METHOD FOR EXTRACTION AND CLEANUP OF 17 2,3,7,8-SUBSTITUTED CHLORODIBENZODIOXINS/FURANS IN FISH

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Since 1981 the U. S. Food and Drug Administration (FDA) has used a method by Niemann et al.[1,2] to determine residues of chlorinated dibenzodioxins (dioxins) and dibenzofurans (furans) in finfish and shellfish. This method was optimized for 2,3,7,8-tetrachlorodioxin (TCDD) and then modified[3] to permit recovery of seven higher chlorinated dioxins (viz., 1,2,4,6,7,9-, 1,2,3,6,7,9-, 1,2,3,6,7,8-, and 1,2,3,7,8,9-hexachloro-, 1,2,3,4,6,7,9- and 1,2,3,4,6,7,8-heptachloro-, and 1,2,3,4,6,7,8,9-octachlorodioxin). The modification required a cleanup time of 10-20 h and was rarely used since TCDD became the target analyte in more than 90% of the analyses. Fehringer et al.[4,5] and Ahlrep et al.[6] also modified the Niemann method to achieve an easier cleanup, but did not increase its application beyond TCDD, which is just one of the seventeen 2,3,7,8-chloro-substituted dioxin and furan congeners considered toxic.[7] To properly assess the human health significance of dioxins and furans in foods, FDA needs methodology that can efficiently determine all 17 toxic congeners.

Firestone[8] surveyed and reviewed methods of analysis for dioxins and furans in foods and biological tissues. Selected techniques cited in that review, including the widely used method of Smith et al.,[9] were investigated for their ability to extract lipids and then isolate the 17 toxic congeners from the lipid material and coextracted pesticides/industrial chemicals. The most efficient techniques were optimized to achieve recoveries of 80% or greater for the 17 dioxins/furans and to produce extracts containing limited amounts of components that would interfere with mass spectrometry (MS) detection. The resultant extraction/cleanup time has been reduced to less than 5 h per determination. This report describes the method and presents recoveries from fish at three fortification levels. TCDD concentrations found in fish tissues analyzed in a six-laboratory round-robin study[10] and by the proposed method are also compared.

Experimental

Principle

Fish tissue is dried with sodium sulfate and extracted twice (without emulsion formation) with 5% dichloromethane (DCM) in hexane. Extracted lipids are partially removed by deactivated and activated silica gel in the extraction column. Remaining lipids are removed by a sulfuric acid/silica gel column. Planar dioxins, furans, and PCBs are isolated from other stable halogenated aromatic compounds by selective elution through activated carbon/silica gel and alumina columns.

Chemicals

Glassware was washed three times with acetone. All solvents were distilled in glass (Baxter Healthcare Corp., Muskegon, MI, USA). ^{12}C- and ^{13}C-labeled dioxin and furan spiking and surrogate standards were obtained from Cambridge Isotope Laboratories (Woburn, MA, USA). Octachlorodioxin and octachlorodibenzofuran were obtained from Ultra Scientific (North Kingston, RI, USA)

Fortification

A 1.00 mL aliquot of ^{12}C-labeled spiking solution in isooctane was added to 50.0 g triply ground skinless fillet. For nonfortification determinations a 1.00 mL aliquot of ^{13}C-labeled surrogate solution was added instead of spiking solution. The ground fillet was allowed to stand for 15 min after the addition of spiking or surrogate solution.

Extraction and cleanup

The 50.0 g ground fish was mixed with 400 g anhydrous sodium sulfate and dried in the hood overnight. The dried mixture was blended for 60 s and poured into a 600 x 50 mm i.d. extraction column containing 40 mm sodium sulfate, 25 g activated silica gel (125°C), and 7 g deactivated silica gel (10% water). Sodium sulfate (25 mm) was added to the column, and the column was eluted with 5% DCM in hexane. The eluate was collected in a 1 L round-bottom flask and the solvent was removed by rotary evaporation (30°C). The adsorbents were removed from the extraction column and dried for 1 h in the hood. The dried adsorbents were added to the extraction column containing 40 mm sodium sulfate and 10 g activated silica gel, and the elution and rotary evaporation steps were repeated.

The residue from the 1 L flask was transferred with three 10 mL hexane rinses to a 25 mm i.d. column containing 7 g 40% sulfuric acid/silica gel with a 100 mL round-bottom flask in place for collection of eluate. The column was eluted with an additional 30 mL hexane, and the solvent was removed by rotary evaporation as before. For catfish, the extract was divided in half, and each half was cleaned up on a separate column as above. Each acid/silica gel column removes 0.75 g lipid, and one column is sufficient for most fish species.

An 85 x 8 mm i.d. column containing 1.3 g 8% activated carbon/silica gel was prepared. The column was connected with Teflon tubing to a 100 mL solvent reservoir and a 4-port 90° valve to allow flow through the column to be reversed.[9] A 125 mL receiving bottle was placed at the valve outlet to collect the eluate.

The residue from the acid/silica gel cleanup was transferred to the carbon/silica gel column with four 5 mL DCM rinses, and the column was eluted with a total of 110 mL DCM. The direction of the column flow was reversed and the column was eluted with 40 mL toluene; a 100 mL round-bottom flask was used to collect the eluate. The toluene was concentrated to near dryness by rotary evaporation (55°C). The residual toluene was removed with a gentle stream of clean dry N_2 and finally by the addition of ca 0.5 mL hexane and re-evaporation to dryness under the N_2 stream.

The contents from the round-bottom flask were transferred to a 5 mm i.d. column containing

0.5 g activated (16 h at 125°C) neutral alumina by rinsing/swirling twice with 1.0 mL and once with 2.0 mL hexane. The eluate (fraction 1) was collected in a 10 mL tube. Elution was continued with 5.0 mL 1% toluene in hexane (fraction 2) and an additional 1.0 mL 1% toluene in hexane (fraction 3). Fractions 2 and 3 were collected in 10 mL receiving tubes. The receiver was changed to a 5.0 mL conical vial and the column was eluted with 2 mL DCM (fraction 4). A keeper solution (1.0 mL) (either 5 μg Parafilm/mL hexane or 25 μg tetradecane/mL hexane) was added to fraction 4. Also 1.0 mL ^{13}C-labeled internal standard solution was added to fortified samples. Fraction 4 (containing dioxins, furans, and planar PCBs) was evaporated to dryness under a stream of clean dry N_2, capped, and stored in the dark until determination.

Determination

Residue and recovery determinations were made using a Fisons Auto Spec high resolution mass spectrometer (Fisons Instruments, Beverly, MA, USA) equipped with an HP-5890 series II gas chromatograph. The mass spectrometer was operated in the electron ionization mode at 10,000 resolution (10% valley definition). Selected ion monitoring (SIM) data were acquired using an Opus 4000 data system with version 2.1 software. The column used was a 60 m x 0.25 mm i.d. DB-5ms fused silica capillary column (J & W Scientific, Folsom, CA, USA) temperature-programmed from 80°C (1 min hold) to 210°C at 70°C/min (12 min hold), to 225°C at 4°C/min (10 min hold), and then to 310°C at 5°C/min (10 min hold).

Approximately 20 g test sample equivalents (2 of 5 μL) were analyzed by gas chromatography/mass spectrometry (GC/MS) using splitless injection. The two most abundant ions from the molecular ion cluster and two ions from the corresponding -COCl loss for each [^{12}C]congener along with the two most abundant ions from the molecular cluster for the ^{13}C-labeled internal standards and a lock mass to compensate for any instrumental changes were monitored. All components that co-eluted with ^{13}C-labeled internal standards and had molecular ion and -COCl loss isotope ratio abundances within 10% of theoretical were quantitated. Concentrations of [^{12}C]congeners were determined from a calibration curve of (^{12}C/^{13}C) relative abundance response ratios versus concentration (pg) of ^{12}C. Concentrations were corrected for background responses obtained for unspiked fish or reagent blanks.

Results and Discussion

Average recoveries (n = 2) of seventeen 2,3,7,8-chloro-substituted dioxins and furans from farm-raised catfish, flounder, and tuna fortified at 50, 10, and 1 ppt (pg/g) are shown in Table 1. Average congener recoveries were 100.6% at 50 ppt, 81.3% at 10 ppt, and 73.6% at 1 ppt. Corresponding relative standard deviations were 3.0, 8.3, and 19.1%. Further investigations are ongoing to improve the recoveries and precision at the 1 ppt level.

A comparison study was also conducted with three fish samples that were analyzed in a previous round-robin study[10] among six laboratories to measure bioincurred TCDD; results are shown in Table 2. Consensus round-robin values (n = 6) for TCDD in lake and rainbow trout were 30.4 and 32.3 ppt, respectively, with no detectable amounts found in ocean haddock. Results of 28.6 ppt (lake trout), 32.8 ppt (rainbow trout), and none detected (haddock) were obtained by using the method described here, except that the sample size for the fatty lake trout was reduced to 25 g.

Extraction and cleanup can be accomplished in approximately 5 h with one apparatus setup. With two apparatus setups, two test portions can be routinely prepared for the determinative step in 1 day. The described method was designed so that each step is optimized for efficient cleanup and recovery. Work is continuing to investigate efficient ways to combine steps and provide an even more rapid approach.

References

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Table 1. Average (n = 2) % recoveries of 2,3,7,8-chloro-substituted dioxins/furans from fish, determined by high resolution GC/MS comparison with external standards

Congener	Farm catfish (50 pg/g)	Flounder (10 pg/g)	Tuna (1pg/g)
2,3,7,8-TCDD	99	92	65
1,2,3,7,8-PeCDD	102	84	67
1,2,3,4,7,8-HxCDD	101	84	81
1,2,3,6,7,8-HxCDD	106	80	80
1,2,3,7,8,9-HxCDD	103	80	73
1,2,3,4,6,7,8-HpCDD	101	79	98
OCDD	95	63	36
2,3,7,8-TCDF	105	90	56
1,2,3,7,8-PeCDF	102	84	69
2,3,4,7,8-PeCDF	104	86	69
1,2,3,4,7,8-HxCDF	96	75	73
1,2,3,6,7,8-HxCDF	99	80	74
1,2,3,7,8,9-HxCDF	101	89	73
2,3,4,6,7,8-HxCDF	101	82	79
1,2,3,4,6,7,8-HpCDF	98	78	86
1,2,3,4,7,8,9-HpCDF	98	81	81
OCDF	100	75	92
Average	100.6	81.3	73.6
RSD, %	3.0	8.3	19.1

Abbreviations: RSD = relative standard deviation; TCDD, PeCDD, HxCDD, HpCDD, and OCDD = tetrachloro-, pentachloro-, hexachloro-, heptachloro-, and octachlorodibenzo-dioxin, respectively; TCDF, PeCDF, HxCDF, HpCDF, and OCDF = tetrachloro-, pentachloro-, hexachloro-, heptachloro-, and octachlorodibenzofuran, respectively.

Table 2. Comparison of TCDD results from analysis of fish (pg/g) used in a previous interlaboratory study[a]

Congener	Lake trout	Rainbow trout	Ocean haddock
2,3,7,8-TCDD			
this study	28.6	32.8	ND[b]
previous study	30.4	32.3	ND
1,2,3,7,8-PeCDD	NC[c]	NC	
1,2,3,6,7,8-HxCDD	4.2	ND	
1,2,3,4,6,7,8-HpCDD	4.0	ND	
OCDD	108.4	17.4	
2,3,7,8-TCDF	38.1	51.6	
1,2,3,7,8-PeCDF	8.6	ND	
2,3,4,7,8-PeCDF	25.0	15.1	

See abbreviations defined in Table 1.

[a]See reference 10; other 2,3,7,8-chloro-substitued dioxins/furans were not determined in the study.

[b]ND = Not detected.

[c]NC = Not confirmed; incorrect isotope ratios.