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Project Summary

Development of Method for Semivolatile Organic Priority Pollutants in Fish

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A method has been developed for determining neutral and acidic priority pollutant compounds in fish tissue. Priority pollutant compounds are extracted from the fish tissue during homogenization of the tissue in acetonitrile. The acetonitrile extract is isolated from the tissue after centrifuging and placed in a tumbling bottle containing salted water buffered to pH 4, methyl t-butyl ether (MTBE), and petroleum ether. The mixture is equilibrated by tumbling and a portion of the organic layer is isolated after drying over sodium sulfate. The acetonitrile partitioning step serves to remove a portion of the extractable fish lipid material from the priority pollutant compounds. The organic extract is concentrated and the majority of the remaining lipid material is removed using a gel permeation chromatography (GPC) procedure. The final extract is concentrated, and priority pollutant compounds are detected and quantified in the extract by gas chromatography-mass spectrometry (GC-MS). The method yielded a concentration factor of 10 and detection limits for the neutral and acidic priority pollutant compounds in fish tissue in the mid to high parts per billion (w/w) range.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, OH, to announce the research to develop an analytical method for semivolatile organic priority pollutants in fish by capillary column GC-MS. The research is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The extraction and cleanup procedures used in the final method were based on the method entitled "Analysis of Fish for General Organics by Solvent Extraction" presented in the EPA publication entitled "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue" (EPA-600/4-81-055). Analysis procedures used in the final method were based on EPA Method 625, which specifies the use of GC-MS. Capillary column GC was used for all analyses. Cleanup procedures investigated were limited to the use of acetonitrile partitioning and GPC. The method was developed to use a fish sample size of approximately 10 to 25 grams and designed for easy inclusion of other semivolatile organic compounds.

Acetonitrile Partitioning

Two acetonitrile partitioning procedures were examined. The first procedure used separatory funnels for the partitioning step as specified in the EPA Interim Method. The second procedure used tumbling to perform the partitioning. Studies were conducted to determine the partitioning characteristics of the priority pollutant compounds between the aqueous and organic phases with and without the presence of fish lipid material. Organic solvents investigated included combinations of hexane, petroleum ether, methylene chloride, and MTBE. Aceton trile content of the resultant organic extract was

also determined as well as the fish lipid removal efficiency observed for each of the organic solvents.

Partitioning studies demonstrated that nonpolar solvents such as hexane or petroleum ether resulted in unacceptable recoveries of polar compounds such as phenols. Use of more polar solvents such as MTBE resulted in higher recoveries of polar priority pollutants. Lipid removal studies indicated that cleanup efficiencies did not vary significantly regardless of the polarity of the extraction solvent. Approximately three-fourths of the lipid material remained in the aqueous phase after partitioning by tumbling. Most of the acetonitrile must be removed from the extract prior to introduction onto the GPC, and acetonitrile is difficult to remove using conventional concentration techniques. In general, the nonpolar extraction solvents contained only a small percentage of acetonitrile after the partitioning step and the polar extraction solvents contained 20 to 30 percent acetonitrile after the partitioning step. The optimum situation was observed when 50 percent MTBE in petroleum ether was used as the extraction solvent. Use of 50 percent MTBE in petroleum ether yielded acceptable recoveries of polar priority pollutant compounds and contained only five percent acetonitrile after the partitioning step.

Recoveries of neutral compounds after separatory funnel partitioning were approximately equivalent to those observed after tumbling partitioning, indicating that the tumbling procedure could be substituted for the more commonly used separatory funnel partitioning technique. Use of the tumbling procedure greatly reduced the amount of time and glassware required for the extraction, avoided the operator-dependent variability associated with separatory funnel extractions, and provided comparable or superior recovery results.

Gel Permeation Chromatography

GPC studies were conducted to evaluate the efficiency of the method for removing fish lipid material from the various semivolatile organic priority pollutant compounds. The GPC column was packed with BioBeads SX-3 resin, and 50 percent methylene chloride in hexane was used as the elution solvent. Relative elution profiles for fish lipid material and the semivolatile organic priority pollutant compounds were determined.

GPC elution studies indicated that greater than 99 percent of the lipid material could be removed from the fish extract without loss of even the long-chain phthalates. Initially, fraction collection was initiated and terminated at the appropriate time, as indicated by the GPC elution study results. This sample collection procedure was suitable during the preliminary method evaluation studies, which used catfish fillets as the fish matrix. During method validation and matrix validation studies, it became apparent that other fish matrixes contained lipid material that eluted later from the GPC column. Initiation of sample collection had to be postponed to avoid excessive amounts of lipid material in the final extract, resulting in lowered recoveries of long-chain phthalates.

Preliminary Method Evaluation

The draft method was performed as written as a preliminary evaluation of the method. Preliminary method evaluation studies were conducted using catfish fillets obtained from a local fish market. Replicate samples were spiked with the priority pollutant compounds at approximately the 2 g/g concentration level and processed immediately after spiking. The resultant fish extracts were analyzed by GC-MS to determine compound recoveries. In an attempt to simulate compound incorporation into the fish tissue, identical samples were spiked and analyzed after they had been stored for a 24-hour period to allow incorporation of the compounds into the tissue.

Most of the priority pollutant compounds demonstrated recoveries greater than 70 percent. Significantly lower recoveries were observed for the more polar phenols; recoveries of 20 percent, 53 percent and 28 percent were obtained for 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, and 4-nitrophenol, respectively. Possibly these components were incorporated into the fish tissue and degraded by or inefficiently removed from the tissue.

A general trend of slightly lower recoveries was observed in samples that had been stored for 24 hours after spiking. The trend may have been due to enzymatic degradation of the compounds or to incomplete extraction of compounds that became incorporated into the fish tissue. The experimental design did not allow for any conclusions regarding these data, but these data do suggest that recoveries observed immediately after spiking may be slightly overstated. Statistically significant decreases in recoveries upon storage were observed for only pentachlorophenol, bis-(2-ethylhexyl) phthalate, and hexachlorocyclopentadiene.

Method Range and Method Detection Limit Studies

Method range and method detection limit studies were conducted to determine how well the analysis procedure performed in fish over a range of concentrations. Method range and method detection limit studies were conducted using a fish matrix consisting of equal parts of ground catfish, scallops, and salmon obtained from a local fish market. Fish tissue was spiked at concentrations ranging from approximately 20 ng/g to 2 g/g with the priority pollutant compounds, processed, and analyzed by GC-MS to determine compound recoveries.

A summary of the average recovery data obtained at each spiking level is given in Table 1. With the exception of the more polar phenols, recoveries obtained from the three highest spiking levels were greater than 50 percent and repeatable over the spiking range. Virtually none of the priority pollutant compounds were detected at the low spiking level and only a few were detected at the medium-low spiking level.

Matrix Validation Studies

Matrix validation studies were conducted as a comparison to the method range studies in order to determine if varying fish matrices might effect the analytical results obtained. The matrix validation studies were conducted using minnows obtained from a local bait store. The fish tissue was spiked at the 2 g/g concentrations level with the priority pollutant compounds, processed, and analyzed by GC-MS to determine compound recoveries.

In most cases, recoveries of priority compounds from the minnow samples did not vary significantly from those observed from the mixed fish matrix. Recovery of di-n-butyl phthalate from the minnow samples was significantly decreased, presumably due to the fact that collection of the GPC fraction was initiated later than was done during the method validation studies.

Table 1. *Summary of Results from Method Range Studies*

Compound	Average Percent Recovery at Given Spike Level (A)				
	High	Med-High	Medium	Med-Low	Low
1,2,4-Trichlorobenzene	63	79	51	75	(C)
1,2-Dichlorobenzene	66	79	57	74	71
1,3-Dichlorobenzene	66	80	52	79	(C)
1,4-Dichlorobenzene	67	86	55	96	124
2,4,6-Trichlorophenol	62	84	71	47	(C)
2,4-Dichlorophenol	62	85	71	(C)	(C)
2,4-Dimethylphenol	171	104	157	(C)	(C)
2,4-Dinitrophenol	13	33	(C)	(C)	(C)
2,4-Dinitrotoluene	54	83	63	(C)	(C)
2,6-Dinitrotoluene	58	82	64	(C)	(C)
2-Chloronaphthalene	61	85	63	81	92
2-Chlorophenol	59	76	57	(C)	(C)
2-Methyl-4,6-Dinitrophenol	36	54	48	(C)	(C)
2-Nitrophenol	56	75	67	(C)	(C)
4,4'-DDD	70	105	88	119	(C)
4,4'-DDE	65	96	106	112	(C)
4,4'-DDT	56	125	141	(C)	(C)
4-Bromophenyl Phenyl Ether	60	86	76	101	(C)
4-Chlorophenyl Phenyl Ether	60	86	63	80	(C)
4-Chloro-3-Methylphenol	65	100	105	(C)	(C)
4-Nitrophenol	15	(C)	(C)	(C)	(C)
Acenaphthene	61	82	78	85	76
Acenaphthylene	64	87	69	88	96
Aldrin	62	87	81	109	140
Alpha-BHC	60	86	67	77	(C)
Alpha-Chlordane	57	84	81	72	(C)
Anthracene	62	89	75	93	(C)
Benzo (A) Anthracene	69	100	90	85	(C)
Benzo (A) Pyrene	61	105	113	102	(C)
Benzo (B) Fluoranthene	63	104	101	85	(C)
Benzo (G,H,I,) Perylene	23	38	44	(C)	(C)
Benzo (K) Fluoranthene	64	104	91	77	(C)
Beta-BHC	61	93	54	(C)	(C)
Bis (2-Chloroethyl) Ether	54	71	59	69	105
Bis-2-Chloroethoxymethane	57	77	68	101	102
Bis-(2-Chloroisopropyl) Ether	53	66	66	79	(C)
Bis-(2-Ethylhexyl) Phthalate	11	16	20	105	238
Butylbenzyl Phthalate	79	114	62	458	655
Chrysene	66	114	122	100	(C)
Delta-BHC	61	80	65	142	(C)
Dibenzo (A,H) Anthracene	91	126	160	(C)	(C)
Dieldrin	61	79	68	(C)	(C)
Diethyl Phthalate	60	83	97	157	263
Dimethyl Phthalate	50	72	53	80	216
Di-N-Butyl Phthalate	50	49	15	145	(C)
Di-N-Octyl Phthalate	8	16	26	(C)	(C)
Endosulfan Sulfate	63	90	80	98	(C)
Endosulfan I	65	102	148	165	(C)
Endosulfan II	62	88	70	110	(C)
Endrin	73	101	213	86	(C)
Endrin Aldehyde	20	31	(C)	(C)	(C)
Fluoranthene	65	96	70	102	(C)
Fluorene	61	82	55	92	44
Gamma-BHC	64	81	72	70	(C)
Gamma-Chlordane	64	103	110	91	(C)
Heptachlor	58	87	79	97	(C)
Heptachlor Epoxide	63	90	87	(C)	(C)
Hexachlorobenzene	58	79	73	87	(C)
Hexachlorobutadiene	61	80	62	71	(C)
Hexachlorocyclopentadiene	13	13	(C)	(C)	(C)
Hexachloroethane	64	78	60	68	(C)
Indeno (1,2,3-Cd) Pyrene	111	142	121	(C)	(C)
Isophorone	52	70	56	73	78
Naphthalene	65	82	58	97	52
Nitrobenzene	60	74	74	(C)	(C)
N-Nitrosodiphenylamine	59	74	32	74	(C)

Table 1. *(Continued)*

Compound	Average Percent Recovery at Given Spike Level (A)				
	High	Med-High	Medium	Med-Low	Lo
N-Nitrosodipropylamine	54	69	86	(C)	(C)
Pentachlorophenol	68	94	115	(C)	(C)
Phenanthrene	60	86	55	107	5
Phenol	38	70	72	(C)	(C)
Pyrene	66	105	94	116	21
Trichlorobiphenyl	60	85	71	(C)	(C)
D10-Acenaphthene (B)	57	76	78	80	9
D10-Fluorene (B)	57	77	83	87	10
D10-Pyrene (B)	62	85	107	98	13
D3-2,4-Dichlorophenol (B)	57	92	83	(C)	(C)

(A) Corrected for the average amount found in the blanks.
(B) Recovery standard added to each fish sample prior to extraction to obtain an indication of method performance.
(C) The compound was not detected.

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The complete report, entitled "Development of Method for Semivolatile Organic Priority Pollutants in Fish," (Order No. PB 86-136 058/AS; Cost: $11.95, subject to change) will be available only from:

National Technical Information Service
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