A CONCERTED ANALYTICAL METHOD FOR DETERMINATION OF VARIOUS HALOGENATED AND RELATED BIOACCUMULATING COMPOUNDS IN FISH AND SEDIMENTS

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ABSTRACT

Analytical procedures have been developed for simultaneously measuring a large group of xenobiotic organic compounds in fish tissue and river sediments. Results obtained with these methods are described.

INTRODUCTION

In collaboration with the U.S. EPA Duluth Laboratory, we have recently worked to develop and evaluate a concerted procedure for simultaneous measurement of some 38 organic compounds in fish tissue and sediments. This set of target analytes was selected by EPA on the basis of toxicity, bioavailability, metabolism and persistence of these chemicals in fish, as well as the quantities of such chemicals produced and used in the U.S. The method developed was intended to utilize minimal sample preparation in order to reduce losses of target analytes. The sample extracts were to be amenable to full-scan capillary-column GC-MS analysis, and identification of analytes was to be based on GC retention time, responses at appropriate indicator masses, correct quantitation-to-confirmation ion intensity ratios, and library matching of mass spectra. The desired GC-MS calibration range was for 1-50 ng/µL of injected analyte.

EXPERIMENTAL

The sample preparation procedures used to isolate the bioaccumulating compounds from the fish and sediment samples evaluated in this study included extraction and fractionation on two liquid chromatographic columns. Following homogenization, 20 g aliquots of each blended fish and sediment sample were mixed with 80 g of sodium sulfate, fortified with three surrogate standards and extracted with hexane/methylene chloride (1/1) for 16 hours in a Soxhlet extraction apparatus. The concentrated sample extracts were fractionated on a 2.5-cm x 45-cm Biobeads SX-3 gel permeation chromatography (GPC) column using cyclopentane/methylene chloride (1/1) as the mobile phase. Because the GPC column separations were more effective for injections of extracts containing 0.5 g or less of lipid, the lipid content of each fish tissue sample was measured prior to GPC fractionation. Since no more than two GPC fractionation sequences were desired for each sample extract, it was necessary to reduce the 20 g fish sample size normally used in the case of fish which contained more than 5% lipid. The GPC eluates for each fish and sediment sample processed were concentrated and these were then fractionated on a 300 mg silica gel column [Silica-Gel 60, 1% (w/w) water deactivated] using 15% methylene chloride-in-hexane as the eluting solvent. The eluates from the silica gel column were concentrated to 90 uL, fortified with three internal standards and refrigerated until just prior to analysis. A set of spiked fish samples prepared by these procedures for purposes of method validation are described in Table 1.

Instrumentation utilized to analyze the extracts prepared as described above included a Kratos MS-25 mass spectrometer equipped with a Carlo-Erba 5300 gas chromatograph and an Extrel ELQ-400 mass spectrometer equipped with a Hewlett-Packard 5890 gas chromatograph. The capillary column employed was a J & W Scientific 60 meter DB-5 with 1.0 micron film

thickness and 0.32 mm I.D. Injections were accomplished in splitless mode (split on at 1 min) using Helium carrier gas (3.2 kg/cm²). Injector, interface, and source temperatures were maintained at 300°C. The column temperature was initially at 60°C for 1 minute, then was increased at 5°C per minute to 300°C, and held at this temperature for 30 minutes.

METHOD VALIDATION

Table 2 shows a summary of the data obtained using these methods in a preliminary validation study with fish samples. These results indicate that the procedures described can successfully detect and quantitate 33 of the 38 target analytes in fish, with detection limits of 12.5 ng/g or lower. The calibration range was found to be linear for these analytes from 12.5 to 1250 ng/g. Pentachlorophenol, dicofol, dieldrin, chlorobenzilate and kepone were not reliably detected in fish by the methods initially applied, although more recent elaborations of these procedures may make it possible to also measure these analytes.

Athough a separate method validation was not initially carried out for sediment samples, over 100 actual fish and sediment samples were analyzed in batches of nine samples, long with one duplicate, one fortified matrix, and one method blank or matrix blank. Results of fortified sediment and fish tissue spikes agreed well with those obtained in the initial method validation.

RESULTS AND CONCLUSIONS

Table 3 shows typical analyte data obtained for selected fish and sediment samples which were obtained from some heavily industrialized rivers in the U.S. In general, this method was found to yield good results on a variety of fish tissues (catfish, large and small mouth bass, smelt, trout and white suckers) and on a variety of river sediments (sandy, silty, and loamy).

A comparison of the analyte data obtained for fish and sediment samples, as shown in Table 3, both of which were identified as having come from one particular geographical region, indicates that there is a correlation of the analytes in these two types of samples.

Currently this methodology is undergoing further validation to extend the total number of target analytes to more than sixty xenobiotic compounds.

TABLE 1: SAMPLES PREPARED FOR VALIDATION
OF METHOD FOR DETERMINING BIOACCUMULATING ORGANICS IN FISH TISSUE

Sample Series[a]	Number Samples	Native Standard Added ng/g	Surrogate Standard Added ng/g	Internal Standard Added ng/g
A	4	12.5	12.5	95.75
B	4	37.5	37.5	93.75
C	4	125.0	125.0	93.75
D	4	1250.0	1250.0	93.75
E	4	0.0	125.0	93.75

a. Sample size was 20 grams.

TABLE 2: SUMMARY OF ANALYTE DATA OBTAINED IN VALIDATION STUDY USING SPIKED FISH SAMPLES

Analyte Name	Retention Time	Quantitation Masses	%RSD of Response Factors	%Recovery Spiked Sample
Internal Standard: D_{10}-Biphenyl				
1,3,5-Trichlorobenzene	15:10	180 182	3.9	85
1,2,4-Trichlorobenzene	16:30	180 182	6.7	84
1,2,3-Trichlorobenzene	17:36	180 182	0.9	85
1,2,3,5-Tetrachlorobenzene	21:03	216 218	6.0	86
1,2,4,5-Tetrachlorobenzene	21:06	216 218	6.0	86
Biphenyl	22:29	154 152	10.0	91
1,2,3,4-Tetrachlorobenzene	22:31	216 218	7.5	85
Pentachlorobenzene	26:34	250 252	4.6	78
Internal Standard: D_{10}-Phenanthrene				
Trifluralin	30:27	306 264	30.8	99
Alpha-BHC	31:12	219 181	8.7	134
Hexachlorobenzene	31:36	284 286	8.5	96
Pentachloroanisole	31:49	280 265	7.7	89
Pentachlorophenol	32:42	266 264	71.6	588
Gamma-BHC (Lindane)	32:45	219 181	11.2	126
Pentachloronitrobenzene	33:01	295 237	52.5	69
Diphenyl Disulfide	34:22	218 109	4.1	158
Heptachlor	36:06	272 100	27.1	136
Chloropyrifos	37:48	197 314	15.1	150
Dicofol (Kelthane)	37:55	250 139	19.9	270
Isopropalin	38:49	280 238	37.8	61
Octachlorostyrene	39:11	380 382	8.1	62
Heptachlor Epoxide	39:22	353 355	7.5	80
Oxychlordane	39:26	185 387	10.9	99
Chlordane, trans	40:24	373 375	12.3	81
Butachlor	40:49	176 161	15.4	57
Chlordane, cis	41:02	373 375	1.5	79
Internal Standard: D_{12}-Chrysene				
Nonachlor, trans	41:15	409 407	15.8	132
DDE -p-p'	41:50	246 318	14.5	139
Dieldrin	42:02	79 263	10.1	307
Nitrofen	42:42	283 285	37.1	68
Perthane	42:49	223 224	16.3	140
Endrin	42:58	317 263	21.0	96
Chlorobenzilate	43:08	251 139	15.1	266
Nonachlor, cis	43:52	409 407	18.8	103
Kepone	44:26	272 274	112.9	1108
Triphenyl Phosphate	45:50	326 325	30.7	131
Methoxychlor	47:22	227 228	22.6	144
Mirex	49:23	272 274	23.9	92
Surrogate Stds:				
$^{13}C_6$-1,2,4,5-Tetrachlorobenzene	21:05	222 224	0.0	86
$^{13}C_6$-Pentachlorophenol	32:46	272 270	56.6	497
D_{10}-Pyrene	40:38	212 106	5.1	67
D_8-DDE	41:46	254 326	11.7	82
D_{12}-Benzo-e-pyrene	54:32	264 265	17.6	46

TABLE 3: MEASURED CONCENTRATIONS OF BIOACCUMULATING COMPOUNDS IN SELECTED FISH AND SEDIMENT SAMPLES FROM HEAVILY INDUSTRIALIZED RIVERS IN THE U.S.

Analyte Name	Fish DEQ7-13	Fish DEQ7-14	Fish DEQ7-20	Sediment DEQ7-22	Sediment DEQ7-27
			ng/g or ppb		
Internal Standard: D_{10}-Biphenyl					
1,3,5-Trichlorobenzene	ND(2.5)	ND(2.5)	ND(2.5)	ND(3.4)	ND(5.8)
1,2,4-Trichlorobenzene	ND(2.5)	ND(2.5)	ND(2.5)	ND(3.4)	ND(5.8)
1,2,3-Trichlorobenzene	ND(2.5)	ND(2.5)	ND(2.5)	ND(3.4)	ND(5.8)
1,2,3,5-Tetrachlorobenzene	ND(2.5)	ND(2.5)	ND(2.5)	ND(3.4)	ND(5.8)
1,2,4,5-Tetrachlorobenzene	ND(2.5)	ND(2.5)	ND(2.5)	ND(3.4)	ND(5.8)
Biphenyl	ND(2.5)	ND(2.5)	15	9.1	39
1,2,3,4-Tetrachlorobenzene	ND(2.5)	ND(2.5)	ND(2.5)	ND(3.4)	ND(5.8)
Pentachlorobenzene	ND(2.5)	ND(2.5)	ND(2.5)	ND(3.4)	ND(5.8)
Internal Standard: D_{10}-Phenanthrene					
Trifluralin	70	154	ND(6.3)	ND(8.6)	ND(15)
Alpha-BHC	ND(6.3)	ND(6.3)	ND(6.3)	25	ND(15)
Hexachlorobenzene	ND(6.3)	15	ND(6.3)	ND(8.6)	ND(15)
Pentachloroanisole	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Gamma-BHC (Lindane)	ND(6.3)	ND(6.3)	18	ND(8.6)	ND(15)
Pentachloronitrobenzene	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Diphenyl Disulfide	ND(6.3)	144	157	ND(8.6)	ND(15)
Heptachlor	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Chloropyrifos	20	ND(6.3)	ND(6.3)	21	ND(15)
Dicofol (Kelthane)	ND(25)	ND(25)	ND(25)	ND(34)	ND(58)
Isopropalin	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Octachlorostyrene	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Heptachlor Epoxide	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Oxychlordane	ND(6.3)	371	88	30	ND(15)
Chlordane, trans	ND(6.3)	ND(6.3)	16	ND(8.6)	ND(15)
Butachlor	20	9.1	ND(6.3)	65	31
Chlordane, cis	7.0	ND(6.3)	26	ND(8.6)	ND(15)
Internal Standard: D_{12}-Chrysene					
Nonachlor, trans	12	ND(6.3)	29	ND(8.6)	ND(15)
DDE -p-p'	1300	712	280	ND(8.6)	ND(15)
Dieldrin	123	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Nitrofen	ND(12)	ND(12)	ND(12)	ND(17)	ND(29)
Perthane	ND(6.3)	11	ND(6.3)	ND(8.6)	ND(15)
Endrin	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	107
Chlorobenzilate	ND(6.3)	9.1	ND(6.3)	ND(8.6)	ND(15)
Nonachlor, cis	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Kepone	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Triphenyl Phosphate	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Methoxychlor	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)
Mirex	ND(6.3)	ND(6.3)	ND(6.3)	ND(8.6)	ND(15)

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