ELSEVIER

Journal of Chromatography B, 757 (2001) 285–293

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

On the isolation of polychlorinated dibenzo-*p*-dioxins and furans from serum samples using immunoaffinity chromatography prior to high-resolution gas chromatography–mass spectrometry

Janice K. Huwe[a,*], Weilin L. Shelver[a], Larry Stanker[b], Donald G. Patterson Jr.[c], Wayman E. Turner[c]

[a]*USDA, ARS, Biosciences Research Laboratory, P.O. Box 5674 University Station, Fargo, ND 58105, USA*
[b]*USDA, ARS, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94170, USA*
[c]*Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Center for Disease Control and Prevention, 4770 Buford Highway N.E., Atlanta, GA 30341-3721, USA*

Received 5 January 2001; received in revised form 2 March 2001; accepted 5 March 2001

Abstract

Immunoaffinity chromatography (IAC) for the purification of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) from biological samples was explored as a means to simplify the cleanup procedure and thereby decrease the time and cost of dioxin analysis. A monoclonal antibody (DD3) was used to produce IAC columns and to isolate the PCDD/Fs from serum. Native and ^{13}C-labeled PCDD/Fs were spiked at the ppq to ppt range into serum. Quantitation of the PCDD/Fs was performed by a standard dioxin analytical method, i.e. high-resolution gas chromatography–mass spectrometry (GC–MS), which was easily compatible with IAC. Five of the most toxic PCDD/Fs consistently showed acceptable recoveries (>25%) and were reliably quantitated. The congeners specifically recovered by this method represent almost 80% of the toxic equivalency of dioxins and furans present in the serum samples. Dioxin-like polychlorinated biphenyls (PCBs) were not recognized by this antibody column. Compared to conventional dioxin cleanup methods, IAC decreased solvent usage by 1.5 l/sample and took only 2 h to process a sample for analysis. Published by Elsevier Science B.V.

Keywords: Polychlorinated dibenzo-*p*-dioxins; Furans

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) are a class of persistent organic pollutants known to cause adverse health effects including immunochemical, developmental, and neurological dysfunctions [1]. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (Fig. 1) is considered the most toxic dioxin and was recently named a known human carcinogen by the World Health Organization [2]. Other dioxins, the closely related furans, and certain polychlorinated biphenyls (PCBs) share a common

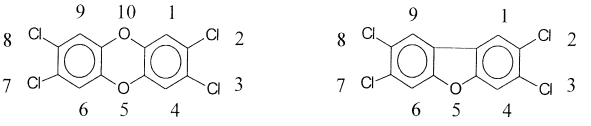

Fig. 1. Structure and numbering of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran.

*Corresponding author. Fax: +1-701-239-1430.
E-mail address: huwej@fargo.ars.usda.gov (J.K. Huwe).

0378-4347/01/$ – see front matter Published by Elsevier Science B.V.
PII: S0378-4347(01)00159-1

mechanism of toxicity with TCDD. Congeners are given toxic equivalency factors (TEFs) relative to that of TCDD whose TEF is assigned a value of 1.0 based on in vitro and in vivo toxicity studies [3]. In total, 17 dioxin and furan congeners have measurable toxicity and are routinely quantitated in dioxin analyses. Thirteen PCB congeners have been assigned TEFs [3] and are often included for quantitation in dioxin analyses.

Dioxins and furans are byproducts of certain incineration, smelting, and chlorophenol manufacturing processes. Environmental contaminations have occurred due to chemical plant accidents such as in Seveso, Italy, and through releases of contaminated products such as the herbicide Agent Orange or the oil spread at Times Beach, MO. Recent contaminations of the food supply by PCDD/Fs occurred in the US poultry industry in July 1997 and in Belgian poultry, pork, and dairy in early 1999 due to adulterated feed additives. All of these instances required widespread testing for PCDD/Fs to remediate sites and monitor exposed populations or livestock.

A major obstacle to performing routine monitoring of PCDD/Fs is the cost of analysis ($800–1200/sample). Because dioxins are present at extremely low levels, i.e. part per trillion (ppt) or even part per quadrillion (ppq), samples require extensive cleanup before detection; this contributes to the cost and also the time required for the analysis. An established cleanup method for serum includes liquid–liquid or C_{18} solid-phase extraction followed by multiple chromatography steps using acid, basic, and neutral silica gel, basic alumina, and carbon columns [4,5]. In our laboratory this procedure takes 1 full day and consumes 1.5 l of organic solvents and 100–200 ml of sulfuric acid per sample. The other cost factor in dioxin analyses involves detection of multiple PCDD/F congeners at the ppt level for which isotope dilution techniques utilizing high-resolution gas chromatography–high-resolution mass spectrometry (HRGC–HRMS) are currently the only acceptable methods (EPA Methods 1613 and 8290).

Several efforts to reduce the cost of dioxin analyses have focussed on the use of immuno-and receptor-based screening assays to replace the expense and complexity of HRGC–HRMS. Polyclonal and monoclonal antibodies have been used to develop radioimmunoassays [6,7] and enzyme immunoassays [8–11]. While detection limits for these assays were in the 10–25 pg range with standards, detection limits were generally over 200 ppt with actual sample matrices even after extensive cleanup. In addition to matrix interferences, other drawbacks to these methods are the need for radioactive or conjugated dioxin-like competitors, complications due to varying cross reactivities of each congener with the antibody, and the lack of quantitation for individual congeners.

Another application of antibodies to analytical methodology is in the purification rather than the detection phase. Immunoaffinity chromatography (IAC) has been applied as a highly specific means of purifying drug or pesticide residues from complex biological fluids [12,13]. Often several members of a multi-component family of compounds can be adequately extracted with a single antibody column.

The use of immunoaffinity columns has the potential to improve throughput, reduce costs, and limit the amount of hazardous solvents needed in PCDD/F cleanups. At the same time, IAC allows detection by HRGC–HRMS thereby maintaining the benefits of isotope dilution, congener-specific quantitation. We have previously shown that IAC using columns generated from a polyclonal antibody was effective in isolating 1,3,7,8-TCDD from serum and milk [14,15]. We now report the use of a monoclonal antibody with specificity towards several of the 2,3,7,8-substituted dioxins as a means of isolating PCDD/Fs from serum samples.

2. Materials and methods

Water and all solvents were HPLC grade. Bovine serum was purchased from Sigma–Aldrich (St. Louis, MO). Quality control serum samples were supplied by CDC. Protein assay reagents were obtained from Bio-Rad Laboratories (Hercules, CA). CNBr-activated Sepharose 4B was purchased from Amersham-Pharmacia (Uppsala, Sweden). ^{3}H-2,3,7,8-TCDD with a specific activity of 28.4 Ci/mmole was purchased from ChemSyn Laboratories (Lenexa, KS). The following ^{13}C-labeled and native PCDD/F solutions for EPA Method 1613 were purchased from Cambridge Isotope Labs (Andover,

MA): labeled compound stock (LCS) containing 15 $^{13}C_{12}$-labeled congeners in nonane, 100–200 ng/ml; precision and recovery standard (PAR) containing 17 native congeners in nonane, 40–400 ng/ml; and internal spiking standard (ISS) containing $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD in nonane, 200 ng/ml.

A hybridoma, DD3, that secreted dioxin antibody has been produced and characterized by Stanker et al. [9]. The antibody was produced either by growing DD3 in culture media or by generating the antibody in ascites from BALB/c mice. Ascites or the cell culture supernatant was used to isolate the immunoglobulin G (IgG). For example, ascites fluid was mixed with 0.1 *M* sodium acetate, pH 5.0, in a ratio of 1:1. The diluted antibody was applied to a 2 ml ImmunoPure IgG purification column (Pierce, Rockford, IL) and the non-bound protein was removed with 10 ml of 0.1 *M* sodium acetate buffer, pH 5.0. The IgG was eluted with 6 ml of 0.1 *M* glycine–HCl, pH 2.8, and immediately neutralized with 0.1 *M* Tris buffer, pH 9. The IgG was quantitated by the method of Bradford [16] and assessed for purity by 10% SDS–PAGE. The IgG fraction was buffer exchanged by passing through a 5 ml Excellulose™ GF-5 desalting column (Pierce, Rockford, IL) and stored at −20°C until used.

Immunoaffinity columns were prepared by immobilizing the DD3 antibody to CNBr-activated Sepharose bead as described previously [14]. Briefly, CNBr-Sepharose gel beads were activated with 1 m*M* HCl. The slurry of the activated CNBr-Sepharose was mixed with DD3 IgG (5 mg protein per ml of gel) in the presence of conjugation buffer, 0.1 *M* sodium bicarbonate, pH 8.3, 0.5 *M* NaCl. The unconjugated IgG was removed by washing with the sodium bicarbonate buffer and the excess active groups in the gel were reacted with 0.1 *M* Tris, pH 8. The conjugated gel was washed three times alternately with 0.1 *M* Tris, pH 8, 0.5 *M* NaCl and 0.1 *M* acetate buffer, pH 4, 0.5 *M* NaCl. The efficiency of the conjugation was determined by measuring protein in wash fractions. The final gel was stored in PBS/0.02% sodium azide at 4°C until used.

A typical dioxin solution for spiking the bovine serum was prepared by combining PAR (5 μl) and LCS (100 μl), evaporating the nonane with a stream of N_2, and reconstituting in acetone (100 μl). Bovine serum was spiked with 10 μl of this solution for a total of 1000 pg each of 14 $^{13}C_{12}$-labeled tetra through hepta congeners, 2000 pg $^{13}C_{12}$-OCDD, 20 pg each of TCDD and TCDF, 100 pg each of 13 penta through hepta congeners, and 200 pg each of OCDD and OCDF. Alternatively, bovine serum was spiked with 200 pg of ^{3}H-2,3,7,8-TCDD in 10 μl acetone. Spiked samples were allowed to equilibrate 30 min at room temperature. Samples prepared in advance were frozen at −20°C until used. The serum samples (1 or 25 g aliquots) provided by CDC were from composite pools which served as quality control standards for dioxin analyses. These samples contained 18 native PCDD/Fs and four co-planar PCBs and were spiked with $^{13}C_{12}$-labeled standards according to CDC protocol before application of IAC [17].

Gravity-flow DD3 columns (0.5 or 2 ml) were packed in disposable glass pipets over a glass wool plug and equilibrated with five column volumes of water or prewashed sequentially with five column volumes of water, 10% acetone, 50% acetone, and water before use. Column flow-rates were <0.5 ml/min. Serum was applied directly to the column. Matrix impurities were removed by washing the column with five column volumes of 10% acetone. PCDD/Fs which bound selectively to the IAC were eluted with five column volumes of 50% acetone. This eluate was concentrated with a stream of N_2 to remove most of the acetone, extracted three times with methylene chloride, passed through dry sodium sulfate (2 g), and concentrated with a keeper solvent (dodecane). ISS was added immediately before analysis by HRGC–HRMS.

HRGC–HRMS was performed on a Carlo-Erba 8000 gas chromatograph coupled to a Micromass Ultima Autospec mass spectrometer according to EPA Method 1613 (Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS). The spectrometer was operated in the electron impact ionization mode using selected ion monitoring at a resolution of 10 000–11 000. Samples were autoinjected in the splitless mode. Chromatographic separation was achieved on a 60 m× 0.32 mm I.D. DB-5MS column (J&W Scientific, Folsom, CA) with the following temperature program: hold at 140°C for 1 min; ramp from 140 to 190°C at 20°C/min; hold at 190°C for 1 min; ramp

from 190 to 300°C at 2°C/min. A fast GC method on a 2 m×0.1 mm I.D. DB-1 column (J&W Scientific, Folsom, CA) was used to analyze the CDC serum samples. This program eluted OCDD in under 10 min but failed to resolve four pairs of hexa and hepta congeners [18].

Non-specific immunoaffinity columns were prepared with IgG antibodies from naive chickens and eluted in the same manner as described above.

Serum samples were applied to the immunoaffinity columns by several methods. Two milliliter samples of serum were equilibrated on 2 ml columns for 30 min, before washing and eluting, by stopping the column flow after the sample was loaded. Twenty-five milliliter samples were equilibrated with 2 ml columns by rotating together in a Teflon container for 1 h and then repacking the column in a glass pipette. For the 0.5 ml columns, all samples were equilibrated simply by loading the sample at a flow-rate of less than 0.5 ml/min.

Radioactivity was measured by liquid scintillation counting in EcoLite cocktail (ICN, Costa Mesa, CA) on a Packard Tri-Carb® analyzer (Meridan, CT).

3. Results

In order to quickly assess the performance of the DD3-IAC, ^{3}H-2,3,7,8-TCDD was used in preliminary studies making detection and recovery determinations rapid. When 2 ml of serum containing ^{3}H-TCDD (200 pg) was applied to a 2-ml DD3 column (n=3), 11.4±2.2% of the radiolabel was recovered in the 10% acetone wash while 86.1±11% bound to the column and was eluted with 50% acetone. The average total recovery was over 97%. Using a non-specific IgG column (n=3), 94.8±6.1% of the radiolabel was not bound but came through in the 10% acetone wash, demonstrating no non-specific binding to the Sepharose columns. Unlike a polyclonal IAC method which we have developed [14,15], the use of non-ionic detergents to remove the dioxins from the DD3 column was unsuccessful. Common elution conditions such as chaotropic agents (3 *M* NaSCN), high salt (1 *M* NaCl), or low pH (0.1 *M* glycine, pH 3.0) were also unsuccessful at removing TCDD from the column. Low percentages of organic solvents such as 30% methanol or 30% acetone removed only small amounts of the ^{3}H-TCDD (1 and 7%, respectively).

The broad-range specificity of the DD3 column was determined with bovine serum (2 ml) spiked with a panel of dioxins and furans ranging from tetra- to octa-substituted. Table 1 shows recoveries of the 15 ^{13}C-labeled congeners in the 50% acetone elution as determined by HRGC–HRMS. According to EPA Method 1613, recoveries of 25–150% are required for acceptable method performance. Five congeners met these recovery requirements. Toxic equivalency factors (TEFs) for each congener are also listed in Table 1 and demonstrate the column's specificity for the three most toxic congeners, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF.

Because of the low levels of PCDD/Fs generally found in serum (low ppt to ppq), typical analytical methods require 25 ml of serum for adequate detection. Table 2 shows initial results from the application of 25 ml spiked serum to a 2 ml DD3 column. Serum I was applied to a column which had been conditioned with water before use and shows recoveries in the 50% acetone eluate of up to 40% for 2,3,7,8-TCDD. Re-equilibration and use of the same column (serum II) resulted in a significant improvement in recoveries, e.g. 69% for 2,3,7,8-TCDD. The increased recoveries may be an artifact due to carryover from the first sample or a result of

Table 1
Recoveries and toxic equivalency factors (TEF) for $^{13}C_{12}$-labeled dioxins and furans; 2 ml serum spiked and applied to new 2 ml DD3 columns

^{13}C-Congener	TEF	% Recovery, n=3
2378-TCDF	0.1	15.8±2.7
12378-PeCDF	0.05	5.9±1.8
23478-PeCDF	0.5	37.8±5.0
123478-HxCDF	0.1	2.7±1.2
123678-HxCDF	0.1	1.2±0.9
234678-HxCDF	0.1	31.5±6.3
123789-HxCDF	0.1	0.9±0.7
1234678-HpCDF	0.01	1.3±0.9
1234789-HpCDF	0.01	0.9±0.7
2378-TCDD	1	53.3±4.4
12378-PeCDD	1	42.7±17.7
123478-HxCDD	0.1	18.0±3.5
123678-HxCDD	0.1	32.4±3.9
1234678-HpCDD	0.01	13.2±1.8
OCDD	0.0001	0.9±1.0

Table 2
Recoveries of $^{13}C_{12}$-labeled dioxins and furans from a DD3 column; 25 ml serum spiked and applied to 2 ml columns

^{13}C-Congener	% Recovery Serum I	% Recovery Serum II	% Recovery no prewash, $n=2$	% Recovery prewash, $n=3$
2378-TCDF	30.9	52.2	24.8±8.7	43.2±37.1
12378-PeCDF	6.7	11.9	4.7±2.8	8.5±1.6
23478-PeCDF	29.1	74.6	27.6±2.1	59.9±63.9
123478-HxCDF	4.9	5.9	2.9±2.8	3.7±0.5
123678-HxCDF	3.6	2.2	2.0±2.3	1.3±0.3
234678-HxCDF	19.9	39.1	15.2±6.7	27.3±28.9
123789-HxCDF	3.5	0.6	1.8±2.4	0.3±0.1
1234678-HpCDF	5.1	2.0	2.7±3.5	0.8±0.2
1234789-HpCDF	3.6	1.0	1.9±2.5	0.5±0.1
2378-TCDD	39.8	69.0	28.0±16.7	40.3±37.9
12378-PeCDD	33.4	60.2	24.3±12.9	38.4±35.4
123478-HxCDD	15.2	27.4	10.2±7.1	14.1±10.2
123678-HxCDD	16.3	29.7	10.4±8.3	11.1±14.1
1234678-HpCDD	9.9	15.1	6.7±4.6	8.1±5.5
OCDD	0	0	0.7±1.0	0.2±0.1

''preconditioning'' the column with the first sample. When blank bovine serum (3 ml) was applied to a previously used DD3 column, incubated, and eluted as before, no dioxins or furans were detected in the 50% acetone eluate by HRGC–HRMS (data not shown). This indicated that carryover was not a problem from run to run.

Preconditioning an IAC column could increase its affinity for PCDD/Fs by removing impurities which interfere with substrate binding or by solvating the beads in a manner which makes the antibody more accessible to binding. Preconditioning with the first sample may also have blocked sites on the columns which led to irreversible binding and low initial recoveries; however, previous results with ^{3}H-TCDD had indicated no irreversible binding to the columns (97% of ^{3}H-TCDD recovered.) We therefore investigated prewashing new DD3 columns with 10% and then 50% acetone solutions as a means to improve recoveries. The results of numerous trials with and without prewashing are shown in Table 2. Although the prewashing appears to provide some increases in recoveries, the variability from run to run obscures this conclusion. This variability may be attributed to the use of different batches of immobilized antibody and/or the inefficiency of the equilibration process which entailed mixing the support and serum on a rotating mixer.

In order to optimize the IAC capacity, minimize variability, and at the same time conserve our supply of antibody, smaller size columns were evaluated for performance and ruggedness using gravity-flow to equilibrate. Table 3 shows recoveries from prewashed 0.5 ml DD3 columns. For first-use columns, four congeners were recovered at over 45%; three other congeners at 13–20%. Compared to recoveries from 2 ml columns, the values were similar but less variable. Because each of the 0.5 ml column replicates utilized a different batch of immobilized antibodies, it appeared that variability did not arise so much from the antibody batches as from the equilibration method. Multiple uses of the 0.5 ml columns (up to three) showed some degradation of the column's performance. Recoveries of the two most toxic congeners, 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, decreased from 45 to 40% and from 45 to 25%, respectively, from the first to the third use. Based on these observations, prewashing may be desirable, but multiple uses of a single column is not recommended for optimal performance.

The precision and accuracy of the DD3-IAC cleanup method were determined by quantitation of the native congeners spiked into 25 ml of serum. Table 4 lists the average congener values for single-use 2 ml columns and single and multiple-use 0.5 ml columns. These values were compared to the actual values in the spiking solutions as determined by HRGC–HRMS and evaluated under our quality

Table 3
Recoveries of $^{13}C_{12}$-labeled dioxins and furans from a prewashed DD3 column; 25 ml serum spiked and applied to 0.5 ml columns multiple times

^{13}C-Congener	% Recovery first use, $n=3$	% Recovery second use, $n=3$	% Recovery third use, $n=3$
2378-TCDF	48.5±8.1	29.5±4.7	30.5±3.7
12378-PeCDF	13.5±3.2	7.1±1.2	7.0±1.2
23478-PeCDF	55.3±5.5	33.5±0.7	31.2±5.3
123478-HxCDF	6.6±2.1	3.2±0.8	3.1±0.7
123678-HxCDF	2.7±1.5	0.8±0.4	0.8±0.2
234678-HxCDF	19.6±5.4	11.4±4.7	13.2±2.9
123789-HxCDF	1.9±1.3	0.4±0.1	0.4±0.1
1234678-HpCDF	1.9±1.3	0.4±0.2	0.4±0.1
1234789-HpCDF	2.2±1.5	0.4±0.1	0.5±0.2
2378-TCDD	45.5±4.2	30.8±11.8	40.4±5.0
12378-PeCDD	45.3±10.0	15.2±4.9	24.7±12.7
123478-HxCDD	17.2±6.3	11.4±3.7	11.6±2.1
123678-HxCDD	4.1±3.6	1.3±0.7	2.7±0.3
1234678-HpCDD	6.7±1.8	4.2±2.0	4.8±0.9
OCDD	1.1±0.9	0.2±0.2	0.2±0.1

assurance–quality control criteria. Acceptance criteria, based on EPA Method 1613, required coefficients of variance less than 25% and mean values of replicates within 25% of the actual value.

Table 4 demonstrates that both 2 and 0.5 ml columns meet QA/QC standards for at least 10 of the 17 congeners. Unacceptable values were due to the low recoveries for those congeners (Tables 2 and 3),

Table 4
Quantitation of spiked native PCDD/Fs isolated from 25 ml serum on a 2 ml or 0.5 ml DD3 columns

Congener	Actual spike (pg) $n=3$	From 2 ml DD3, $n=5$ (pg)	C.V.[a] (%)	RSE[b] (%)	Actual spike (pg)	From 0.5 ml DD3, $n=7$ (pg)	C.V.[a] (%)	RSE[b] (%)
2378-TCDF	22.2±2.0	24.1±0.7	2.9	8.5	30.3	29.4±1.1	3.7	−2.9
12378-PeCDF	118.6±12.4	140.0±8.8	6.3	18.0	150.2	147.2±7.1	4.8	−2.0
23478-PeCDF	107.5±8.6	118.1±3.1	2.6	9.9	142.2	137.7±6.0	4.4	−3.1
123478-HxCDF	100.9±9.2	117.0±6.5	5.5	16.1	130.1	130.7±6.0	4.6	0.5
123678-HxCDF	98.1±5.1	128.1±18.3	14.3	30.6	132.5	134.0±12.7	9.5	1.1
234678-HxCDF	114.6±10.8	126.1±2.2	1.7	10.1	133.3	131.4±4.0	3.0	−1.4
123789-HxCDF	107.7±8.3	186.8±111.5	59.7	73.4	129.2	129.7±18.2	14.0	0.4
1234678-HpCDF	95.1±12.4	157.1±45.2	28.8	65.1	131.4	164.0±18.0	11.0	24.9
1234789-HpCDF	102.2±13.9	195.2±103.2	52.9	91.0	136.1	127.3±28.8	22.6	−6.5
OCDF	209.1±43.7	739.3±624.8	84.5	253.6	295.8	476.5±486	102.0	61.1
2378-TCDD	19.0±3.6	22.2±2.4	10.8	16.4	28.2	25.7±2.4	9.3	−9.1
12378-PeCDD	91.4±12.2	107.1±3.7	3.5	17.2	114.0	112.4±4.2	3.7	−1.4
123478-HxCDD	93.9±10.7	106.6±2.1	2.0	13.6	115.7	115.4±7.2	6.2	−0.3
123678-HxCDD	101.1±7.3	117.5±10.9	9.3	16.3	134.5	108.0±50.6	46.9	−19.7
123789-HxCDD	98.1±7.8	90.1±26.5	29.4	−8.2	123.0	200.8±302	150.4	63.2
1234678-HpCDD	116.7±15.6	159.3±70.8	44.4	36.5	152.9	112.1±11.6	10.3	−26.7
OCDD	205.1±39.3	93568±2.1×10^5	222.3	456043	289.8	1609.0±1240	77.1	455.5

[a] C.V., coefficient of variance; SD/mean.
[b] RSE, relative standard deviation; mean-actual value/actual value.

and the inclusion of columns used more than once did not adversely affect the quantitation of the PCDD/Fs.

A more rigorous check of performance was provided by using CDC serum samples which contained PCDD/Fs and PCBs in the ppq range. Table 5 shows recoveries of the ^{13}C-labeled surrogates from 1 and 25 g samples applied to prewashed, single-use, 0.5 ml DD3 columns. Recoveries of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF from the 25 g samples were over 50%. Five other congeners were recovered at over 10% which is considered acceptable by CDC criteria [17]. Recoveries from the 1 g samples were lower, but still over 10% for six PCDD/Fs. Because the 1 and 25 g samples contained the same amounts of PCDD/Fs and PCBs, it appears that the shorter residence time of the 1 g samples on the column led to less binding of substrate to antibody. In light of this observation, IAC flow-rates could be critical to sample recovery and reproducibility of results.

Table 5 also compares quantitation of the native congeners to on-going quality control data collected at CDC using standard purification methods [4]. Seven of the congeners fell within the 95% confidence limits of the experiment; however, OCDD had a recovery which precluded its acceptance. Two other pairs of congeners (1,2,3,4,7,8-HxCDD/1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD/1,2,3,4,6,7,9,-HpCDD) fell within the 95% confidence limit sums for the pairs but could not be evaluated due to lack of resolution in the ''fast'' GC program used in this particular analysis [18]. Overall the method met performance and accuracy limits for six congeners: 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, and 1,2,3,7,8,9-HxCDD. These congeners have toxic equivalency factors $\geq$0.1, account for

Table 5
Recovery and quantitation for PCDD/F/PCBs isolated from serum with prewashed, single-use, 0.5 ml DD3 columns

Congener	% Recovery, n=3		Quantitation for 25 g sera, n=3		Mean spike target[a], n>114	95% Confidence limits for 25 g sera[a], n>114	
	1 g sera	25 g sera	ppq	C.V.[b] (%)	ppq	ppq	In limits
2378-TCDF	14.1±1.5	32.9±3.0	200.3±5.1	2.6	217.2±23.08	172.0–262.4	yes
12378-PeCDF	13.5±3.0	14.1±2.3	100.3±14.3	14.3	213.3±25.18	164.0–262.6	
23478-PeCDF	18.4±4.4	56.2±10.0	223.3±14.0	6.3	201.5±22.99	156.4–246.6	yes
123478-HxCDF/					197.8±26.43	146.0–249.6	
123678-HxCDF	6.1±2.0	7.0±0.4	121.7±13.1	11.5	210.0±25.62	159.8–260.2	
123789-HxCDF	3.7±1.3	2.7±0.7	nd		199.6±24.25	152.1–247.1	
234678-HxCDF	11.8±3.3	37.9±8.2	235.0±2.0	0.9	213.3±26.90	160.6–266.0	yes
1234678-HpCDF/					561.0±97.77	369.4–752.6	
1234789-HpCDF	1.0±0.4	1.0±0.1	nd		209.7±34.12	142.8–276.6	
OCDF	0.8±0.4	0.7±0.1	nd		304.8±196.7	−80.8–690.4	
2378-TCDD	18.4±4.4	53.3±8.5	238.3±8.5	3.6	203.2±23.87	156.4–250.0	yes
12378-PeCDD	14.2±2.7	54.4±9.4	249.0±18.2	7.3	217.4±24.72	168.9–265.9	yes
123478-HxCDD/					177.6±22.02	134.4–220.8	
123678-HxCDD	3.6±1.4	12.7±1.8	655.3±91.1	13.9	494.1±49.59	396.9–591.3	?
123789-HxCDD	6.3±1.7	11.6±0.8	173.7±8.6	5.0	209.4±25.86	158.7–260.1	yes
1234678-HpCDD/					1077.0±162.0	759.4–1394.6	
1234679-HpCDD	1.7±0.4	5.5±0.4	1032.0±19.7	1.9	141.7±31.40	80.2–203.2	?
OCDD	0.7±0.2	0.7±0.1	7485.0±3034	40.5	8324.2±1911.9	4576.9–12071.5	yes
3344-PCB	12.1±2.1	3.0±0.7	341.7±148	43.3	1068.2±136.6	800.4–1336.0	
3445-PCB	7.2±1.1	4.1±1.1	434.3±77.2	17.8	1044.1±112.8	822.9–1265.3	
33445-PCB	8.3±0.9	8.2±1.6	772.3±129.5	16.8	1351.9±162.6	1033.2–1670.6	
334455-PCB	3.6±0.2	3.7±0.7	nd		948.2±116.3	720.2–1176.2	

[a] Values determined from on-going conventional analysis using a method similar to EPA Method 1613 (4).
[b] C.V., coefficient of variance; SD/mean.

78% of the toxic equivalency (TEQ) of the sample due to PCDD/Fs, and represent the major congeners found in human serum on a toxicity basis [1]. The DD3 column had no specificity for the dioxin-like PCBs.

4. Conclusion

Immunoaffinity chromatography has the potential to become a practical alternative method for dioxin cleanup. It reduces the time and solvent consumption involved in traditional methods, and it is easily compatible with isotope dilution HRGC–HRMS analysis. For a 25 ml serum sample, total solvent usage with the IAC method (0.5 ml column) was 3 ml acetone and 3 ml methylene chloride compared to 100 ml sulfuric acid and 1500 ml organic solvents used in a conventional cleanup. A sample can be processed for analysis by HRGC–HRMS in about 2 h using IAC instead of 24 h by conventional methods.

Currently only one monoclonal antibody has been incorporated into the affinity column. These DD3 columns provide specificity for a number of the most toxic congeners; however, total congener analysis is not possible due to low recoveries of some of the higher chlorinated compounds. Until further antibodies are available which recognize these higher chlorinated congeners and the dioxin-like PCBs, these columns will be useful in screening samples, in instances where congener patterns are dominated by those congeners specific to DD3, and in cases were estimates of total toxicity are sufficient.

Improvements in recoveries and reproducibilities may be made by optimizing and controlling the flow-rates of the IAC procedure. Slower rates of application may improve binding by increasing residence time for small sample sizes. We are also investigating the use of automated solvent delivery systems to alleviate variability caused by flow-rate irregularities. As improvements are made to the IAC method and new antibodies are available to broaden the column's selectivity, this technique may eventually satisfy all the performance requirements for a full dioxin congener analysis. At this point it provides a simple, rapid screening method for dioxin TEQs in serum by meeting performance-based criteria for six of the most toxic dioxins and furans.

Acknowledgements

The authors would like to acknowledge Mr Richard Zaylskie for mass spectrometry services and Ms Vandi Anderson for technical assistance. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

References

[1] A. Schecter (Ed.), Dioxins and Health, Plenum Press, New York, 1994.
[2] Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans, IARC Monograph, Vol 69, Lyon, France, 1997.
[3] M. Van den Berg, L. Birnbaum, A.T.C. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, Environ. Health Persp. 106 (1998) 775.
[4] W.E. Turner, S.G. Isaacs, D.G. Patterson Jr., L.L. Needham, in: C. Rappe, H.R. Buser, B. Dodet, I.K. O'Neill (Eds.), Environmental Carcinogens Methods of Analysis and Exposure Measurement, Vol. 11, International Agency for Research on Cancer, Lyon, France, 1991, p. 343, Method 7.
[5] W.E. Turner, E.S. DiPietro, T.P. Cash, P.C. McClure, D.G. Patterson Jr., H. Shirkhan, Organohalogen Comp. 19 (1994) 31.
[6] J. Sherry, J. ApSimon, L. Collier, R. Wilkinson, P. Albro, B. Afghan, Chemosphere 19 (1989) 255.
[7] P.W. Albro, M.I. Luster, K. Chae, S.K. Chaudhary, G. Clark, L.D. Lawson, J.T. Corbett, J.D. McKinney, Toxicol. Appl. Pharmacol. 50 (1979) 137.
[8] J.R. Sanborn, S.J. Gee, S.D. Gilman, Y. Sugawara, A.D. Jones, J. Rogers, F. Szurdoki, L.H. Stanker, D.W. Stoutamire, B.D. Hammock, J. Agric. Food Chem. 46 (1998) 2407.
[9] L. Stanker, B. Watkins, M. Vanderlaan, W.L. Budde, Chemosphere 16 (1987) 1635.
[10] M. Vanderlaan, L.H. Stanker, B.E. Watkins, P. Petrovic, S. Gorbach, Environ. Toxicol. Chem. 7 (1988) 859.
[11] R.O. Harrison, R.E. Carlson, Chemosphere 34 (1997) 915.
[12] S.E. Katz, M. Siewierski, J. Chromatogr. 624 (1992) 403.

[13] L.A. Van Ginkel, J. Chromatogr. 564 (1991) 363.
[14] W.L. Shelver, G.L. Larsen, J.K. Huwe, J. Chromatogr. B 705 (1998) 261.
[15] W.L. Shelver, J.K. Huwe, J. Liq. Chrom. Rel. Technol. 22 (1999) 813.
[16] M. Bradford, Anal. Biochem. 72 (1976) 248.
[17] D.G. Patterson Jr., S.G. Isaacs, L.R. Alexander, W.E. Turner, L. Hampton, J.T. Bernert, L.L. Needham, in: C. Rappe, H.R. Buser, B. Dodet, I.K. O'Neill (Eds.), Environmental Carcinogens Methods of Analysis and Exposure Measurement, Vol. 11, International Agency for Research on Cancer, Lyon, France, 1991, p. 299, Method 6.
[18] D.G. Patterson Jr., J.R. Barr, E.S. DiPietro, J. Granger, V.E. Green, C.R. Lapeza Jr., V.L. Maggio, P.C. McClure, S. Sirimanne, W.E. Turner, Organohalogen Comp. 27 (1996) 309.