ PCBs analysis[2,3].

Methods and Materials

Immunoaffinity column preparation: The procedure for immunoaffinity column preparation followed the previously published method except that cell culture supernatant was used instead of mouse ascites as the source of the antibody[2]. Monoclonal antibody producing cell lines that recognize dioxins were grown in the cell culture[4]. After the cells were pelleted by centrifugation, the supernatant was passed through a protein-G column for IgG isolation and a size exclusion column for buffer exchange. The IgG fraction was conjugated to CNBr-Sepharose beads following the manufacture's protocol (Pharmacia Biotech, Uppsala, Sweden) and packed into glass columns. A column of 0.5 ml was used for the experiment, which is equivalent of 2.5 mg IgG per column.

Immunoaffinity column procedure: Calibrated standards that contained known quantities of PCDDs/PCDFs/PCBs were added to serum samples for IAC recovery tests. Serum samples weighing either 1g or 25 g were spiked with the same amounts of standard and used for the study. The procedures for the IAC recovery test were modified from a previously described method. Briefly, after spiking each sample with carbon-13-labeled internal standards 1g or 25 g samples were passed through a 10% acetone washed IAC (0.5 ml) with gravity flow. The unbound materials in IAC were washed off using 5 column volumes of 10% acetone and the desired dioxin-containing fraction was eluted using 5 column volumes of 50% acetone. The PCDDs/PCDFs/PCBs were then extracted from the column eluate into dichloromethane and passed through anhydrous sodium sulfate. The final solutions were concentrated and mixed with external recovery standards dissolved in dodecane.

Analysis of recoveries: Sample analyses were performed by high resolution GC-high resolution-MS[5]. Satisfactory recovery was considered to be between 25-150%, as defined by EPA method 1613, and was used for the calculation of total TEQ.

Results and Discussion

The 25-g serum sample produced satisfactory results with the following congeners falling between 25-150% range: 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD. These congeners account for 80% of the TEQ. All five congeners that had a satisfactory recovery also gave analyte concentrations that fell within the quality control samples 95% confidence limit. The assay was found to be very reproducible with standard deviations for congener recoveries of less than 10% and with most of congeners having less than 20% coefficient of variation (Table 1).

One gram spiked serum samples have approximately 1/3 of the recovery compared to the 25 g serum samples for most of the PCDD and PCDF congeners. However, the PCB congeners showed equal or enhanced recovery in the 1 g serum samples compared with the larger sample size (Table 1).

Recoveries appeared to be dependent on column flow-rate and allowing sufficient time for the analyte to bind to the immobilized antibody. Comparison of the recoveries of the 25 g serum samples with the 1 g serum samples demonstrates the importance of sample loading rate. The higher recovery from the 25 g samples is probably due to slower sample loading allowing more effective interaction with the antibody binding site. Previously, when 2 ml samples of bovine serum were equilibrated with the immunoaffinity gel beads for 30 min prior to the washing and elution steps, the congener recoveries were comparable with the 25 g serum samples reported here.

Decreasing the IAC size from 2 ml to 0.5 ml did not change the recovery (data not presented). Since a 0.5-ml column still has sufficient binding sites for the analytes it was not expected that the recovery would be decreased because of the smaller column size. Decreasing the size of the column reduced the amount of antibody needed and decreased the solvent needed for washing, elution, and extraction steps; providing a more economical approach to the analysis.

Enzyme-linked immunosorbtion assays (ELISA) can roughly predict the recovery of the congener from the column. Table 1 shows ELISA I_{50} values for the PCDD, PCDF, and PCB congeners. If the I_{50} was below 2.5 ng, the immunoaffinity columns showed strong binding of the congeners. However, the correlation of I_{50} vs recovery is not quantitative. The dioxin series 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD had I_{50}'s differing by a factor of three but showed almost

equal recovery from the IAC. Prediction between the dioxin and dibenzofuran series is also not good, 2,3,7,8-TCDD has only 10% of the sensitivity of 2,3,4,7,8-PeCDF in the ELISA, however, with the IAC these two congeners exhibited nearly equal recoveries. The antibody showed minimal binding to the PCBs as demonstrated by both ELISA cross-reactivity and immunoaffinity column recovery.

Future plans include generation of cocktail antibody-based IAC in order to retain all of the congeners. Automation of the IAC is currently underway to further improve the efficiency of serum sample cleanup procedures.

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Table 1: Recovery of PCDDs/PCDFs/PCBs congeners from 1g or 25 g serum samples (n=3)

Congener	% Recovery (1 gram serum sample)[a]	% Recovery (25 gram serum sample)[a]	Analyte Concentration ppq (25 g serum)	Within 95% CL[b]	I_{50} (ng)[c]
Dibenzofurans					
2378-TCDF	14.1 ± 1.5	32.9 ± 3.0	200 ± 5.1	Yes	0.7
12378-PeCDF	13.5 ± 3.0	14.1 ± 2.3	100 ± 14.3	No	ND[d]
23478-PeCDF	18.4 ± 4.4	56.2 ± 10	223 ± 14.0	Yes	0.3
123478-HxCDF	6.1 ± 2.0	7.0 ± 0.4	110 ± 13.1	No	ND
123678-HxCDF	6.1 ± 2.0	7.0 ± 0.4	122 ± 13.1	No	ND
234678-HxCDF	11.8 ± 3.3	38.0 ± 8.2	235 ± 2.0	Yes	ND
123789-HxCDF	3.7 ± 1.3	2.7 ± 0.7	~		ND
1234678-HpCDF	1.0 ± 0.4	1.0 ± 0.1	~		ND
OCDF	0.8 ± 0.4	0.7 ± 0.1	~		>200
Dibenzo-*p*-dioxins					
2378-TCDD	18.4 ± 4.4	53.3 ± 8.5	238 ± 8.5	Yes	2.5
12378-PeCDD	14.2 ± 2.7	54.4 ± 9.4	249 ± 18.2	Yes	0.8
123478-HxCDD	3.6 ± 1.4	12.7 ± 1.8	655 ± 91.1	No	20
123678-HxCDD	3.6 ± 1.4	12.7 ± 1.8	655 ± 91.1	No	ND
123789-HxCDD	6.3 ± 1.6	11.6 ± 0.8	174 ± 8.5	Yes	>200
1234678-HpCDD	1.7 ± 0.4	5.5 ± 0.4	1032 ± 20.0	Yes	ND
OCDD	0.7 ± 0.2	0.7 ± 0.1	7489± 3040	Yes	>200
PCB's					
3344-PCB	12.1 ± 2.0	3.0 ± 0.7	342 ± 148	No	200
3445-PCB	7.2 ± 1.0	4.1 ± 1.0	435 ± 76.9	No	ND
33445-PCB	8.3 ± 0.9	8.2 ± 1.6	773 ± 129	No	ND
334455-PCB	3.6 ± 0.2	3.7 ± 0.7	~		ND

[a] Per cent recovery computed by EPA method 1613; [b]The amount found falls within the 95% confidence limits of the current 25g serum standards of CDC samples (n=125 – 131); [c]ELISA data that was obtained from reference 4. [d]ND= not determined. ~=not detected.