SIMPLIFIED SAMPLE PREPARATION METHODS FOR RAPID IMMUNOASSAY ANALYSIS OF PCDD/Fs IN FOODS

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Introduction

Immunochemical analysis of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) has been an important research goal for more than two decades, but without significant success until the late 1990's[1]. One recently developed enzyme immunoassay (EIA) for PCDD/Fs has demonstrated correlation with TEQ to low pg/g levels in soils and fly ash samples[2,3,4]. Broad application of this immunoassay method to food samples would be greatly facilitated by rapid sample preparation methods which take full advantage of the speed and simplicity of the EIA. This paper describes the development of rapid sample preparation methods designed specifically for this EIA. The key component of these sample preparation methods is a proprietary activated carbon column which uses polytetrafluoroethylene (PTFE) column materials to reduce adsorptive losses. This inexpensive disposable column is used as a first step for retention of the total aromatic portion of a sample while the bulk of the sample lipid and other matrix materials are passed through the column. The eluate is then treated with concentrated sulfuric acid and exchanged to methanol for EIA analysis. The procedure for loading, washing and elution is much simpler and faster than conventional carbon column chromatography. This carbon column method, when coupled to the acid treatment, comprises a rapid and simple two step cleanup procedure for EIA use. When combined with the EIA, this cleanup yields a complete method for low ppt PCDD/F analysis. The entire sample preparation and EIA analysis can be performed in approximately 10 hours for a batch of 8 to 16 samples.

Materials and Methods

The PCDD/F immunoassay was performed as described previously[4]. Activated carbon columns were made from commercially available PTFE column components and a PX-21 activated carbon based packing. Columns contained 150 mg of activated carbon adsorbed to Celite 545 (8% w/w). Lard and cream were purchased locally and were used without prior PCDD/F analysis. Fish oil samples were tested for PCDD/Fs by conventional HRGC-HRMS and were used without further processing. Toluene was Burdick & Jackson residue analysis grade; other solvents were HPLC grade. Analytical standard grade 2,3,7,8-TCDD was purchased from Ultra Scientific.

Prior to EIA analysis, samples were prepared by the following method. A 25 mL glass reservoir was rinsed with acetone and loaded with 6 g of anhydrous sodium sulfate. An activated carbon column was attached to the tip of the reservoir and the reservoir and column were washed with 5 mL of hexane, forced through at 1-2 mL/min by slight manual pressurization using a rubber stopper and stopcock assembly. Lard samples (melted with a hot air stream) or fish oil samples were dissolved in 25 mL solvent and added to the reservoir. Slight pressure was applied as before until the entire sample had passed through the carbon column at 1-2 mL/min. Clean solvent (7-25 mL of the same solvent used for loading) was added to the reservoir and the column was pressurized as before for washing in the forward direction. The column was removed from the 25 mL reservoir and placed on a clean 7 mL reservoir for additional washing and elution in the reverse direction. After washing with 7-14 mL of the load solvent, the sample was eluted with 7-10 mL toluene. The toluene was evaporated under a stream of nitrogen at 50°C, hexane was added, and the sample was shaken vigorously for 5 to 15 minutes with concentrated sulfuric acid. The supernatant hexane was recovered and evaporated using a sample keeper system based on Triton X-100 nonionic detergent and tetraethylene glycol. The keeper-sample residue was dissolved in methanol and added to the EIA. The protocol used allows for recovery of 80% of the original sample volume for a single EIA analysis (if 5 g sample is introduced to the carbon column, then 4 g sample equivalent is recovered for introduction to the EIA tube).

Results and Discussion

Initial method development was performed with lard. Because lard in hexane and hexane:dichloromethane (1:1) did not produce a completely clear solution, a small amount of isopropanol was added to fully dissolve the lard samples. The final solvent mixture for carbon column loading and washing of the lard samples was dichloromethane:hexane:isopropanol (25:25:1). Lard samples (10 g) were spiked with 50 or 200 pg of 2,3,7,8-TCDD and processed as described above. Early attempts at direct EIA analysis of exchanged toluene eluates clearly indicated that an additional acid treatment was required. Samples added to the EIA without acid treatment caused clouding due to excessive lipid content. After acid treatment, nearly all samples gave a clear solution when added to the aqueous EIA system. With the combined carbon column and acid treatment, statistically significant differences were observed between unspiked and 5 pg/g spiked samples (uncorrected mean±SD = 0.5±0.2 pg/g vs. 1.4±0.4 pg/g; Figure 1).

The data of Figure 1 show that the recovery corrected concentration of the higher spike group (9.2±1.8 pg/g) is significantly below the actual spike level of 20 pg/g. The fact that this difference exists for recovery corrected samples indicates that the large reduction from the expected response is due to a false negative interference rather than low recovery of analyte from the carbon column. Similar results were obtained for one fish oil sample spiked at 5 or 20 pg/g and analyzed by the same method, except using dichloromethane:hexane:acetone (1:1:1) as the load and wash solvent. Discrimination between unspiked and 5 pg/g spiked oil was observed, but quantitative recovery values were consistently low (data not shown). Four fish oil samples which had been analyzed by GC-MS (ranging from 2 to 21 pg/g) were tested by EIA without spiking to determine method correlation. These samples gave a high correlation coefficient ($r = 0.97$; other data not shown), indicating a useful relationship between EIA result and TEQ. However, the

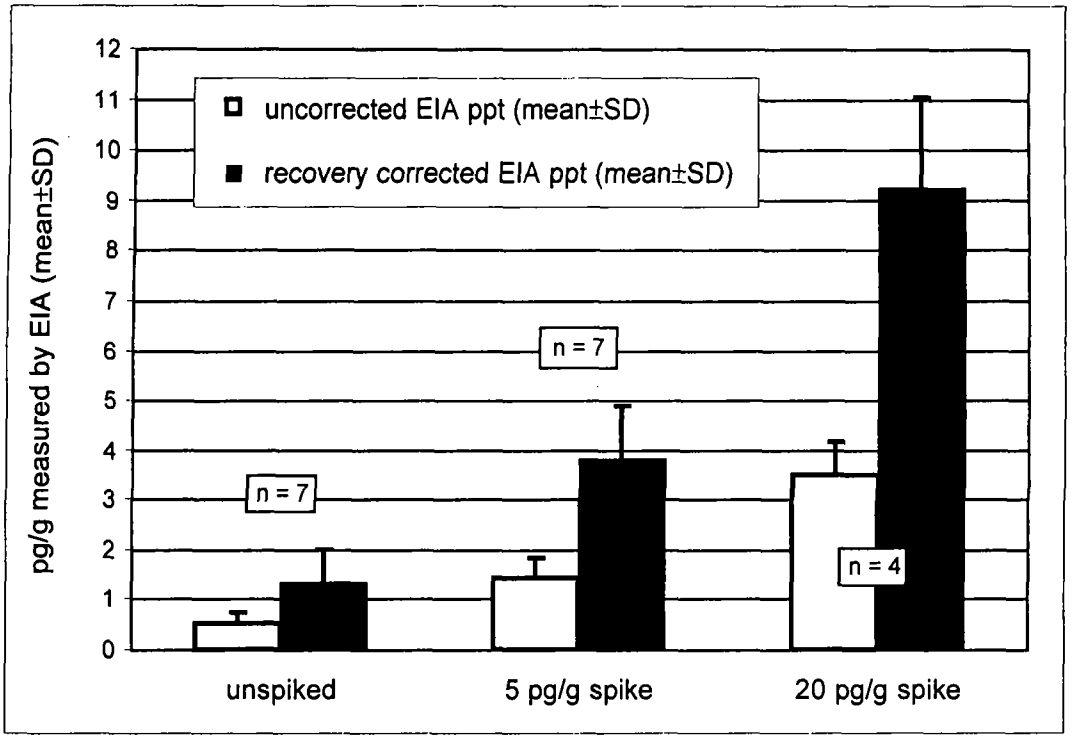

Figure 1. Recovery of 2,3,7,8-TCDD spiked into lard using rapid sample preparation and EIA analysis. Lard aliquots (10 g per EIA analysis) were melted, spiked, and analyzed as described in the materials and methods section. Replicate analyses were performed over 5 days. Spiked method blanks were used to determine recovery for each run. Based on these recovery values, a corrected concentration was calculated for each group of replicates.

slope of the regression line was 0.2, again indicating low recovery of dioxin by the EIA. This result was consistent with the low recovery (≤10%; data not shown) of 50 pg TCDD spikes from all four fish oil samples.

Cream was chosen to test the feasibility of analyzing samples such as milk and milk products, which can not be processed directly over the carbon columns because of their high water content. Heavy cream was spiked with 2,3,7,8-TCDD and mixed gently overnight with hexane and conc. HCl. After centrifugation, the hexane was removed, washed briefly with aqueous NaCl, and loaded directly to the carbon column as described above. The eluted sample was treated with sulfuric acid as described above and analyzed by EIA, giving values of 1.3, 3.9, and 6.2 pg/g, respectively, for spike levels of 0, 5, and 20 pg/g. This performance demonstrates the ability to recover dioxin from cream with a very simple sample preparation method. However, the same low recovery at higher spike levels was observed as for lard and fish oil.

Based on these results, additional experiments were performed with two goals. The first goal was to demonstrate recovery of analyte from the carbon columns irrespective of wash procedure and other factors. The second goal was to improve the wash procedure and reduce the potential for both false positive and false negative interferences. For the first goal, ^{14}C-2,3,7,8-TCDD was added to lard and fish oil matrices, then loaded onto carbon columns, washed, and eluted as described above. Load, wash, and elution solvents were counted directly in a liquid scintillation counter to determine dioxin concentrations. These radiotracer studies gave recoveries from the carbon column of 81-93% for all tested combinations of matrix, load solvent, and non-aromatic wash solvent. Attainment of the second goal requires a combination of radiotracer and EIA analysis to validate the final column wash protocol. Preliminary data indicate that a wash in the forward direction with 15-20 mL of 30% toluene in non-aromatic solvents gives acceptable removal of matrix interferences while retaining over 95% of the 2,3,7,8-TCDD spike. Subsequent elution in the reverse direction gave nearly quantitative spike recovery with less than 7 mL of toluene. These initial results based on radiotracer studies have been confirmed by EIA using spikes as low as 20 pg of 2,3,7,8-TCDD. Testing by EIA shows reduction of interferences to minimal levels. Work toward a final method is presently focused on refinement of this column wash protocol. All of the above results are consistent with immunoassay interferences as the primary cause of low recovery. The process described above of development of an immunoassay specific sample preparation method illustrates the need to treat the total EIA method as separate components- EIA and sample preparation.

The sample preparation and EIA analysis described here can be performed in a simple field lab with minimal equipment. Requirements for the EIA are limited to 2 or 3 widely used specialty pipettors and a small portable differential photometer. Requirements for sample preparation include an orbital shaker, tabletop centrifuge, small fume hood, and a system for evaporation of less than 10 mL of solvent from several samples at a time. The sample preparation scheme described here relies on a familiar method, modified in a way that would likely be unacceptable for GC-MS analysis, but which works well with the EIA. The low cost, rapid turnaround time, and portability of this system offer a completely different approach to the analysis of dioxin, which should provide an excellent complement to conventional methods.

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