Separation of PCDDs from biomatrices using an immunoaffinity column generated from a monoclonal antibody

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Introduction

Polychlorodibenzo-*p*-dioxins are persistent environmental contaminants which pose health concerns due to their immunotoxicity, teratogenicity, and carcinogenicity. Because of the minute amount in environmental or biological matrices and the high lipophilicity, dioxin samples require extensive sample cleanup before quantification can be made by high resolution GC-MS (HRGC-MS). This study explores the feasibility of using an immunoaffinity column to shorten the dioxin sample clean up time and decrease the volume of potentially toxic organic solvents used in common dioxin extractions.

A monoclonal antibody has been shown to specifically bind 2,3,7,8-TCDD and have cross activity toward 1,3,7,8-TCDD, and 1,2,3,7,8-PCDD[1]. Immobilization of the antibody to CNBr-Sepharose provided a convenient method for the immunoaffinity purification of dioxins. The sample loading, column washing, and elution conditions will be described for dioxin-containing biological fluids.

Material and Methods

1. Immunoaffinity column application

An immunoaffinity column (IAC) was generated by immobilizing 5 mg of dioxin-specific antibody (DD3) to 1 ml of CNBr-Sepharose beads as described by the manufacturer (Pharmacia Biotech, Uppsala, Sweden). Congeners (^{14}C-1,3,7,8-TCDD, specific activity of 63.4 mCi/mmole; ^{3}H-2,3,7,8-TCDD, specific activity of 28.4 Ci/mmole; ChemSyn Laboratories, Lenexa, KS, USA) were spiked into 2 ml bovine serum or milk and then applied to the immunoaffinity column. Either 100 ng/ml of ^{14}C-1,3,7,8-TCDD in serum or milk, or 100 pg/ml ^{3}H-2,3,7,8-TCDD in serum was applied to the immunoaffinity column. Each sample was allowed to incubate for 30 min with the IAC before washing the column

with 5 column volumes of 10% acetone. Elution of the affinity-bound dioxins was carried out with 50% acetone. Protein concentrations were measured by the Bradford method [2] (Bio-Rad Laboratories, Hercules, CA, USA) and radioactivity was quantitated by liquid scintillation counting.

2. High-Resolution GC-MS quantitation

The immunoaffinity column eluent peak (total volume 5ml) obtained from the ^{3}H-2,3,7,8-TCDD application was collected, the solvent removed by a stream of N_2, and the sample extracted into hexane. The hexane was evaporated and an internal standard, ^{13}C-1,2,3,4-TCDD (ChemSyn Laboratories, Lenexa, KS, USA), was added for HRGC-MS quantitation.

Results and Discussion

The immunoaffinity column used for this study showed the ability to retain both 1,3,7,8-TCDD and 2,3,7,8-TCDD congeners. Using ^{14}C-1,3,7,8-TCDD, a non-toxic congener, for method development, we found serum samples could be applied directly to the column with minimal break through of TCDD in the wash. However, the results obtained with spiked milk samples, even at a 1:20 dilution (Table I), were unsatisfactory. The reduced binding of dioxins to the immunoaffinity column, when applied in milk, may be due to interfering substances such as lipids and proteins. Thus, further sample preparation was required prior to applying milk samples to the immunoaffinity column.

Table I. Percent recovery of ^{14}C-1,3,7,8-TCDD from spiked samples applied to a DD3-CNBr-Sepharose column.

Matrix	10% acetone wash	50% acetone elution
Whole serum (n=3)	11.5±1.2	91.6±2.5
Milk 1:10 (n=1)	74.8	24.2
Milk 1:20 (n=1)	59.6	38.9

The 2,3,7,8-TCDD congener was tested at the ppt level to simulate actual enviromental samples. To assure the immunoaffinity column was binding through antibody-antigen recognition, non-specific chicken antibodies were also immobilized to CNBr-Sepharose to serve as a negative control. As data in Table II show, over 90% of the radioactivity does not bind to the non-specific column while the dioxin-specific column binds ^{3}H-2,3,7,8-TCDD. The protein measurements indicate that serum proteins were detectable at the beginning of the 10% acetone wash. Protein was not detected in the 50% acetone elution fractions. For the dioxin-specific column, radioactivity was eluted with 50% acetone demonstrating that the immunoaffinity column effectively separates interference from TCDD.

Table II. Percent recovery of ^{3}H-2,3,7,8-TCDD in serum applied to DD3 and negative control columns.

IAC	10% acetone	50% acetone
Negative control (n=3)	94.8±6.1	2.8±1.4
DD3 (n=3)	11.4±2.2	86.1±11

Compared to our previous study using polyclonal antibodies isolated from egg yolks[3], this monoclonal antibody showed higher affinity and eliminated the need for pre-affinity column treatment of serum samples. While 0.5% non-ionic detergent was able to elute dioxins from our previous polyclonal immunoaffinity column, this elution system was unable to elute TCDD from the monoclonal immunoaffinity column. Common affinity column elution conditions such as chaotropic agent (3M NaSCN), high salt (1M NaCl), and low pH (0.1M glycine pH3.0) were tried for this column with no success. In addition, low percentages of organic solvents such as 30% methanol or 30% acetone had minimal effect on the elution of TCDD from the IAC. This may be due to strong hydrophobic binding, which would be unaffected by agents which predominately interrupt ionic bonding.

From HRGC-MS analysis, the overall recovery of ^{3}H-2,3,7,8-TCDD from spiked serum samples was 90 and 71.5% for two repetitions. These recoveries are well within EPA dioxin sample cleanup requirements. Samples with multiple congeners will be tested for the dioxin specific IAC column in order to assess the congeners binding specificity.

Conclusion

The current immunoaffinity column generated from monoclonal antibodies shows the ability to retain both 1,3,7,8-TCDD and 2,3,7,8-TCDD congeners. Due to the antibody's high affinity, no preaffinity column treatment was needed for serum samples. Changing from non-ionic detergent to 50% acetone as the elution solvent simplified the post-affinity column cleanup procedure by avoiding solid phase extraction steps, which were necessary with the polyclonal column[3]. The immunoaffinity column can simplify dioxin serum sample cleanup and decrease solvent consumption, thereby decreasing the analysis costs.

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Names are necessary to report factually on available data; however, the USDA neither gurantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

References

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