ELSEVIER

Journal of Chromatography A, 923 (2001) 177–185

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Protocol for the determination of selected neutral and acidic semi-volatile organic contaminants in fish tissue

Roy Y. Araki, Gerald H. Dodo, Steven H. Reimer, Margaret M. Knight*

United States Environmental Protection Agency, Manchester Environmental Laboratory, 7411 Beach Drive East, Port Orchard, WA 98366, USA

Received 9 January 2001; received in revised form 21 May 2001; accepted 21 May 2001

Abstract

Progress toward the development of a protocol for the determination of a broad spectrum of organic compounds in fish tissue is reported. Finely ground and homogenized fish tissue samples were Soxhlet extracted. Phenolic compounds in the extracts were acetylated and the derivatized extract containing the acetates and neutral semi-volatiles was cleaned up with silica gel and size-exclusion column chromatography. These semi-volatile organic compounds were determined by gas chromatography–mass spectrometry. The method is evaluated for recovery and precision of selected analytes during the analysis of over 300 fish tissue samples of varying species in support of contaminant determination in fish tissue from the Columbia/Snake River watershed. Published by Elsevier Science B.V.

Keywords: Fish; Food analysis; Phenolic compounds; Polynuclear aromatic hydrocarbons; Chlorobenzenes; Nitrotoluenes; Chlorophenols

1. Introduction

A great deal of information exists on persistant organic chemicals in fish. The overwhelming bulk of information is on those chemicals amenable to separation from the sample matrix and to rigorous cleanup procedures, for example, on polychlorinated biphenyls (PCBs), chlorinated furans and dioxins, non-polar chlorinated pesticides, polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons. Determination of a broad spectrum of organic compounds at a low level would be preferable for the purposes of either assessing risk from organic contaminants to fish consumers or assessing the status of organic contaminants in the aquatic environment through determination of the bioaccummulator. Consequently, there have been efforts to develop unified protocols capable of examining several classes of compounds simultaneously. As an example, in 1986 a screening study which investigated selected bioaccummulative pollutants in fish was begun by the United States Environmental Protection Agency (EPA) [1]. In that study the choice of target analytes was determined by bioaccumulation potential, human toxicity, exposure potential, persistence in the aquatic environment, and biochemical fate in fish. The targeted compounds included chlorinated diox-

*Corresponding author. Fax: +1-360-871-748.

E-mail address: knight.peggy@epa.gov (M.M. Knight).

0021-9673/01/$ – see front matter Published by Elsevier Science B.V.

PII: S0021-9673(01)00997-9

ins and furans, chlorinated pesticides, PCBs, chlorinated benzenes, biphenyl, diphenyldisulfide, and octachlorostyrene. Other examples of ongoing studies involving a suite of xenobiotic chemicals are the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects, through the National Oceanic and Atmospheric Administration (NOAA), which includes PAHs, coprostanol, DDT and metabolites, other chlorinated pesticides, and PCBs [2]. The EPA recommended target list to be used in contaminant determination for fish advisories consists of selected organochlorine pesticides, organophosphorous pesticides, PCBs, chlorphenoxy herbicides, PAHs, and chlorinated dibenzodioxins and -furans [3].

During a survey of contaminants in over 300 fish tissue samples from the Columbia River Basin which runs through the states of Washington, Idaho, and Oregon, there was interest in widening the normal scope of organic compounds determined. In the analyte category amenable to gas chromatography–mass spectrometry (GC–MS) analysis, of particular interest were chlorinated free phenolic compounds and low levels of PAHs. Although it is recognized that the phenols [4] and PAHs [5] are readily metabolized, the emphasis during the survey was directed toward assessing exposure to ingested contaminants from fish rather than assessing exposure of the fish to contaminants. Thus the metabolites were not targeted in the scope of the work.

Interest in phenolic compounds formed during the manufacture of wood pulp led to the use of acetylation as an analysis technique [6,7]. A few studies exist which use this technique in the analysis of fish [8–11]. The purpose of this document is to report our progress in the development of a protocol for the analysis of phenolic compounds and low level PAHs demonstrated by analysis of over 300 fish tissue samples from the Columbia River Basin.

The contaminants were Soxhlet extracted from fish tissue, the extract was acetylated, and the derivatized extracts were cleaned with silica gel chromatography followed by size-exclusion chromatography (SEC). The acetates were determined by full scan GC–MS and the remaining semi-volatile compounds by selected ion monitoring (SIM) GC–MS to allow detection at a lower level.

2. Experimental

2.1. Reagents

Chemicals used were all pesticide or analytical-reagent grade unless otherwise specified. They were used without further purification with the following exceptions: sodium sulfate was heated to 430°C overnight and stored at 105°C until use; silica gel was stored at 130°C until use. All glassware was washed with detergent, rinsed with purified water and heated to 430°C for 30 min. Soxhlet thimbles were precleaned by extracting with acetone for 3 h. Solvents were from Burdick and Jackson (Muskegon, MI, USA), acetic anhydride from Fisher Scientific (Fair Lawn, NJ, USA), standards from Ultra Scientific (North Kingstown, RI, USA) or Accustandard (New Haven, CT, USA), purified water from a Milli-Q system (Waters, Milford, MA, USA), silica gel and sodium sulfate from J.T. Baker (Phillipsburg, NJ, USA). All volume reductions with the exception of Kuderna–Danish evaporations on a steam table were done under a gentle stream of dry nitrogen with an N-Evap apparatus (Organomation, Berlin, MA, USA).

2.2. Samples

Fish species collected included chinook and coho salmon (*Oncorhynchus tshawytscha* and *O. kisutsch*), steelhead and rainbow trout (*O. mykiss*), mountain whitefish (*Prosopium williamsoni*), lake whitefish (*Coregonus clupeaformis*), white sturgeon (*Acipenser transmontanus*), walleye (*Stizostedion vitreum*), largescale and bridgelip sucker (*Catostomus macrocheilus* and *C. columbianus*), Pacific lamprey (*Lampetra tridentata*), smelt (*Thaleichthys pacificus*), channel catfish (*Ictalurus punctatus*), and smallmouth bass (*Micropterus dolomieu*). The fish were bisected, wrapped in aluminum foil, and frozen before shipment to a laboratory for sample homogenization.

2.3. Procedure

2.3.1. Sample homogenization

Whole fish, fish fillets, and eggs were homogen-

ized with a commercial meat grinder. Large fish samples were first cut into cubes about 2.5 cm on an edge and the cubes from all the fish comprising the composite combined and ground. After the first grinding, the ground material was divided into quarters, opposite quarters mixed together, and the halves then mixed together. The grinding and mixing was repeated until the composite sample appeared to be homogeneous. At a minimum, each composite sample was ground and mixed three times.

2.3.2. Tissue extraction

All samples were received at the analytical laboratory previously homogenized and frozen. The samples were analyzed in batches of 20. One batch at a time was thawed at 4°C overnight. A 5–10-g amount of fish tissue was weighed into a beaker containing 50 g anhydrous sodium sulfate and mixed. The mixture was transferred to an extraction thimble, placed in a Soxhlet extractor and the surrogate compounds [$^{2}H_{4}$]1,2-dichlorobenzene, 2-fluorobiphenyl, [$^{2}H_{5}$]nitrobenzene, [$^{2}H_{10}$]pyrene, and [$^{2}H_{14}$]terphenyl and the phenolic internal standard 3,4,5-trichlorophenol were added. The surrogate compounds were added to help monitor the efficiency of the preparation procedure. 3,4,5-Trichlorophenol also served as an internal standard for calculation of phenols. The mixture was extracted with methylene chloride–hexane (1:1, v/v) [12]. The extract was reduced to approximately 10 ml in a Kuderna–Danish concentrator on a steam table.

2.3.3. Clean up

The extract was added to 100 ml of water and stirred. A 2-ml volume of 25% aq. K_2CO_3 was added followed by 2 ml of acetic anhydride. The reaction mixture was vigorously stirred for 15 min, diluted to 500 ml with water, and extracted three times with 100 ml of methylene chloride. The extract volume was reduced under nitrogen to 5 ml and then washed two times with 2 ml saturated aqueous K_2CO_3. Silica gel chromatography was done before the SEC to remove most of the lipid. A 50×2.2 cm chromatography column was prepared with 10 g of silica gel topped with 2 g of Na_2SO_4. The column was washed with 25 ml pentane, the extract applied to the column, and the analytes eluted with 50 ml methylene chloride. The eluent was reduced to 10 ml and further cleaned up by SEC.

2.3.4. Size-exclusion chromatography [13]

SEC was performed at room temperature using one 10 cm×22 mm and two 50 cm×22 mm, 100 Å Jordi-Gel DVB (Jordi Associates, Bellingham, MA, USA) columns in series. The 10 cm×22 mm column was used as a guard column. A Rheodyne 7125 manual injector (Cotati, CA, USA) with a 1.0-ml sample loop was used for injections with a Hewlett-Packard 1050 series (Palo Alto, CA, USA) high-performance liquid chromatography (HPLC) pump for delivery of the mobile phase (methylene chloride) at a rate of 2.0 ml/min. Pump pressures were about 900 p.s.i. (1 p.s.i.=6894.76 Pa). A Hewlett-Packard 1050 series ultraviolet (UV) detector was used at 254 nm in series with a Waters 410 differential refractive index (RI) detector. Upchurch Scientific (Oak Harbor, WA, USA) stainless steel tubing of

Table 1
Neutral semi-volatile compound SIM window parameters

Window	Time[a] (min)	*m/z* (nominal)
1	9.5–12.8	41, 45, 51, 54, 77, 82, 93, 113, 117, 119, 121, 123, 128, 146, 148, 150, 152, 199, 201, 202
2	12.8–14.6	64, 102, 126, 127, 128, 129, 134, 135, 136, 137, 145, 147, 180, 182, 184, 224, 226, 257, 260, 261
3	14.6–16.5	63, 70, 71, 81, 85, 86, 115, 126, 127, 139, 141, 142, 143, 162, 163, 164, 170, 171, 172, 173
4	16.5–18.0	63, 77, 78, 89, 90, 119, 121, 139, 148, 151, 152, 153, 154, 163, 164, 165, 166, 167, 168, 169
5	18.0–19.9	77, 105, 141, 142, 163, 165, 166, 167, 168, 169, 170, 182, 204, 206, 248, 249, 250, 282, 284, 286
6	19.9–21.5	88, 89, 92, 94, 150, 151, 152, 158, 160, 175, 176, 177, 178, 179, 184, 185, 186, 187, 188, 189
7	21.5–24.5	100, 101, 102, 200, 201, 202, 203, 204, 210, 211, 212, 213, 219, 220, 234, 235, 240, 243, 244, 245
8	24.5–28.9	101, 113, 114, 126, 226, 227, 228, 229, 236, 239, 240, 241, 249, 250, 251, 252, 253, 263, 264, 265
9	28.9–end	111, 112, 113, 123, 124, 125, 126, 135, 136, 137, 138, 139, 272, 273, 274, 275, 276, 277, 278, 279

[a] Times are approximate. The windows are adjusted to accommodate actual target retention times.

1 m×1/16 in. O.D.×0.010 in I.D. was used for all column connections (1 in.=2.54 cm). A Perkin-Elmer Turbochrom 4 workstation (Norwalk, CT, USA) was used to generate and store chromatograms for each injection. The collection time was determined by separate injections of a non-fortified fish extract and a SEC calibration mixture of 25 mg/ml corn oil, 1 mg/ml bis(2-ethylhexyl)phthalate

Table 2
Quantitation and qualifying ions for neutral semi-volatile target compounds

Compound	Window	Quantitation (qualifying) ion
1,3-Dichlorobenzene	1	146 (113; 148; 150)
1,4-Dichlorobenzene	1	146 (113; 148; 150)
[$^{2}H_{4}$]1,2-Dichlorobenzene	1	152 (117; 150)
1,2-Dichlorobenzene	1	146 (113; 148; 150)
2,2′-Oxybis(1-chloropropane)	1	45 (41; 121; 123)
Hexachloroethane	1	117 (119; 121; 199; 201)
[$^{2}H_{5}$]Nitrobenzene	1	82 (54; 128)
Nitrobenzene	1	77 (51; 123)
1,2,4-Trichlorobenzene	2	180 (145; 147; 182; 184)
[$^{2}H_{8}$]Naphthalene	2	136 (134; 137)
Naphthalene	2	128 (64; 102; 126–27; 129)
Hexachlorobutadiene	2	225 (224; 226; 258; 260; 262)
2-Methylnaphthalene	3	142 (115; 141)
1-Methylnaphthalene	3	142 (115; 141)
2-Fluorobiphenyl	3	172 (115; 170–1; 173)
2-Chloronaphthalene	3	162 (63; 126–7; 164)
2,6-Dinitrotoluene	4	165 (63; 77–8; 119; 121; 166)
Acenaphthylene	4	152 (151; 153)
[$^{2}H_{10}$]Acenaphthene	4	164 (163; 165)
Acenaphthene	4	154 (151–3)
Dibenzofuran	4	168 (139; 169)
2,4-Dinitrotoluene	4	165 (63, 77–8; 119; 121; 166)
Fluorene	5	166 (163; 165; 167)
4-Chlorophenyl phenyl ether	5	204 (77; 141–2; 168–9; 206)
N-Nitrosodiphenylamine	5	169 (141; 167–8; 170)
1,2-Diphenylhydrazine	5	77 (105; 182)
4-Bromophenyl phenyl ether	5	248 (77; 141; 168–9; 249–50)
Hexachlorobenzene	5	284 (142; 282; 286)
[$^{2}H_{10}$]Phenanthrene	6	188 (94; 187)
Phenanthrene	6	178 (88–9; 150–2; 175–7; 179)
Anthracene	6	178 (88–9; 150–2; 175–7; 179)
Fluoranthene	7	202 (100–1; 200–1; 203–4)
[$^{2}H_{10}$]Pyrene	7	212 (210–11; 213)
Pyrene	7	202 (100–1; 200–1; 203–4)
[$^{2}H_{14}$]Terphenyl	7	244 (243; 245)
Retene	7	219 (202–4; 219–20; 234–5)
Benz[*a*]anthracene	8	228 (101; 113–4; 226–7; 229)
[$^{2}H_{12}$]Chrysene	8	240 (236)
Chrysene	8	228 (101; 113–4, 226–7; 229)
Benzo[*b*]fluoranthene	8	252 (113; 126, 249–51; 253)
Benzo[*k*]fluoranthene	8	252 (113; 126; 249–51, 253)
Benzo[*a*]pyrene	8	252 (113; 126; 249–51; 253)
[$^{2}H_{12}$]Perylene	8	264 (262; 265)
Indeno[1,2,3-*cd*]pyrene	9	276 (123–125; 136–8; 272–277)
Dibenz[*a*,*h*]anthracene	9	278 (139; 279)
Benzo[*ghi*]perylene	9	276 (123–125; 136–8; 272–277)

(BEHP), 0.2 mg/ml methoxychlor, 0.02 mg/ml perylene, and 0.08 mg/ml sulfur in methylene chloride. From this comparison it was determined that the sample collection period should begin at 115 min from the time of injection which is at the front end of the BEHP peak. The collection end time was determined by injection of a 40 μg/ml standard mixture containing the semi-volatile target analytes. Collections of fractions at 25 min intervals and GC–MS analyses of each fraction after concentrating determined a collection end time of 190 min from the time of injection. These SEC conditions result in a chromatographic run which is significantly longer than is usual in environmental analyses. The long program was necessary to remove enough of the fish lipid from the extract to allow low level analysis by GC–MS.

The fraction containing the analytes of interest was concentrated on a steam bath in a Kuderna–Danish apparatus and to a final volume of 1 ml. To the semi-volatiles extract was added 20 μg of the internal standard 2,2′-difluorobiphenyl (for determination of 3,4,5-trichlorophenol recovery) and 0.2 μg each of the internal standards [$^{2}H_{8}$]naphthalene, [$^{2}H_{10}$]acenaphthene, [$^{2}H_{10}$]phenanthrene, [$^{2}H_{12}$]chrysene, and [$^{2}H_{12}$]perylene (for determination of the SIM analytes).

2.3.5. Gas chromatography–selected ion mass spectrometry – neutrals

The extracts were determined using a HP-5890 gas chromatograph interfaced to a Hewlett-Packard 5971 or 5972 mass-selective detector. A 30 m×0.25 mm I.D., 0.25 μm film thickness 5% phenyl–95% methylpolysiloxane column (J&W DB-5 MS, Folsom, CA, USA) was used with helium as the carrier gas and a temperature program of 5 min at 35°C to 320°C at 12°C/min, then held at 320°C for 5 min. A 2-μl pulsed pressure injection was used (0.01 min at 8 p.s.i. to 35 p.s.i. at 35 p.s.i./min, hold 0.10 min, then to 8 p.s.i. at 35 p.s.i./min, the remainder of the run at a constant flow of 1 ml/min).

Neutral compounds were determined using SIM. Windows were selected to allow several *m/z* for each analyte. The *m/z* ratios selected for each window are shown in Table 1. All dwell times were 16 ms.

Identification was made on the basis of matching the mass spectrum and the retention time of the compound to that of a known standard. Quantitation was against calibration curves developed from the response of the target compounds compared to that of the internal standards in eight standards ranging

Table 3
Recoveries from fortified fish tissue – neutral semi-volatile compounds

Compound	Recovery (%)	SD (%)	*n*
Acenaphthene	86.1	22.3	39
Acenaphthylene	67.0	20.4	39
Anthracene	66.6	22.6	39
Benz[*a*]anthracene	76.2	18.6	39
Benzo[*b*]fluoranthene	78.4	20.3	39
Benzo[*k*]fluoranthene	75.7	18.4	39
Benzo[*a*]pyrene	63.1	28.0	39
Benzo[*ghi*]perylene	74.3	38.3	39
Di(2-chloroisopropyl) ether	63.5	31.3	38
Bromophenyl phenyl ether	73.9	20.3	39
2-Chloronaphthalene	68.5	16.4	39
Chlorophenyl phenyl ether	68.2	17.9	39
Chrysene	73.3	14.6	39
Dibenz[*a,h*]anthracene	92.8	54.0	39
1,2-Dichlorobenzene	55.7	13.9	39
1,3-Dichlorobenzene	53.1	14.6	39
1,4-Dichlorobenzene	54.0	14.4	39
2,4-Dinitrotoluene	157	198	39
2,6-Dinitrotoluene	102	48	31
1,2-Diphenylhydrazine	60.4	36.4	31
Fluoranthene	79.4	24.4	39
Fluorene	71.3	18.5	39
Hexachlorobenzene	80.8	26.9	39
Hexachlorobutadiene	72.2	23.4	39
Hexachloroethane	64.0	18.9	37
Indeno[1,2,3-*cd*]pyrene	93.6	52.7	39
n-Nitrosodiphenylamine	43.6	56.4	39
Naphthalene*	96.3	22.2	8
Nitrobenzene	72.1	28.9	39
Phenanthrene	77.7	16.4	39
Pyrene	67.5	12.8	39
1,2,4-Trichlorobenzene	66.5	18.4	39
[$^{2}H_{4}$]1,2-Dichlorobenzene (SS)	57.8	12.9	39
2-Fluorobiphenyl (SS)	75	15.5	39
[$^{2}H_{5}$]Nitrobenzene (SS)	75.9	30.3	38
[$^{2}H_{10}$]Pyrene (SS)	74.3	12.5	39
[$^{2}H_{14}$]Terphenyl (SS)	75.5	14.7	39

Compounds spiked at 80 ng/g wet tissue. *Data not included where matrix interferences precluded analysis of the compound. Napthalene was excluded in sample sets which contained appreciable amounts in the associated blanks. SS, Surrogate standard.

from 0.02 to 2.0 μg/ml and adjusted for the amount of tissue extracted. Quantitation ions used for the target analytes are listed in Table 2.

2.3.6. Gas chromatography–mass spectrometry – phenols

The phenol acetates were determined by scanning from m/z 40–m/z 540 in ~0.5 s using the same instrumentation and injection pressure program as listed above. A 1-μl injection was used and an oven temperature program of 1 min at 50°C then 8°C/min to 220°C, then 30°C/min to 320°C. The column was then held at the final temperature of 320°C for 1 min. Analytes elute within the first temperature ramp. The second ramp serves to rid the column of late eluting components which otherwise might be observed in succeeding chromatographic runs.

Identification was made on the basis of matching the mass spectrum and the retention time of the compound to that of a known standard. Quantitation was against calibration curves developed from the response of the target compounds compared to the internal standard 3,4,5-trichlorophenol from five standards ranging from 2 to 50 μg/ml and adjusted for the amount of tissue extracted. Recovery of the internal standard was calculated against 2,2′-difluorobiphenyl added immediately before injection. Standards were prepared by spiking known amounts of the analytes into water, derivatizing and preparing as described above for samples, with the exception of the SEC and silica gel clean-up steps.

3. Results and discussion

For each analysis batch, nominally 20 samples, two method blanks and two matrix spikes were extracted and analyzed. The precision and accuracy for the average of all matrix spike data for neutral semi-volatile compounds are listed in Table 3 and for phenolic compounds in Table 4. The 39 samples spiked included 12 different matrix types (either species or body portion). Results for the majority of compounds tested was generally greater than 70% with the exception of highly volatile analytes which may have been due to the lengthy preparation protocol. High standard deviations are observed for the late eluting PAHs which is speculated to be due to variability in interferences occurring from residual lipid. The compounds 2,4- and 2,6-dinitrotoluenes were variably recovered.

Tables 5 and 6 show results for method detection limits (MDLs) [14] for compounds determined by GC–MS determined by analysis of replicates of one

Table 4
Recoveries from fortified fish tissue – phenolic semi-volatile compounds

Compound	Recovery (%)	SD (%)	*n*
4-Chloroguaiacol	99	35	36
4-Chloro-3-methylphenol	118	32	36
2-Chlorophenol	115	47	36
3,4-Dichloroguaiacol	103	20	36
4,5-Dichloroguaiacol	97	19	36
4,6-Dichloroguaiacol	100	19	36
2,4-Dichlorophenol	121	35	36
2,4-Dimethylphenol	96	33	36
Pentachlorophenol	90	30	36
Phenol	98	39	36
Tetrachloroguaiacol	88	25	36
2,4,5-Trichlorophenol*	105	14	21
2,4,6-Trichlorophenol	138	31	36
3,4,5-Trichloroguaiacol	92	22	36
3,4,6-Trichloroguaiacol	101	18	36
4,5,6-Trichloroguaiacol	94	20	36
3,4,5-Trichlorophenol (I.S.)	73	19	36

Compounds spiked at 5.0 μg/g wet tissue. *Only 21 spiked samples included this compound. I.S., Internal standard.

Table 5
Calculated method detection limit – neutral semi-volatile compounds

Compound	Average concentration[a] (ng/g)	SD (ng/g)	MDL (ng/g)
Acenaphthene	11.4	1.2	3.6
Acenaphthylene	10.0	1.1	3.2
Anthracene	10.0	0.88	2.6
Benz[*a*]anthracene	11.2	0.74	2.2
Benzo[*b*]fluoranthene	10.5	0.86	2.6
Benzo[*k*]fluoranthene	9.72	0.84	2.6
Benzo[*ghi*]perylene	11.3	1.1	3.4
Benzo[*a*]pyrene	8.78	1.5	4.4
Di(2-chloroisopropyl) ether	7.48	1.0	3.2
Bromophenyl phenyl ether	12.5	1.1	3.4
2-Chloronaphthalene	10.0	0.98	3.0
Chlorophenyl phenyl ether	11.5	1.4	4.0
Chrysene	10.4	0.92	2.8
Dibenz[*a*,*h*]anthracene	13.9	1.3	3.8
1,2-Dichlorobenzene	8.42	0.82	2.4
1,3-Dichlorobenzene	7.62	0.96	2.8
1,4-Dichlorobenzene	8.02	0.86	2.6
2,4-Dinitrotoluene	17.8	2.7	8.0
2,6-Dinitrotoluene	15.0	1.7	5.2
1,2-Diphenylhydrazine	6.40	0.82	2.4
Fluoranthene	13.6	1.3	4.0
Fluorene	11.0	1.1	3.4
Hexachlorobenzene	15.1	1.3	4.0
Hexachlorobutadiene	9.68	1.2	3.8
Hexachloroethane	8.48	1.5	4.4
Indeno[1,2,3-*cd*]pyrene	12.6	1.2	3.8
Naphthalene	26.6	6.7	20
Nitrobenzene	10.4	2.0	6.0
Phenanthrene	14.4	2.6	7.8
Pyrene	9.16	1.0	3.0
1,2,4-Trichlorobenzene	10.1	1.3	4.0

[a] Eight replicates of fish tissue spiked at 16.0 ng/g per component, wet mass.

homogeneous matrix type, largescale sucker (*C. macrocheilus*), fillet with skin, spiked at a low level.

Over 300 tissue samples were analyzed using this protocol. In general, chlorinated phenolic compounds were not detected in the tissues, though pentachloroanisole, a metabolite of pentachlorophenol, was found in several samples at sub μg/kg levels during the separate GC–electron-capture detection analysis of pesticides in these same samples. A few neutral semi-volatile compounds, PAHs, were found at very low levels. For example, 1- and 2-methylnaphthalenes, the most frequently encountered compounds, were found in fewer than a third of the samples and were typically 5–50 μg/kg wet tissue mass.

Naphthalene was common in the batch blanks compromising the results for this analyte for those batches. Under these analytical conditions the determination of naphthalene in matrix spikes was also complicated by the 50-fold spiking level difference of closely eluting naphthalene and the acetate of 2-chlorophenol.

Although the fractions were kept together to maximize quantitation limits by not dividing the sample extract, it would be worthwhile investigating the effect of splitting the extract before derivatization. This would allow a more rigorous silica gel clean-up for the neutral organics and decrease interferences arising in the SIM analysis of the neutral

Table 6
Calculated method detection limit – phenolic semi-volatile compounds

Compound	Average concentration[a] (μg/g)	SD (μg/g)	MDL (μg/g)
4-Chloroguaiacol	0.44	0.24	0.70
4-Chloro-3-methylphenol	0.96	0.10	0.32
2-Chlorophenol	0.94	0.10	0.32
3,4-Dichloroguaiacol	0.78	0.17	0.50
4,5-Dichloroguaiacol	0.82	0.17	0.52
4,6-Dichloroguaiacol	0.88	0.16	0.48
2,4-Dichlorophenol	1.08	0.088	0.26
2,4-Dimethylphenol	0.80	0.14	0.44
Pentachlorophenol	1.14	0.098	0.30
Phenol	0.54	0.096	0.30
Tetrachloroguaiacol	1.06	0.090	0.28
2,4,5-Trichlorophenol	1.18	0.082	0.24
2,4,6-Trichlorophenol	1.30	0.076	0.24
3,4,5-Trichloroguaiacol	1.04	0.14	0.44
3,4,6-Trichloroguaiacol	1.06	0.11	0.34
4,5,6-Trichloroguaiacol	1.00	0.15	0.44

[a] Eight replicates of fish tissue spiked at 1.0 μg/g per component, wet mass.

semi-volatile compounds from the acetic anhydride derivatization. Further, the chlorinated derivatized phenols could be determined with a more sensitive detection, e.g., electron-capture detection or SIM-MS.

4. Conclusion

In general the protocol including the acetylation step was found to be effective in reducing the amount of lipid material injected into the GC–MS system allowing determination of selected neutral semi-volatile and phenolic compounds in the variety of fish tissues analyzed. Calculated MDLs were generally less than 0.5 μg/g for phenolic compounds and less than 0.01 μg/g for neutral semi-volatile compounds. The combined results for these organic compounds as well as the results obtained for metals, chlorinated dibenzodioxins and -furans, pesticides, Aroclors and co-planar PCBs in these same fish tissue samples will be used to develop risk criteria for consumption of fish in the Columbia River Basin.

5. Note

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Acknowledgements

The authors would like to acknowlege the laboratory assistance of K. Donnegan, D. Wood, and T. Gilfoil and helpful discussions with J. Blazevich.

References

[1] National Study of Chemical Residues in Fish, EPA 823-R-92-008a, US EPA Office of Science and Technology, Washington, DC, September 1992.
[2] G.G. Lauenstein, A.Y. Cantillo (Eds.), Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984–1992: Comprehensive Descriptions of Trace Organic Analytical Methods, Vol. IV, NOAA Technical Memorandum NOS ORCA 71, Silver Spring, MD, 1993.
[3] Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, 2nd ed, Fish Sampling and Analysis, EPA 823/R-95-007, Vol. I, 1995.
[4] M. Söderström, C.A. Wachtmeister, L. Förlin, Chemosphere 28 (1994) 1701.
[5] U. Varanasi, J.E. Stein, M. Nishimoto, in: U. Varanasi (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbon in the Aquatic Environment, CRC Uniscience Series, CRC Press, Boca Raton, FL, 1989, p. 93.

[6] R.T. Coutts, E.E. Hargesheimer, F.M. Pasutto, J. Chromatogr. 179 (1979) 291.
[7] R.H. Voss, J.T. Wearing, A. Wong, in: L.H. Keith (Ed.), Advances in the Identification and Analysis of Organic Pollutants in Water, Ann Arbor Sci, Ann Arbor, MI, 1981, p. 1059, Chapter 53.
[8] I.H. Rogers, J.A. Servizi, C.D. Levings, Water Pollut. Res. J. Can. 23 (1988) 100.
[9] A. Morales, D.A. Birkholz, S.E. Hrudey, Water Environ. Res. 64 (1992) 669.
[10] D.D. MacDonald, M.G. Ikonomou, A.-L. Rantalaine, I.H. Rogers, D. Sutherland, J. Van Oostdam, Environ. Toxicol. Chem. 16 (1997) 491.
[11] C.M. Couillard, P. Nellis, Environ. Toxicol. Chem. 18 (1999) 2545.
[12] Analytical Procedures and Quality Assurance Plan for the Determination of Xenobiotic Chemical Contaminants in Fish, EPA 600 3-90-023, US EPA, Environmental Research Laboratory, Deluth, MN, March 1990.
[13] G. Dodo, M. Knight, J. Chromatogr. A 859 (1999) 235.
[14] J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave, W.L. Budde, Environ. Sci. Technol. 15 (1981) 1426.