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Methods for Determination of Organochlorine Pesticides, Polychlorinated Biphenyl Congeners and Chlorinated Dibenzo-p-dioxins and furans in Fish

S.Y. Huestis, M.R. Servos, D.B. Sergeant
M. Leggett, and D.G. Dixon

Great Lakes Laboratory for Fisheries and Aquatic Sciences
Canada Centre for Inland Waters
867 Lakeshore Road, P.O. Box 5050
Burlington, Ontario L7R 4A6

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Methods for determination of organochlorine pesticides, polychlorinated biphenyl congeners, and chlorinated dibenzo-p-dioxins and furans in fish.

by

S.Y. Huestis, M.R. Servos, D.B. Sergeant, M. Leggett
and D.G. Dixon[1]

Great Lakes Laboratory For Fisheries
And Aquatic Sciences,
Canada Centre for Inland Waters,
867 Lakeshore Road, P.O. BOX 5050,
Burlington, Ontario
L7R 4A6

[1]Dept. of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1

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ABSTRACT

Huestis, S.Y., M.R. Servos, D.B. Sergeant, M. Leggett and D.G. Dixon. 1995. Methods for determination of organochlorine pesticides, polychlorinated biphenyl congeners, and chlorinated dibenzo-p-dioxins and furans in fish: Can. Tech. Rep. Fish. Aquat. Sci. 2044: 30 p.

Methodologies for the separate determination of organochlorine pesticides (OCs), polychlorinated biphenyl (PCB) congeners, and non-ortho substituted PCBs/chlorinated dibenzo-p-dioxins (PCDDs) and furans (PCDFs) in lake trout are presented. Samples were whole fish homogenate reference materials stored in sealed glass ampules at room temperature. All samples were extracted by grinding with sodium sulfate, packing into glass columns, and eluting with methylene chloride. Lipids were removed from samples via gel permeation chromatography. OC/PCB extracts were fractionated on silica gel columns, and analysed by a combination of high resolution gas chromatography (GC)-electron capture detection (ECD), and GC-mass selective detection (MSD) with detection limits in the low ng/g. Recoveries of blanks spiked with analytical standards of the native compounds ranged from 92% to 108% for OC's, 98% to 135% for total PCBs, and 83% to 104% for selected PCB congeners. Non-ortho PCB/PCDD/PCDF extracts were cleaned up on alumina and silica gel columns, and non-ortho PCBs were separated from PCDDs and PCDFs by carbon chromatography. Analysis and quantification was by GC-high resolution mass spectrometry (MS). Detection limits were in the low pg/g range for non-ortho PCBs and PCDD/PCDFs. Recoveries of samples spiked with ^{13}C-labelled standards ranged from 72% to 76% for non-ortho PCBs, and from 54% to 83% for PCDDs. Certified reference materials (CRMs) of lake trout, salmon and herring tissues were analyzed, and results compared favourably to those predicted from interlaboratory studies.

RESUME

Huestis, S.Y., M.R. Servos, D.B. Sergeant, M. Leggett and D.G. Dixon. 1995. Methods for determination of organochlorine pesticides, polychlorinated biphenyl congeners, and chlorinated dibenzo-p-dioxins and furans in fish: Can. Tech. Rep. Fish. Aquat. Sci. 2044: 30 p.

Nous faisons état de méthodes pour le dosage séparé de pesticides organochlorés (OC), de congénères de biphényles polychlorés (BPC) ainsi que de dérivés de substitution non-ortho de BPC/polychlorodibenzo-p-furanes (PCDF)/polychlorodibenzo-p-dioxines (PCDD) trouvés dans les tissus de touladis. Nous avons pris comme échantillons des matériaux de référence constitués d'homogénat de poisson conservés dans des ampoules de verre scellées et conservées à température de la pièce. Nous avons pratiqué une extraction sur tous les échantillons par broyage avec du sulfate de sodium, garnissage de colonnes de verre avec le produit et élution au chlorure de méthylène. Les lipides ont été éliminés des échantillons par chromatographie par perméation de gel. Les extraits d'OC/BPC ont été fractionnés sur colonne de gel de silice et analysés par chromatographie en phase gazeuse (CG) à haute résolution et détection par capture d'électrons (DCÉ) ainsi que par chromatographie en phase gazeuse et détection par discrimination de masse, technique dont les limites de détection sont vers le bas de la plage des valeurs de l'ordre du $ng.g^{-1}$. La récupération à partir de blancs dopés par des étalons d'analyse des composés non marqués variait entre 92 % et 108 % pour les OC, 98 % et 135 % pour les BPC totaux et 83 % et 104 % pour certains congénères des BPC. Les extraits des dérivés de substitution non-ortho de BPC/PCDD/PCDF ont été purifiés sur colonnes à gel de silice et d'alumine, et les dérivés de substitution non-ortho de BPC ont été séparés des PCDD et des PCDF par chromatographie sur colonne de charbon actif. L'analyse et le dosage ont été assurés par CG-spectrométrie de masse (SM) à haute résolution. Les limites de détection se sont situées dans le bas de la plage des valeurs de l'ordre du $pg.g^{-1}$ dans le cas des dérivés de substitution non-ortho de BPC et des PCDD et PCDF. La récupération dans des échantillons enrichis de solutions-étalons marquées au ^{13}C a été de 72 % à 76 % dans le cas des dérivés de substitution non-ortho de BPC, et de 54 % à 83 % dans celui des PCDD. Les matériaux de référence certifiés (CRM) de touladi, de saumon et de hareng ont été analysés, et les résultats se comparaient favorablement à ceux que donnent à prévoir des études interlaboratoires.

INTRODUCTION

Organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs) and chlorinated dibenzo-p-dioxins (PCDDs) and furans (PCDFs) are groups of chemicals which have been found in the water, sediment and fish of Lake Ontario (Niimi and Oliver, 1989, Borgmann and Whittle, 1991, 1992, Oliver et al, 1989, DeVault et al, 1989). While levels of many of these chemicals have declined in Lake Ontario fish since the 1970s, recent data shows that concentrations, specifically those of total PCBs, have in recent years stabilized at levels which are generally above fish consumption guidelines (Baumann and Whittle, 1988).

PCBs vary greatly in toxicity, and number and positioning of chlorine substituents plays a large role in determining toxicity. Some of the 209 PCBs are stereochemically similar to 2,3,7,8-TCDD, and elicit toxic effects similiar to that of TCDD. These PCBs, known as non-ortho or coplanar PCBs due to their lack of chlorines in the "ortho" positions which allows them to assume a planar configuration, occur at very low concentrations in the environment relative to other PCB congeners. Detection is difficult due to their extremely low levels, and co-elution problems with other compounds have caused traditional high resolution gas chromatography (GC)-electron capture detection (ECD) analysis methods to be ineffective at separating and identifying PCB congeners. The use of GC - high resolution mass spectrometry (MS) and GC-mass selective detection (MSD) techniques for the determination of PCBs minimizes co-elution problems through the use of selected ion monitoring (SIM) techniques, providing a much higher confidence level in the resultsthan results generated by GC-ECD.

The analytical techniques to isolate and quantify the non-ortho PCBs from other compounds requires the use of highly specialized techniques and instrumentation that has become available only in recent years. The objective of this study was to develop, validate and document a method to determine simultaneously non-ortho PCBs, PCDDs and PCDFs in fish tissue. Techniques to separate and quantify non-ortho PCBs in conjunction with PCDDs and PCDFs by GC-MS in lake trout are described. Organochlorine pesticides and other PCB congeners in the same samples were determined by GC-ECD and GC-MSD respectively. The techniques used to prepare and analyze samples for these compounds are also described in this document.

EXPERIMENTAL

SAMPLES

Samples used in this study were certified reference materials (CRMs) (Cambridge Isotope Laboratories (CIL), Andover, MA, USA) for PCDDs, PCDFs and non-ortho PCBs, and consisted of homogenized whole lake trout, herring and salmon samples. All CRMs were stored in clear glass ampoules at room temperature, inside a sealed aluminum foil packet, until utilized.

MATERIALS

All solvents used were distilled-in-glass, pesticide residue grade (Baxter-Canlab Division, Mississauga, ON, and Caledon Laboratories, Georgetown, ON). All glassware items were rinsed with acetone and hexane prior to use. Anhydrous sodium sulfate (Na_2SO_4), 30-60 mesh (Anachemia, Mississauga, ON) was heated at 400°C for 18 hours, cooled and stored in a glass bottle inside a dessicator. Alumina (Woelm N-Super 1, type W200; InterSciences Inc., Markham, ON), and silica (ICN Silica 100-200, aktiv 60A; ICN Biomedicals, St-Laurent, QE) were heated at 120°C for 18 hours, and left heated until needed. Dry silica gel was removed from a 150°C oven just prior to use, cooled to room temperature inside of a dessicator, and used as is. A 3% water-deactivated silica gel mixture was prepared by mixing 3 g of deionized, distilled toluene-extracted water with 97 g of dry silica gel.. Acidic silica gel was prepared by mixing 50 g of concentrated sulfuric acid with 100 g of dry silica gel. Basic silica gel was prepared by mixing 35 g of 1 N sodium hydroxide and 100 g of dry silica gel. Silanized glass wool (Supelco, Mississauga, ON) was rinsed with hexane and allowed to air dry prior to use. Carbon fibre column material was prepared by combining 600 mg of shredded filter paper (Toyo glass fibre type GA200, 142 mm Nucleopore prefilter, InterSciences Inc.) with 50 mg of activated carbon (Amoco PX-21, Anderson Development Co., Adrian, MI), adding methylene chloride, and blending at high speed until thoroughly mixed. All gases used in this method were ultrapure grade, from CANOX Oxygen Services, Mississauga, ON.

STANDARDS

All OC and PCB congener standards were obtained as individual stocks from Supelco(Mississauga, ON), Accustandard (New Haven, CT), Ultra Scientific (North Kinston, RI), CIL and the US-EPA. A mixed stock solution was prepared by combining appropriate aliquots of each individual stock solution and diluting with isooctane. All stock solutions were stored in the dark in a freezer. Working standards were prepared by further dilution of the mixed stock solutions. Dioxin

and furan analytical, surrogate and performance standards were obtained from CIL as premixed, ready-to-use standards, and were stored similarily. Native and labelled coplanar PCB standards were purchased from Ultra Scientific and CIL respectively as individual stock solutions, and mixed stock solutions of varying concentrations were prepared from these.

SAMPLE EXTRACTION AND CLEANUP - OC and PCBs

SAMPLE EXTRACTION

Homogenates of whole fish were thawed to room temperature (3-4 hours) when necessary, and the homogenate mixed thoroughly to recombine any separated lipid with the tissue. A 5 gram (g) portion of the homogenate was weighed into a solvent-rinsed aluminum weighing boat. Samples were transferred qualitatively to large (750 milliliter (mL)) mortars, and 200 g of anhydrous Na_2SO_4 was added. The sample mixture was ground manually until a free-flowing mixture was obtained. This mixture was transferred into a large chromatography column (22 millimeter (mm) inner diameter x 500 mm length) plugged with silanized glass wool. The samples were eluted with 300 mL of methylene chloride, at a rate of approximately 10 mL/minute (min). Samples were concentrated by a combination of rotary evaporation (Buchi rotary evaporator, Baxter-Canlab, Mississauga, ON) and nitrogen evaporation (Pearce Reacti-Therm evaporator, Chromatographic Specialties Inc., Brockville, ON) prior to gel permeation chromatography (GPC) for bulk lipid removal.

BULK LIPID REMOVAL

Sample extracts were diluted with 1:1 DCM:hexane to 7 mL. To remove suspended particulates, the sample extracts were centrifuged, and a pre-filter (25 mm filter unit, 0.5um PTFE sterile, Chromatographic Specialties Inc.) used while loading the samples onto the GPC. The GPC unit was an automated Analytical Biochemistry Laboratories (ABC) Autoprep model 1002A, and has the capacity to process up to 23 individual samples per run. The samples were injected into 5 mL loops (1.5 mL of sample is needed to fill from the injection port to the start of the sample loop; and initial sample volume of 7mL ensures that as each sample is loaded, any remaining liquid from the previous sample is pushed entirely through the sample loop). The flow rate was set to 5 mL/min. The column was packed with 60 grams (g) of Bio Beads S-X3, 200-400 mesh (Bio-Rad Laboratories, Richmond, CA) in a 25 mm X 600 mm glass column. The elution solvent was 300 mL of DCM:hexane (1:1). Lipids and other biogenic molecules were eluted in the first 150 mL, which was automatically discarded; compounds of interest were eluted in the second 150 mL fraction,

which was collected, concentrated and solvent exchanged into 2,2',4-trimethylpentane (isooctane) prior to silica gel separation. Calibration of the GPC occured by injecting known standards of OCs, PCBs, PCDDs and PCDFs, and determing their elution times.

SILICA GEL CLEANUP/SEPARATION COLUMNS

Both 3% water deactivated and dry silica gel columns were prepared by packing glass chromatography columns (33 cm length X 1.6 cm width) with 3 cm of Na_2SO_4, 10 cm of silica gel, and 3 cm of Na_2SO_4. The columns were prewashed with 40 mL of hexane. The eluant from the GPC was concentrated to 1 mL, added to a 3% silica gel column, and eluted with 70 mL of 1% benzene in hexane (collected as the A fraction), and 50 mL of benzene (B fraction). The A fraction was concentrated, applied to a dry silica gel column, and eluted with 60 mL of hexane (C fraction) and 50 mL of benzene (D fraction). The B, C and D fractions were concentrated and solvent-exchanged into 10 mL of isooctane prior to analysis.

The B fraction contained the bulk of the OCs. The C fraction contained hexachlorobenzene, octachlorostyrene, aldrin, photomirex, mirex, and approximately half of the PCB congeners. The D fraction contained p,p'-DDE, o,p'-DDT and the remainder of the PCB congeners.

INSTRUMENTATION

OC and total PCB analyses were performed on two instruments, a Varian 6000 GC equipped with an 8000 autosampler, and a Varian 3600 GC equipped with an 8200 autosampler (Varian Canada Inc., Mississauga, ON). An on-column injector was used, and injection size was 2 µL in isooctane, onto a 1 m X 0.53 mm deactivated fused silica guard column joined by a glass Press-Tight connector to a 2.5 m X 0.25 mm deactivated fused silica retention gap. The injectors were maintained at a constant temperature of 240°C, and helium was used as a carrier gas at 30 psi. The sample was split after injection via a glass Y-connector, into dual capillary columns; a Restek Rt_x5, 60 m X 0.25 mm X 0.25 µm, and a Restek Rt_x1701, 60 m X 0.25 mm X 0.25 µm (Chromatographic Specialties Inc, Brockville, ON). Both systems had dual electron capture detectors, heated to 330°C. The make-up gas was nitrogen at 30 ml/min. The GC columns were maintained at 80°C for 1 min, ramped to 180°C at 15°C/min, to 260°C at 2°C/min and held for 2 min, and lastly to 270°C at 10°C/min where they were held for 13 min.

Data was collected and processed by a 486/33 Mhz personal computer equipped with a Varian Star GC Workstation software package. Quantification of the analytes was via an external standard method. Criteria for compound identification included matching retention times, acceptable peak

shapes, and dual column confirmation. Individual OC standards were analyzed by GC-ECD at ng/µL levels to confirm standard purity, and at pg/µL levels to characterize and determine the elution order of individual OC compounds. A five-point calibration curve of the analytical standard at varying concentrations was generated on an as needed basis. Analytical standard concentration for OCs was 50 pg/uL (50 ppb) each component, and the response of this standard was compared on a daily basis to the five point calibration curve. OCs analyzed included the benzene hexachloride (BHC) group, α and γ chlordane and the DDT group, and a complete listing is given in Table 6. Total PCBs were quantitated against a standard containing a 1:1:1 mixture of Aroclors 1242, 1254 and 1260, with an analytical standard concentration of 500 pg/uL for each Aroclor.

For congener specific PCB analyses, a Hewlett-Packard (HP) 5890 GC coupled by direct interface to an HP 5970 mass selective detector (MSD) was used (Hewlett-Packard, Mississauga, ON). The carrier gas was helium at a flow rate of 1.4 ml/min, and was maintained at a constant flow throughout the run; the vacuum compensation feature was enabled. An HP 7673A autosampler configured for on-column injection delivered a 2 ul injection onto a 1m X 0.53 mm deactivated fused silica guard column, coupled to a 2.5 m X 0.25 mm deactivated fused silica retention gap, which in turn was joined to the capillary column. The column was a 60 m X 0.25 mm X 0.25 µm Restek Rt_x5. The GC column was maintained at 80°C for 2 min, then ramped to 160°C at 16°C/min, followed by an increase to 280°C at 2°C/min. The injector was held at 90°C for 1 min, then ramped to 280°C at 40°C/min, and held for 63 min. The GC-MS interface temperature was 270°C. The MSD was operated at 70 electron volts in the electron impact mode. GC-MSD data was acquired in the selected ion monitoring (SIM) mode by monitoring characteristic ions of the PCBs.

Quantitation of analytes was accomplished by comparing the samples to known standards of PCBs, and criteria for compound identification included correct retention times, acceptable peak shape, and correct confirmation ion ratios. Quantitation and confirmation ions are given in Table 1, which also gives the retention windows for each congener group (there is a large amount of overlap between congener groups). A mixed standard (IPCB std) containing the PCB congeners listed in Table 2, in their elution order within congener groups, was prepared from individual PCB congener standards. The IPCB analytical standard concentration ranged from 25-100 pg/uL per congener. All IPCB standard concentrations and identities were verified internally, and against standards obtained from other laboratories performing similiar analysis.

Table 1: Mass spectral ions and retention time windows for determination of congener-specific PCBs by GC-MSD.

Congener group	Quantitation ion	Confirmation ion	Retention Window (min)
Biphenyl	154	76	10.0-17.0
Monochlorobiphenyl	188	190	10.0-17.0
Dichlorobiphenyl	222	224	17.0-24.0
Trichlorobiphenyl	256	258	22.0-31.0
Tetrachlorobiphenyl	292	290	24.0-42.0
Pentachlorobiphenyl	326	324	29.0-51.0
Hexachlorobiphenyl	360	362	31.0-54.0
Heptachlorobiphenyl	394	396	42.0-57.0
Octachlorobiphenyl	430	428	49.0-60.0
Nonachlorobiphenyl	464	466	56.0-66.0
Decachlorobiphenyl	498	500	60.0-66.0

Table 2: PCB congeners; BZ #'s and structures of congeners analyzed by GC-MSD, listed by congener group.

Di		Penta		Hepta	
BZ15	4,4'-	BZ84	2,2',3,3',6-	BZ178	2,2',3,3',5,5',6-
Tri		BZ89/	2,2',3,4,6'-/	BZ187	2,2',3,4',5,5',6-
BZ18	2,2',5-	101	2,2',4,5,5'-	BZ183	2,2',3,4,4',5',6-
BZ32	2,4',6-	BZ99	2,2',4,4',5-	BZ185	2,2',3,4,5,5',6-
BZ31	2,4',5-	BZ97	2,2',3',4,5-	BZ177	2,2',3,3',4',5,6-
BZ28	2,4,4'-	BZ87	2,2',3,4,5'-	BZ171	2,2',3,3',4,4',6-
Tetra		BZ85	2,2',3,4,4'-	BZ172	2,2',3,3',4,5,5'-
BZ50	2,2',4,6-	BZ110	2,3,3',4',6-	BZ180	2,2',3,4,4',5,5'-
BZ52	2,2',5,5'-	BZ118	2,3',4,4',5-	BZ193	2,3,3',4',5,5',6-
BZ49	2,2',4,5'-	BZ105	2,3,3',4,4'-	BZ170	2,2',3,3',4,4',5-
BZ47/48	2,2',4,4'-/ 2,2',4,5-	BZ126	3,3',4,4',5-	Octa	
/75	/ 2,4,4',6-	Hexa		BZ199	2,2',3,3',4,5,6,6'-
BZ44	2,2',3,5'-	BZ136	2,2',3,3',6,6'-	BZ201	2,2',3,3',4',5,5',6-
BZ42	2,2',3,4'-	BZ151	2,2',3,5,5',6-	BZ196/	2,2',3,3',4,4',5,6- /
BZ64	2,3,4',6-	BZ149	2,2',3,4',5',6-	203	2,2',3,4,4',5,5',6-
BZ40	2,2',3,3'-	BZ153	2,2',4,4',5,5'-	BZ195	2,2',3,3',4,4',5,6-
BZ74	2,4,4',5-	BZ141	2,2',3,4,5,5'-	BZ194	2,2',3,3',4,4',5,5'-
BZ70	2,3',4',5-	BZ137	2,2',3,4,4',5-	Nona	
BZ76	2',3,4,5-	BZ138	2,2',3,4,4',5'-	BZ206	2,2',3,3',4,4',5,5',6-
BZ66	2,3',4,4'-	BZ158	2,3,3',4,4',6-	Deca	
BZ55	2,3,3',4-	BZ129	2,2',3,3',4,5-	BZ209	2,2',3,3',4,4',5,5',6,6'
BZ56/60	2,3,3',4'-/2,3,4,4'-	BZ128	2,2',3,3',4,4'-		
BZ81	3,4,4',5-	BZ156	2,3,3',4,4',5-		
BZ77	3,3',4,4'-	BZ169	3,3',4,4',5,5'-		

SAMPLE EXTRACTION AND CLEANUP - PCDDs, PCDFs, and Coplanar PCBs

SAMPLE EXTRACTION

The whole fish homogenate was thawed to room temperature (3-4 hours) when neccesary, and the homogenate was mixed thoroughly to recombine any separated lipid with the tissue. A 10 g portion was weighed into a solvent-rinsed aluminum weighing boat. Samples were transfered qualitatively to large (750 mL) mortars, and 200 g of anhydrous Na_2SO_4 was added. The sample mixture was ground until a free-flowing mixture was obtained. This mixture was transferred into a large (22mm ID x 500 mm long) chromatography column plugged with silanized glass wool. Labelled surrogate spiking solutions, consisting of 50 µL of a 20-60 pg/uL solution of ^{13}C-PCDDs, and 20 µL of a 100 pg/µL solution of ^{13}C-non-ortho PCBs, were spiked into the samples after they were packed in the columns. The samples were eluted with 300 mL of methylene chloride, at an approximate rate of 10 mL/min. Samples were concentrated prior to GPC separation via rotary evaporation.

BULK LIPID REMOVAL

Sample extracts were diluted with 1:1 DCM:hexane so that lipid levels were equivalent to 0.5 g of lipid per 5 mL of solvent. As for OC/PCB analyses, the sample extracts were centrifuged, and pre-filters were used to remove suspended particulates while loading the samples onto the GPC system. The samples were injected into 5 mL loops, and most samples required more than one loop. The flow rate was set to 5 mL/min. The elution solvent was 300 mL of DCM:hexane (1:1) per loop, of which the first 150 mL was discarded, and the second 150 mL collected and concentrated to about 1 mL.

ALUMINA CLEANUP

Alumina columns were prepared by inserting a plug of glass wool into the bottom of a 22.8 cm long-stemmed disposable glass pipet. To the pipet was added 5 cm of activated alumina, topped with 0.5 cm Na_2SO_4. The column was prerinsed with 5 mL hexane. The sample extract was applied with rinsings to the head of the column, and eluted with 5 mL of hexane (discarded), followed by 10 mL of toluene. The toluene fraction was concentrated and solvent exchanged into methylene chloride, for a final volume of 1 mL, prior to either acid/base silica gel or carbon column cleanup.

ACID/BASE SILICA CLEANUP (when necessary)

This step was only neccesary for samples that were still ooloured after coming through the microalumina cleanup. Acid/base silica gel columns were prepared by plugging a disposable pipet with glass wool, and adding 2 cm of basic silica gel, 1 cm of Na_2SO_4, 4 cm of acidic silica gel, and topping with 0.5 cm of Na_2SO_4. Columns were prewashed with 10 mL of DCM. Sample extracts were applied quantitatively to the columns, and eluted with 12 mL of 1:1 DCM:hexane. This sample eluant was collected and concentrated to 1 mL prior to carbon column cleanup.

CARBON CHROMATOGRAPHY

A semi-automated high performance liquid chromatography (HPLC) system consisted of a Waters Model 590 programmable solvent delivery module, equipped with an automated column switching valve (used to change flow directions), a Waters U6K injector, and an Autochrom solvent switching valve (Millipore Waters Inc., Mississauga, ON). The column consisted of an 8 mm X 0.3 mm section of glass tubing, packed firmly with carbon fibre column material. The column end fittings were stainless steel reducing unions, equipped with 2 micron stainless steel sintered frits. Samples were injected onto the HPLC system in 1 mL of 1:1 DCM:cyclohexane, and eluted using the program in Table 3.

Table 3: Flow rates and elution volumes for HPLC carbon chromatography.

Time	Flow direction	Flow rate	Solvent
0-22 min	normal	2 mL/min	1:1 methylene chloride:cyclohexane (discarded)
22-56 min	normal	3 mL/min	1:1 ethyl acetate: benzene (collect for non-ortho PCBs)
56-87 min	reverse	3 mL/min	toluene (collect for PCDDs and PCDFs)
87-111 min	normal	4 mL/min	1:1 methylene chloride:cyclohexane (discard)

Both the benzene:ethyl acetate, and toluene fractions were collected, concentrated and ultimately transferred to autosampler micro-vials using methylene chloride. The solvent was evaporated and the extract re-dissolved in 20 uL of a 100 pg/µL solution of the instrument performance standard.

ANALYTICAL INSTRUMENTATION

High resolution GC/MS analyses of non-ortho PCBs, PCDDs and PCDFs was carried out on a VG AutoSpec-Q mass spectrometer (Fisons, VG Analytical, Manchester, UK) connected to a Hewlett-Packard 5890 GC (Hewlett Packard, Palo Alta, California, USA), equipped with a CTC A200s autosampler (Leap Technologies, Chapel Hill, North Carolina, USA). The GC injection port was configured for 1 uL on-column injections, with a start temperature of 80°C, held for 1.0 min, then ramped at 40°C/min to 280°C and held for up to 55 min. The carrier gas was ultrapure helium, and injections were into a retention gap consisting of a 1 m piece of 0.53 mm I.D. deactivated fused silica connected to a 2.5 m length of 0.25 mm I.D. deactivated fused silica, connected to the capillary column. The GC capillary column was a fused silica DB-5, 60 m X 0.25 mm I.D., with a 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA). A 1 m length of 0.25 mm I.D. deactivated fused silica was connected to the end of the column, and inserted through the heated (280°C) interface into the source.

The GC temperature program for non-ortho PCBs had an initial temperature of 90°C, held for 1 min, then increased at 15°C/min to 180°C, followed by a slower increase of 5°C/min to 283°C, and held for 3 min. For PCDDs and PCDFs, the initial oven temperature was 80°C, held for 1 min, then increased by 30°C/min to 210°C, followed by an increase at 2°C/min to 235°C and held for 15 min, then a final ramp at 8°C/min to 290°C and held for 15 min. For both analyses, ionization of the samples was performed under electron impact (EI) conditions, at an electron voltage ranging from 30 to 40 eV depending on the optimization parameters of the instrument. The source temperature was 260°C. The resolving power of the analyzer was 10000:1. Data processing was with a VG Opus software package. Compounds were detected in the SIM mode, using the ion groups given in Tables 4 and 5. Chlorinated diphenylethers (CDPEs), which are known to interfere with the determination of PCDFs, were also monitored during the PCDD/PCDF runs, although the described method is known to remove CDPE interferences (Huestis and Sergeant, 1992).

Quantitation of samples was by an internal standard quantitation method. Criteria for peak determination included correct retention times (± .05 min.), acceptable peak shape, correct confirming ion ratios (± 15%), and acceptable surrogate spike recoveries (40-120%). Congener quantitation was based on the sum of the quantitation and confirmation ions. Instrument performance was monitored by addition of performance standard (^{13}C-1234-TCDD) just prior to injection of samples. Quantification of native compounds was based on the relative response of the corresponding surrogate and native congeners in the calibration standard(s). Sample concentrations were corrected for calculated ^{13}C-non-ortho PCB and PCDD surrogate recoveries, which in turn were

based on the response of the performance standard. The method detection limit for each congener was defined as 3 times the signal to background noise in the region of the ^{13}C-non-ortho PCB and PCDD surrogate quantification peaks.

Table 4: Mass Spectral Ions for Determination of Non-ortho PCBs

Compound	Quantification Ion (Q)	Confirmation Ion (C)	Mass Ratio	Retention Window (min.)
PCB 77	291.9195	293.9597	0.49	10.0-22.3
PCB 81	291.9195	293.9597	0.49	10.0-22.3
PCB 126	325.8805	323.8834	0.61	22.3-27.0
PCB 169	359.8415	361.8386	0.82	27.0-30.0
^{13}C-PCB 77	303.9597	301.9626	0.77	10.0-22.3
^{13}C-PCB 126	337.9507	335.9237	0.61	22.3-27.0
^{13}C-PCB 169	371.8817	373.8788	0.82	27.0-30.0
^{13}C-1,2,3,4-TCDD[a]	333.9339	335.9237	0.20	22.3-27.0

[a] Instrument performance standard
[b] Theoretical mass ratio of the confirmation ion to the quantitation ion

Table 5: Mass Spectral Ions for Determination of PCDDs and PCDFs

Congener	Quantification Ion (Q)	Confirmation Ion (C)	Mass Ratio	Retention Window (min.)
2,3,7,8-TCDF	305.8987	303.9016	0.77[b]	15.0-26.4
2,3,7,8-TCDD	321.8936	319.8965	0.77	15.0-26.4
1,2,3,7,8-PeCDF	341.8568	339.8597	1.55	26.4-35.4
2,3,4,7,8- PeCDF	341.8568	339.8597	1.55	26.4-35.4
1,2,3,7,8-PeCDD	357.8517	355.8546	1.55	26.4-35.4
1,2,3,4,7,8-HxCDF	375.8178	373.8207	1.24	35.4-41.2
1,2,3,6,7,8- HxCDF	375.8178	373.8207	1.24	35.4-41.2
1,2,3,7,8,9- HxCDF	375.8178	373.8207	1.24	35.4-41.2
2,3,4,6,7,8- HxCDF	375.8178	373.8207	1.24	35.4-41.2
1,2,3,4,7,8- HxCDD	375.8178	373.8207	1.24	35.4-41.2
1,2,3,6,7,8- HxCDD	375.8178	373.8207	1.24	35.4-41.2
1,2,3,7,8,9-HxCDD	391.8127	389.8156	1.24	35.4-41.2
1,2,3,4,6,7,8-HpCDF	409.7788	407.7818	1.04	41.2-47.0
1,2,3,4,6,7,8-HpCDD	425.7737	423.7767	1.04	41.2-47.0
OCDF	443.7398	441.7428	0.89	47.0-55.0
OCDD	459.7348	457.7377	0.89	47.0-55.0
^{13}C-1,2,3,4-TCDD[a]	333.9339	331.9368	0.77	15.0-26.4
^{13}C-2,3,7,8-TCDD	333.9339	331.9368	0.77	15.0-26.4
^{13}C-1,2,3,7,8-PeCDD	369.8918	367.8949	1.55	26.4-35.4
^{13}C-1,2,3,6,7,8-HxCDD	403.8530	401.8559	1.24	35.4-41.2
^{13}C-1,2,3,7,8,9-HxCDD[a]	403.8530	401.8559	1.24	35.4-41.2
^{13}C-1,2,3,4,6,7,8-HpCDD	437.8140	435.8169	1.04	41.2-47.0
^{13}C-OCDD	471.7750	469.7780	0.89	47.0-55.0
HxCDPE	375.8364			15.0-26.4
HpCDPE	409.7974			26.4-35.4
OCDPE	445.7555			35.4-41.2
NCDPE	479.7165			41.2-47.0
DCDPE	513.6775			47.0-55.0

[a] Instrument performance standard

[b] Theoretical mass ratio of the confirmation ion to the quantitation ion

RESULTS AND DISCUSSION

OC, PCB, IPCB ANALYSES

Levels of OC's and total PCB in a lake trout composite material were determined over a period of two years, and are given in Table 6. Mean recoveries of samples spiked with a mixed OC standard solution, or with PCB and IPCB standard solutions are also given in Table 6. The lake trout composite material is available as a certified reference material for PCDDs, PCDFs and non-ortho substituted PCBs, but no certification has been provided for OCs and total PCBs; it is not known how these results compare to other laboratories. Method detection limits for OCs and PCB congeners was 2 and 2-10 ng/g, respectively. Chromatograms for the OC and mixed Aroclor PCB, and total ion chromatograms (TICs) for the IPCB standard, as well as representative chromatograms and TICs of the lake trout CRMs, are given in Figures 1, 2 and 3, with retention times given along the x axis.

NON-ORTHO PCB, PCDD, and PCDF ANALYSES

An existing methodology for the analyses of PCDDs and PCDFs in fish tissue used at the Great Lakes Laboratory for Fisheries and Aquatic Sciences (GLLFAS) Ultratrace Laboratory was adapted to include the analyses of non-ortho PCBs. It was decided to separate the non-ortho PCBs from the PCDDs and PCDFs, so as to minimize potential interferences at the analysis stage. ^{13}C-non-ortho PCBs and ^{13}C-PCDD/PCDFs were used to track recoveries. The existing methodology consisted of a column extraction with DCM, followed by lipid removal on a GPC column, cleanup on a silica gel column, and fractionation of the planar compounds on a carbon column. Results of testing of the alumina column cleanup step for non-ortho PCB recoveries (Table 7) show good recovery of the ^{13}C-PCBs in the toluene fraction of the microalumina column (98 to 108%), and co-elution with PCDDs and PCDFs. Testing of the acid/base silica gel column (Table 7) also demonstrated good recoveries of all of the ^{13}C-PCBs (85 to 91%), and co-elution with PCDDs and PCDFs.

Problems with the existing method were encountered at the carbon column cleanup step. Preliminary results showed ^{13}C-PCBs in both the benzene:ethyl acetate and toluene fractions, instead of a separation of the ^{13}C-PCBs into the benzene:ethyl acetate fraction, and the PCDDs and PCDFs into the toluene fraction. A literature search of existing non-ortho PCB methodologies revealed a method that used a similiar carbon chromatography column separation, and achieved the desired separation by using a larger volume of solvent for the benzene:ethyl acetate wash (Tanabe et al, 1987). Increasing the amount of benzene:ethyl acetate used, from 44 mL to 102 mL, significantly decreased the carryover of ^{13}C-PCBs into the toluene fraction. Excellent recoveries of

^{13}C-PCBs in the benzene:ethyl acetate fraction, and ^{13}C-TCDDs in the toluene fraction were achieved using the modified method (Table 8). Results of testing of the overall method using ^{13}C-labelled spikes are also shown in Table 8. Lower than expected recoveries for TCDD and PCDD spiked samples were found to be caused from flow problems on the carbon column HPLC systems, and these problems were corrected in future analyses.

Table 6: Concentrations of OCs and PCBs in a lake trout composite material, and recoveries of OC and PCB spiked samples.

Compound	Lake trout material Concentration (ng/g) Mean +/- std dev[1]	OC and PCB spikes % Recoveries Mean +/- std dev[2]
α-BHC	5 +/- 1	100.2 +/- 9.8
β-BHC	ND	105.2 +/- 11.6
Δ-BHC	ND	103.7 +/- 10.3
γ-BHC	ND	100.6 +/- 9.8
HEPTACHLOR	ND	91.8 +/- 8.8
HEPTACHLOR EPOXIDE (HEPT.EPOX.)	18 +/- 3	102.5 +/- 9.3
HEXACHLOROBENZENE (HCB)	30 +/- 8	94.8 +/- 11.6
OCTACHLOROSTYRENE (OCS)	29 +/- 5	101.0 +/- 9.8
ρ,ρ'-DDE	610 +/- 66	108.3 +/- 12.5
ρ,ρ'-TDE	136 +/- 74	102.9 +/- 9.8
σ,ρ'-DDT	11 +/- 8	102.7 +/- 11.8
ρ,ρ'-DDT	5 +/- 4	100.0 +/- 15.1
α-CHLORDANE	54 +/- 2	102.1 +/- 10.2
γ-CHLORDANE	21 +/- 6	102.5 +/- 10.3
ALDRIN	ND	96.5 +/- 8.0
DIELDRIN	81 +/- 8	103.7 +/- 10.3
ENDRIN	8 +/- 1	102.9 +/- 10.2
PHOTOMIREX	160 +/- 23	102.6 +/- 11.6
MIREX	235 +/- 47	107.2 +/- 9.7
BZ 28	NA	93.1 +/- 6.8
BZ 52	NA	83.1 +/- 4.4
BZ 118	NA	99.4 +/- 9.4
BZ 137	NA	93.0 +/- 3.9
BZ 138	NA	100.0 +/- 11.5
BZ 170	NA	104.0 +/- 10.2
BZ 180	NA	100.7 +/- 10.5
Total PCB	2492 +/- 641	114.2 +/- 14.2

[1] N=11 for lake trout composite material
[2] N=14 for OC spikes, and N=10 for PCB spikes;
[3] ND = none detected
[4] NA = result not available

Table 7 : Recoveries of ^{13}C-PCBs from alumina columns, and acid/base silica gel columns.

Compound	Alumina ^{13}C % Recovery N=5	A/B Silica ^{13}C % Recovery N=4
^{13}C-PCB77	103.6 +/- 8.3	83.8 +/- 6.0
^{13}C-PCB126	98.4 +/- 12.0	88.8 +/- 7.5
^{13}C-PCB169	107.5 +/- 8.8	90.8 +/- 9.1

In addition to verifying the method using spikes, several fish certified reference materials (CRMs) were also analyzed. These CRMs consisted of a lake trout composite material from fish collected from Lake Ontario, a herring composite material, and a salmon composite material, both from fish collected from B.C. Surrogate spike recoveries of ^{13}C-non-ortho PCBs and ^{13}C-PCDD/PCDFs spiked into the CRMs, and measured concentrations of non-ortho PCBs, PCDDs and PCDFs for three CRMs are given in Tables 8 and 9, respectively. Predicted values for the CRMs were obtained from an interlaboratory study involving laboratories in the USA and Canada (Sergeant, 1993). Detection limits were on the order of 0.1 to 3 pg/g for tetra to octa-substituted PCDDs and PCDFs, and 1-3 pg/g for non-ortho PCBs. CRM-#1 was a composite lake trout material, CRM-#2 was a salmon composite material with non-ortho PCBs and PCDDs/PCDFs spiked into it, and CRM-#3 was a herring composite material. Representative ion chromatograms of the non-ortho PCB standard and of the corresponding chromatograms for the lake trout CRM are given in figure 4. Ion chromatograms for the PCDD/PCDF standard, and the lake trout CRM, are shown by congener group (tetra through octa) in figures 5 to 9. Specific congeners analyzed are identified on the chromatograms.

The CRMs contained 3 varying levels of compounds. CRM-#3, the herring composite, contained materials at extremely low levels (none detected to 2 pg/g for PCDD/PCDFs, 2 to 24 pg/g for non-ortho PCBs). CRM-#1, the lake trout, had naturally occuring levels of non-ortho PCBs ranging from 80 to 2000 pg/g, and PCDD/PCDFs at levels from 1 to 23 pg/g. CRM-#2, a salmon composite material, had non-ortho PCBs spiked into it at levels from 600 to 1400 pg/g, and PCDD/PCDFs spiked at levels of 17 to 190 pg/g. Using CRMs with such widely differing levels of contaminants shows that the method is effective for samples that have a range of contamination levels.

The two methodologies described in this report provide an accurate and precise technique for the determination of a variety of OCs, PCB, PCDD and PCDF congeners in fish samples. The CRMs were analyzed over a period of 2 years, and the relatively low standard deviation demonstrates the

stablility of the utilized method over time. As well, a comparison of the mean determined concentration, with the predicted concentration, demonstrates the accuracy of the process used to analyze these samples. Future method development work should explore the possibility of merging the two methods used in this report.

Table 8: % Recoveries of ^{13}C-PCBs and PCDD/PCDFs, mean +/- standard deviation.

Compound	N	Carbon column recoveries	N	Method recoveries	N	CRM recoveries
^{13}C-PCB 77	9	99.6 +/- 16.0	16	72 +/- 4	8	65 +/- 16
^{13}C-PCB 126	9	104.2 +/- 15.1	16	73 +/- 3	8	65 +/- 18
^{13}C-PCB 169	9	88.6 +/- 19.1	14	76 +/- 6	8	60 +/- 16
^{13}C-T4CDD	8	110.3 +/- 15.6	19	54 +/- 2	8	56 +/- 15
^{13}C-P5CDD	8	100.4 +/- 7.9	19	68 +/- 2	8	68 +/- 8
^{13}C-H6CDD	8	102.2 +/- 7.6	19	78 +/- 2	8	82 +/- 12
^{13}C-H7CDD	8	100.2 +/- 8.5	19	83 +/- 3	8	77 +/- 9
^{13}C-OCDD	8	94.6 +/- 7.5	19	72 +/- 3	8	78 +/- 18

Table 9: Levels of non-ortho PCBs, PCDDs and PCDFs in several fish CRMs, mean +/- std dev, pg/g.

Compound	CRM-1, N=8	Predicted	CRM-2, N=8	Predicted	CRM-3, N=8	Predicted
2378-TCDD	19.8 ± 3.1	17.0 ± 1.4	18.0 ± 5.4	19 ± 1.4	ND[1]	ND
12378-PCDD	5.0 ± 1.8	0.57 ± .57	38.6 ± 13	40 ± 3.1	0.20 ± 0.1	ND
123478-HxCDD	2.0 ± 1.7	0.77 ± .27	54.5 ± 16	60 ± 4.8	ND	ND
123678-HxCDD	3.5 ± 1.9	3.0 ± 1.2	49.6 ± 14	56 ± 4.8	0.58 ± 0.4	ND
123789-HxCDD	1.6 ± 1.8	0.79 ± 0.26	59.5 ± 15	60 ± 4.4	0.25 ± 0.1	ND
1234678-HpCDD	2.8 ± 2.8	1.4 ± 0.53	67.2 ± 16	76 ± 5.9	1.08 ± 0.3	ND
OCDD	17.6 ± 18.0	7.2 ± 3.7	180 ± 50	192 ± 14	5.11 ± 3.2	ND
2378-TCDF	22.6 ± 1.9	22.0 ± 1.6	15.6 ± 4.4	17 ± 1.5	2.18 ± 0.6	2.5 ± .16
12378-PCDF	5.8 ± 1.9	4.9 ± 0.56	43.9 ± 5.7	40 ± 3.7	ND	ND
23478-PCDF	15.5 ± 2.8	14.0 ± 1.3	42.6 ± 6.9	38 ± 3.5	ND	ND
123478-HxCDF	7.6 ± 3.1	8.2 ± 3.7	70.6 ± 23	80 ± 8.4	ND	ND
234678-HxCDF	3.6 ± 1.7	2.7 ± 1.2	53.6 ± 19	63 ± 5.5	ND	ND
123678-HxCDF	2.5 ± 1.9	0.76 ± 0.35	59.0 ± 16	58 ± 7.0	ND	ND
123789-HxCDF	2.6 ± 1.1	2.3 ± 1.9	60.2 ± 13	60 ± 5.5	ND	ND
1234678-HpCDF	2.6 ± 1.6	4.4 ± 6.0	63.3 ± 23	83 ± 9.2	0.97 ± 1.2	ND
OCDF	6.9 ± 7.8	2.6 ± 1.3	187 ± 68	190 ± 22	0.43 ± 0.3	ND
PCB 77	2025 ± 134	2376± 672	566 ± 50	619 ± 107	16 ± 13	24 ± 2
PCB 126	672 ± 57	834 ± 277	577 ± 62	1140 ± 485	7.19 ± 5.5	6.5 ± 2.8
PCB 169	84 ± 22	181 ± 264	770 ± 113	1416 ± 593	1.37 ± 1.2	2.5 ± 1.2

[1] Detection limits ranged from 0.1-1 pg/g, depending on the congener group.

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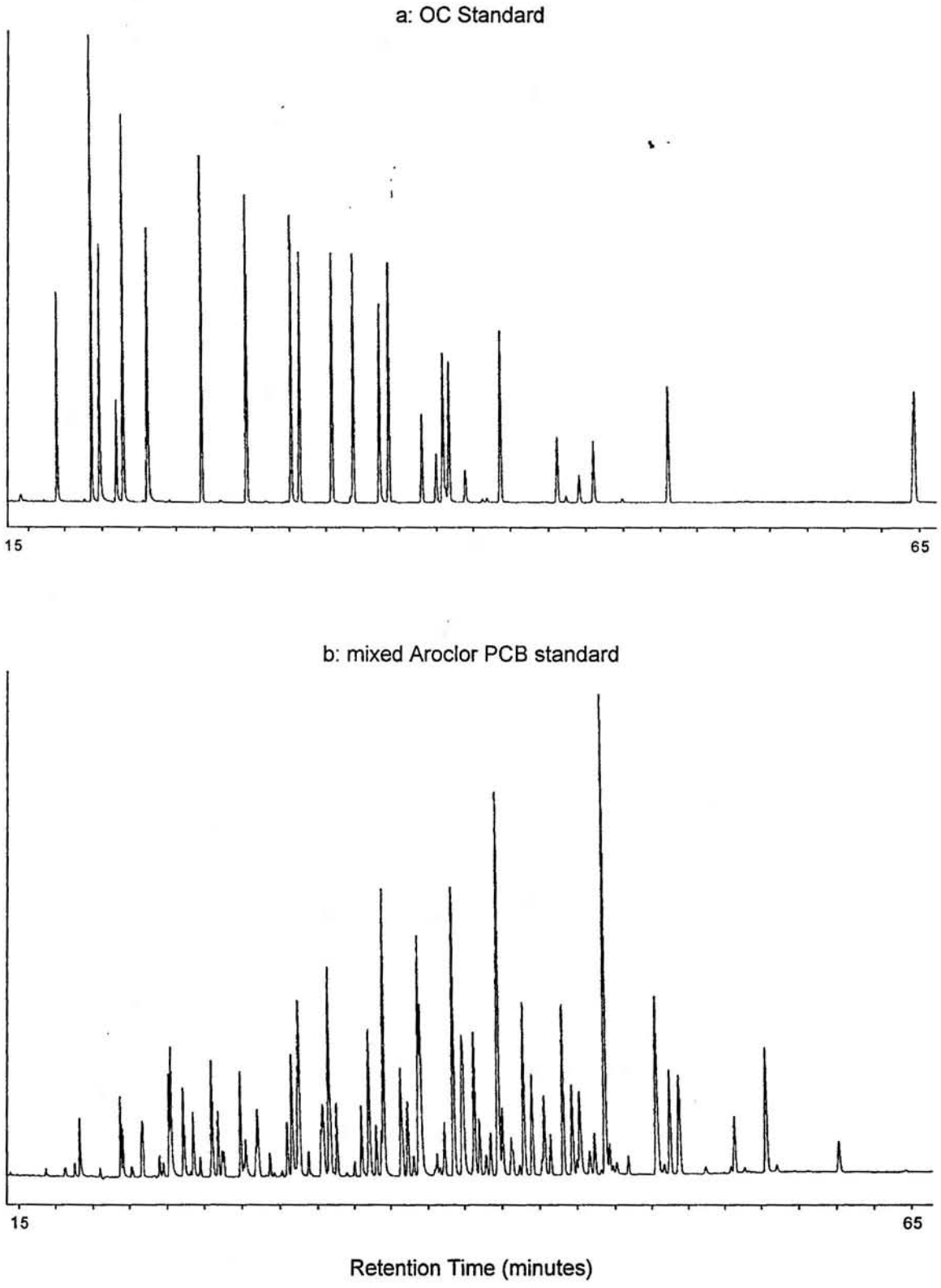

FIG. 1 a, b : OC and PCB standard chromatograms, DB-5 column

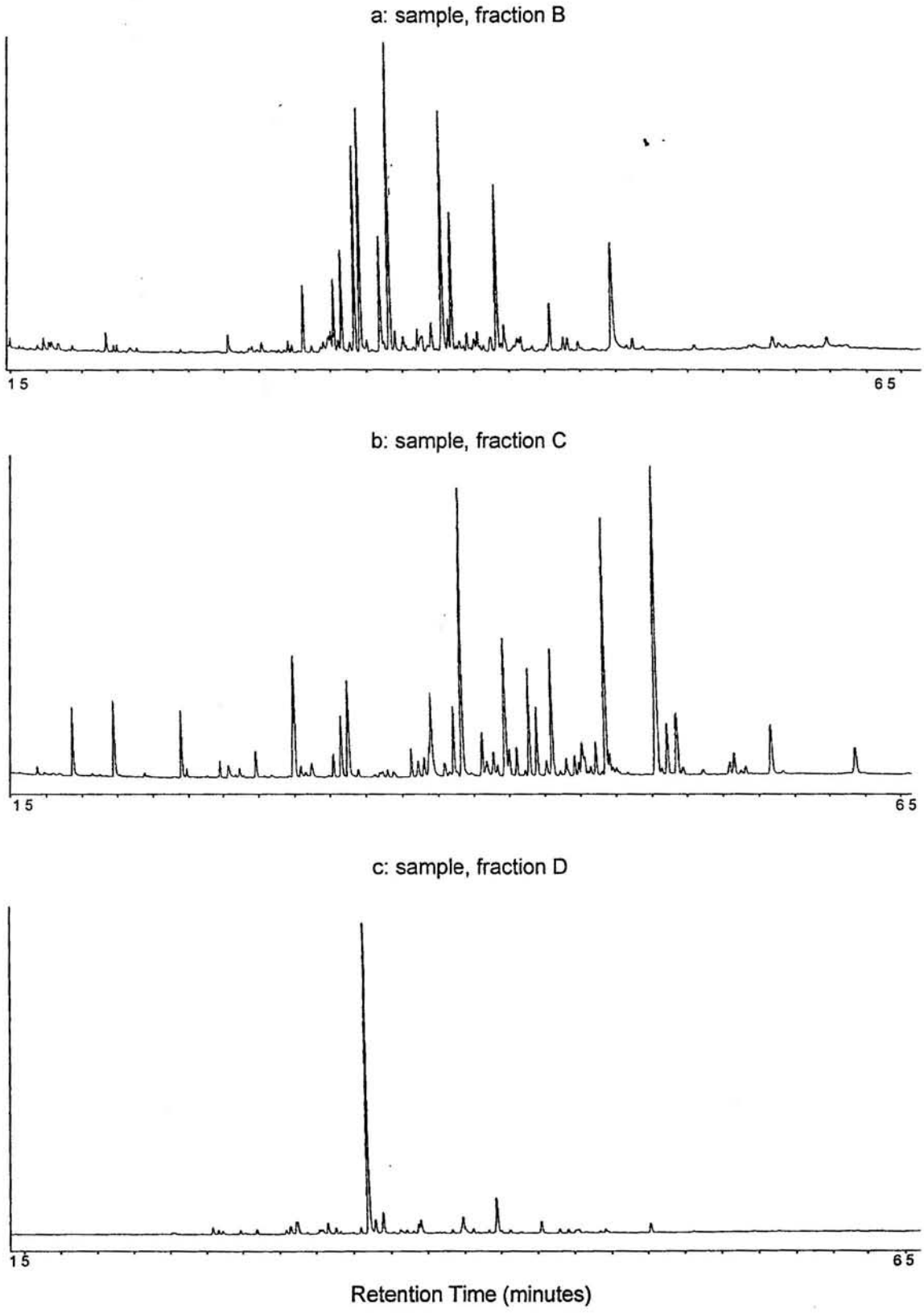

FIG. 2 a, b, c : lake trout sample chromatograms, DB-5 column

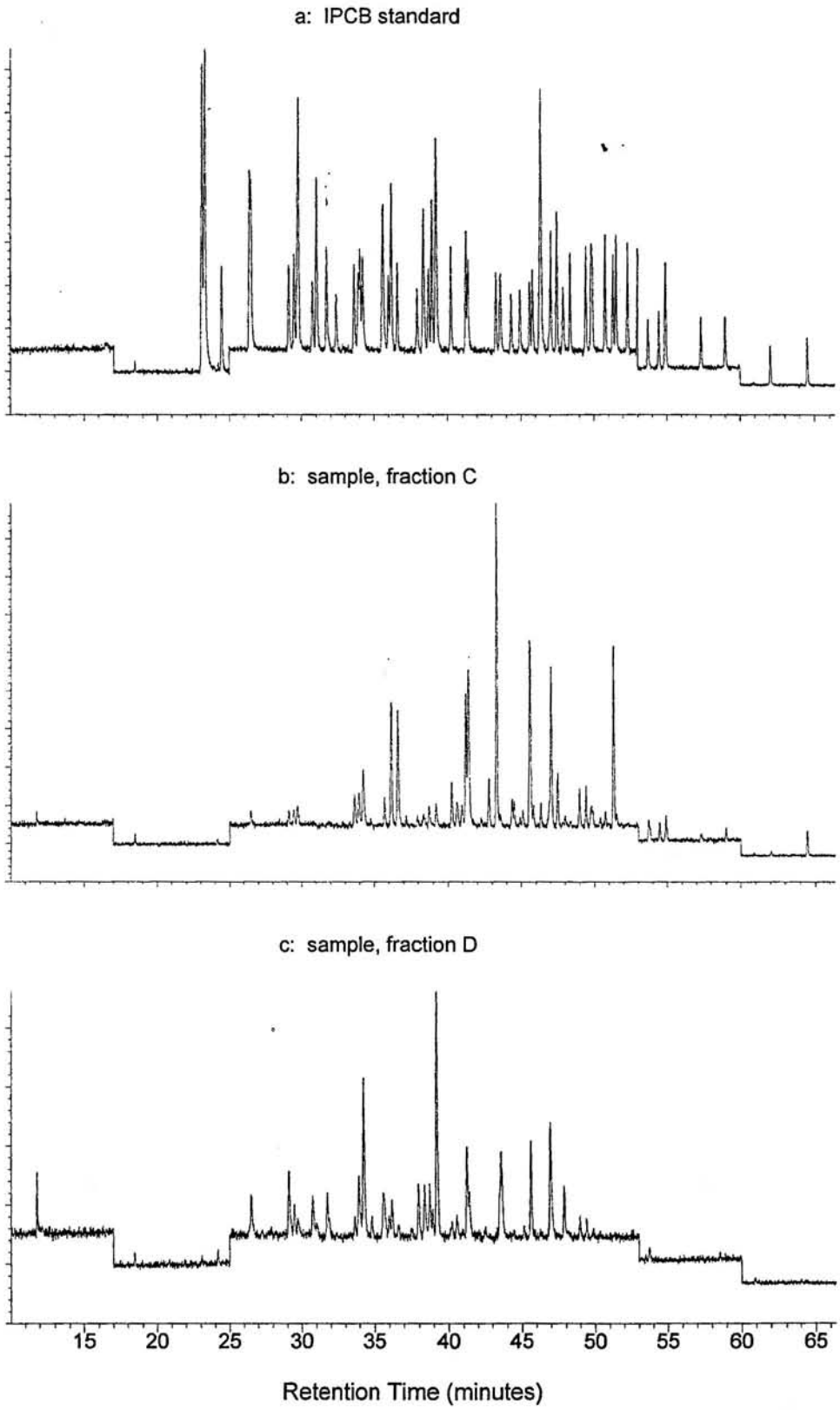

FIG. 3 a, b, c: TICs for Congener-specific PCBs, DB-5 column

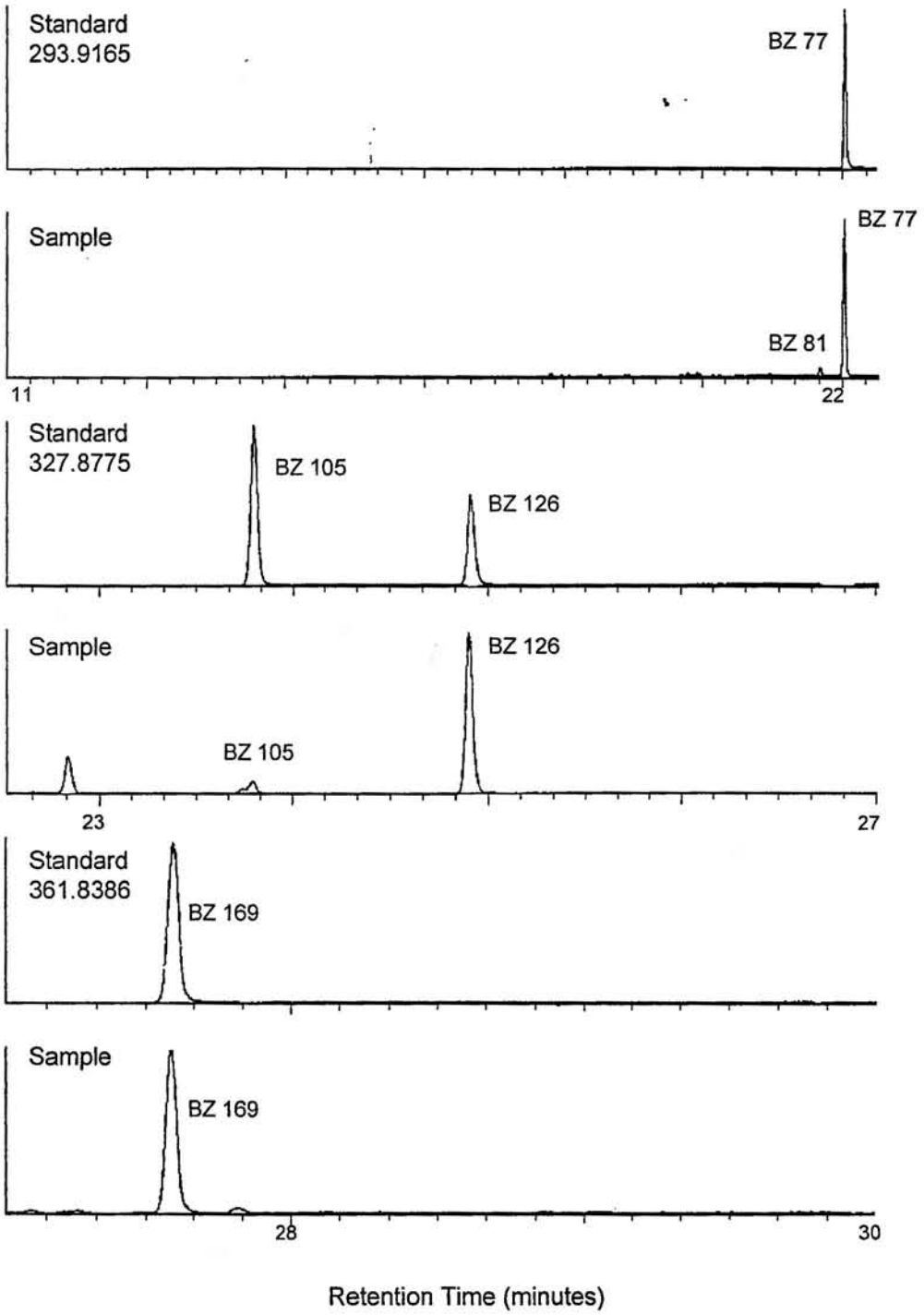

FIG. 4 : Ion chromatograms for non-ortho PCBs

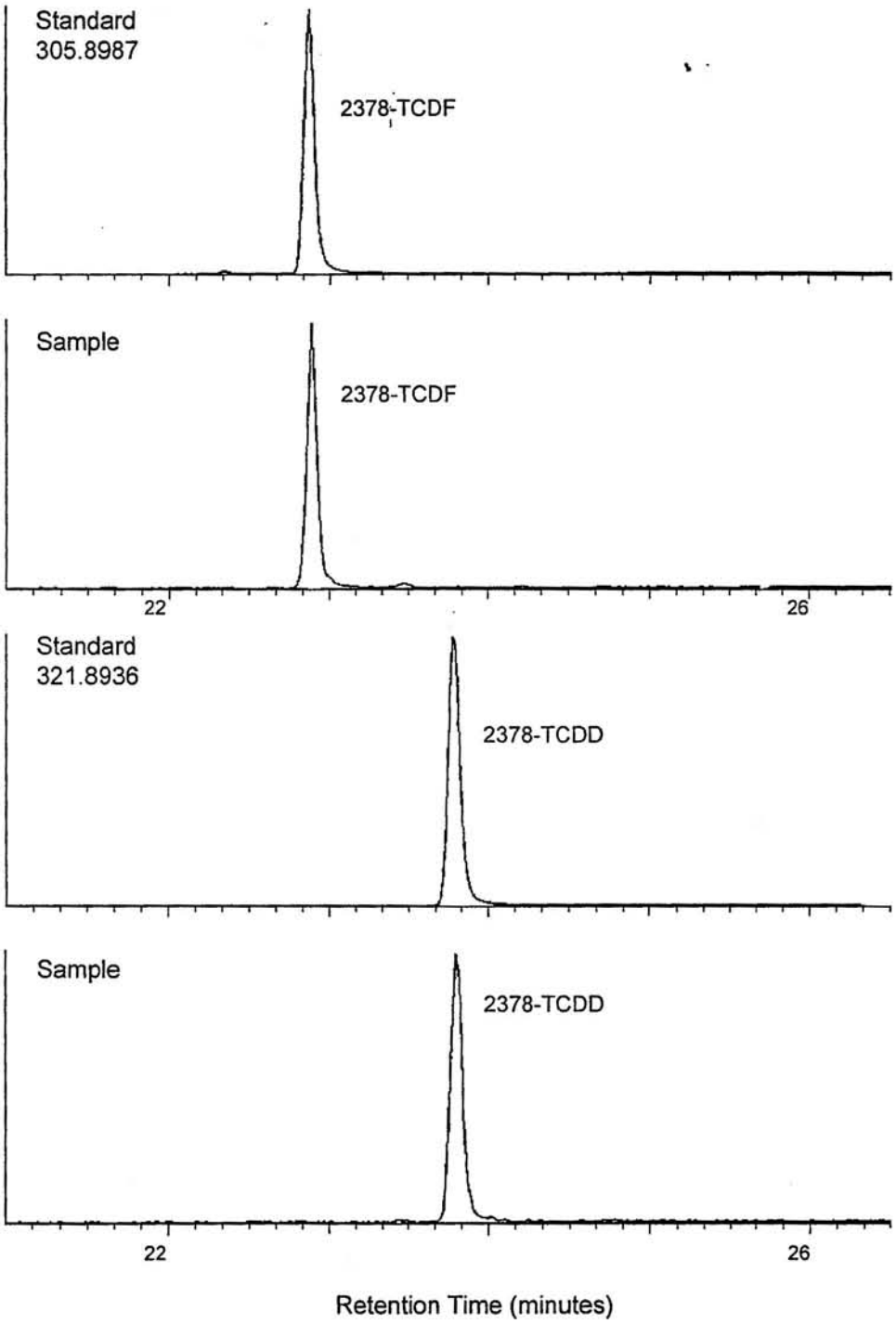

FIG. 5 : Ion chromatograms for tetra-substituted PCDDs / PCDFs

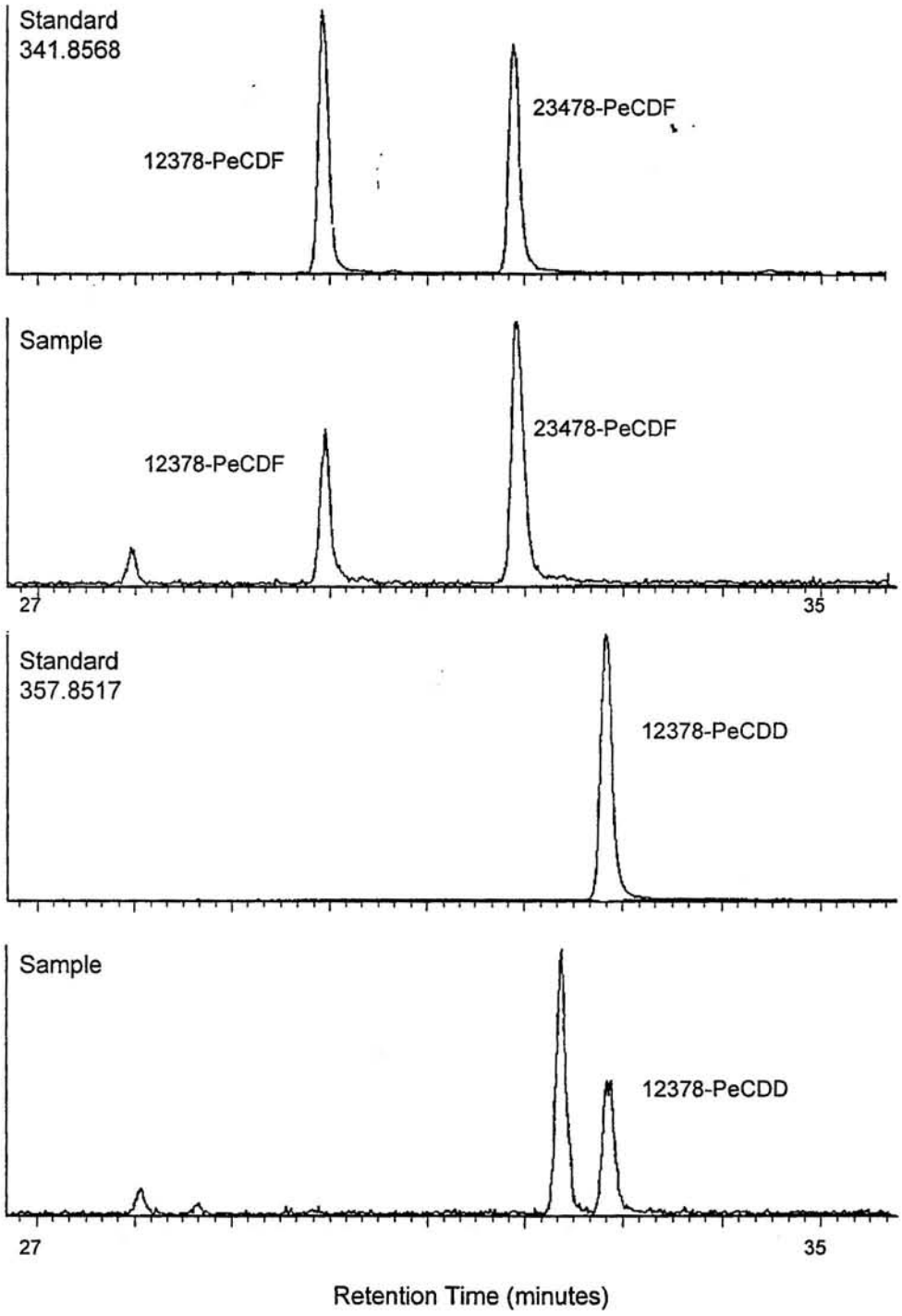

FIG. 6 : Ion chromatograms for penta-substituted PCDDs / PCDFs

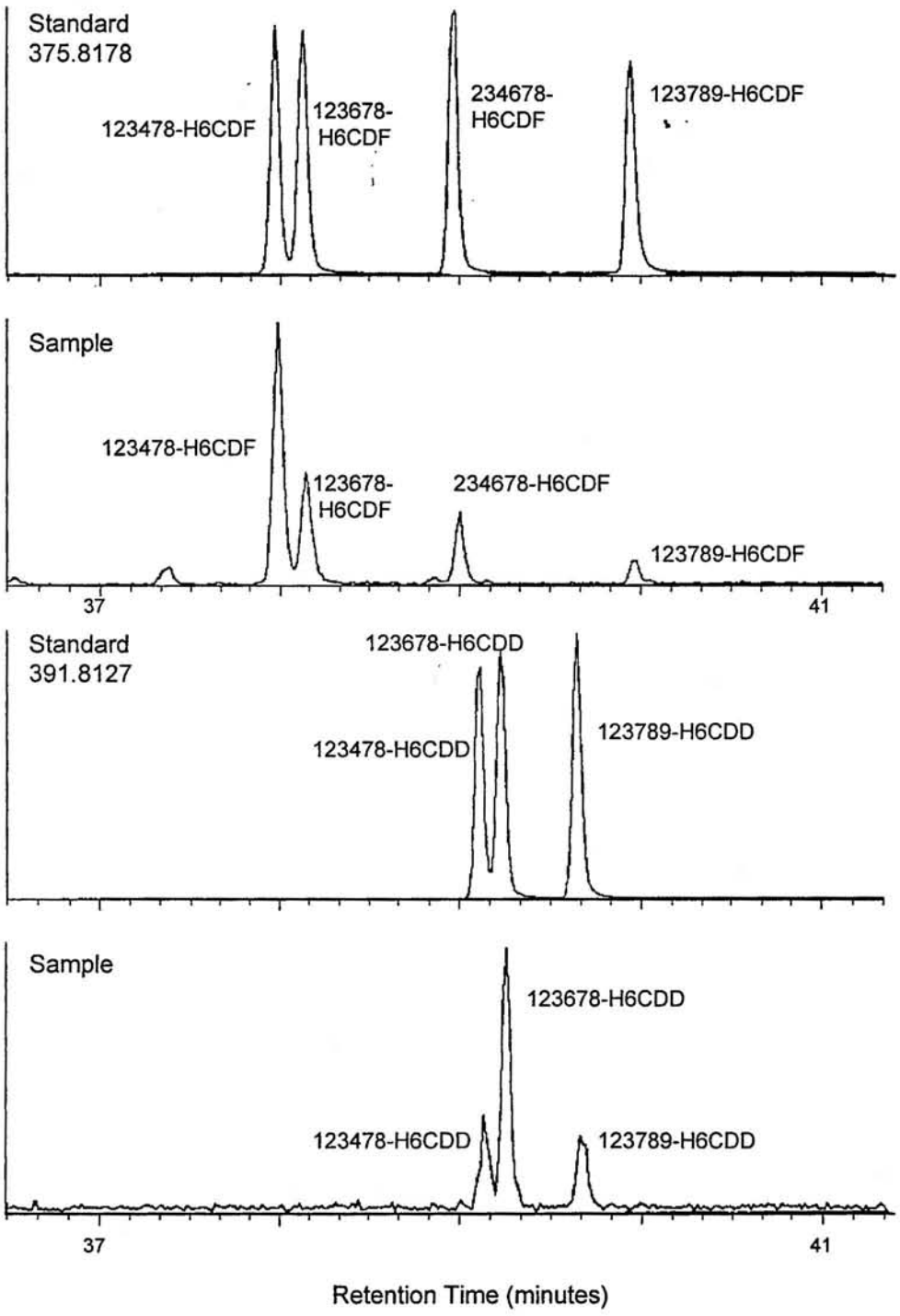

FIG. 7 : Ion chromatograms for hexa-substituted PCDDs / PCDFs

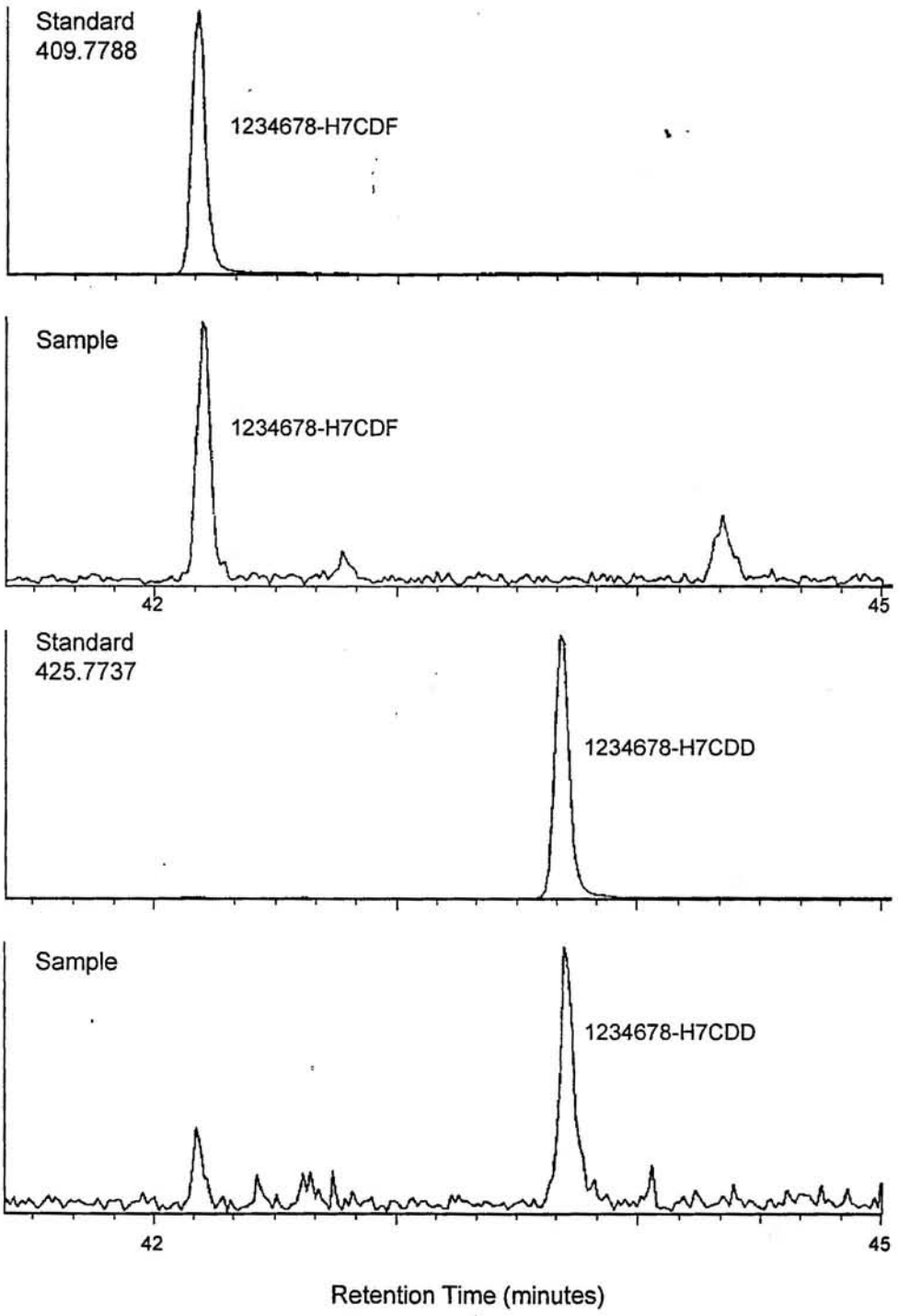

FIG. 8 : Ion chromatograms for hepta-substituted PCDDs / PCDFs

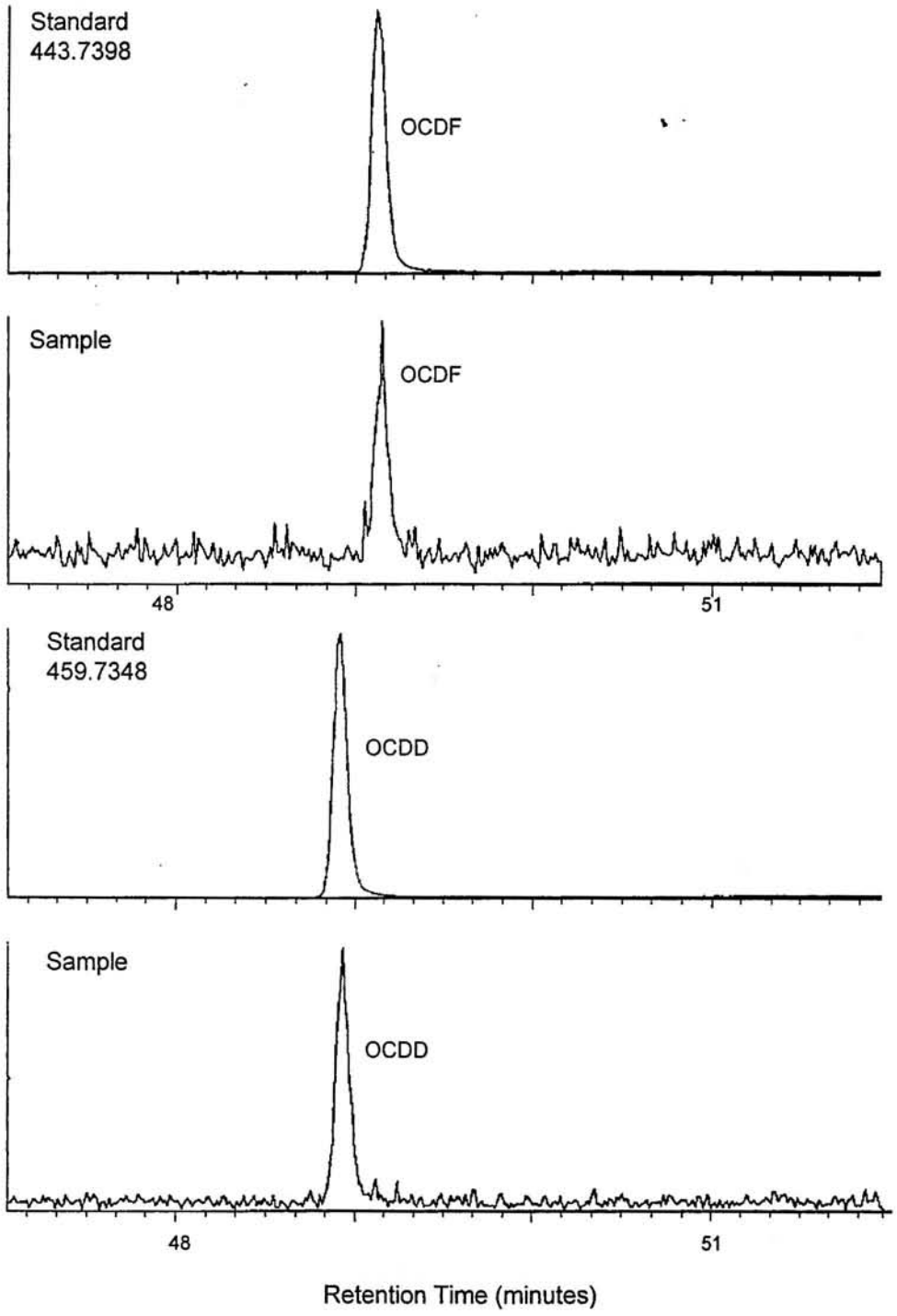

FIG. 9 : Ion chromatograms for octa-substituted PCDDs / PCDFs