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Short communication

Application of polydivinylbenzene liquid chromatography columns to remove lipid material from fish tissue extracts for the analysis of semivolatile organics

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Abstract

Liquid chromatography columns of 100% polydivinylbenzene (DVB) (packing) were used to remove lipid material from fish extracts before analysis of several semivolatile organic pollutants by gas chromatography–mass spectrometry (GC–MS). This packing material was found to be durable as the columns could be operated to about 900 p.s.i. resulting in high efficiency separation. Recoveries and relative standard deviations for 18 polynuclear aromatic hydrocarbons fortified into a fish extract and cleaned up by multiple DVB columns in series were in the range of 86 to 123% and 4 to 11%, respectively. Published by Elsevier Science B.V.

Keywords: Poly(divinylbenzene); Lipids; Polynuclear aromatic hydrocarbons

1. Introduction

The analysis of organic pollutants at trace levels from fish tissue requires extensive clean-up of the sample extract before analysis by sensitive instrumentation such as gas chromatography–mass spectrometry (GC–MS). Lipid material, particularly the high-molecular-mass triglyceride fraction, can significantly reduce performance of GC–MS due to residue accumulation in the injection port, column and source. Size-exclusion or gel-permeation chromatography (GPC) is a useful technique for the separation of high boiling or macromolecular compounds from semivolatile organic pollutants in sample extracts to improve overall GC or GC–MS performance [1,2]. Additional steps after GPC clean-up with polystyrene–divinylbenzene type columns (e.g., Bio-Beads SX-3) have been found necessary for the analysis for certain organic pollutants due to residual macromolecular material in the collected fraction that could interfere with analysis [3–7]. Highly crosslinked polystyrene–divinylbenzene type columns designed for use at high pressures have been applied to extracts of food products and environmental matrices [8–12].

In this study a method using columns of 100% polydivinylbenzene (DVB) packing material was developed for the removal of lipid material in fish extracts before the analysis of semivolatile organic compounds including polynuclear aromatic hydrocarbons (PAHs). This material was chosen because of its ability to be used in small pore sizes (100 Å

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for this study) at high pressures. The higher number of theoretical plates for DVB over the more commonly used polystyrene–divinylbenzene packing was expected to increase resolution of the high-molecular-mass fraction containing lipid material from selected semivolatile organic compounds. The method was developed to be isocratic with methylene chloride as the mobile phase to allow concentration of the collected fraction to 1–2 ml using only Kuderna–Danish evaporators with steambaths followed by volume adjustments with a stream of nitrogen.

2. Experimental

2.1. Chemicals

Analytical standards were purchased from Ultra Scientific (North Kingstown, RI, USA). Pesticide residue grade solvents used for sample preparation and analyses were purchased from Burdick and Jackson (Muskegon, MI, USA).

2.2. Sample preparation

A concentrated methylene chloride–hexane (1:1) extract prepared from homogenized whole body Steelhead trout (*Oncorhynchus mykiss*) was used as a test matrix for recovery experiments. The fish sample used was previously analyzed for the semivolatile organic compounds listed in Table 1 to allow accurate recovery calculations. The percent of the high-molecular-mass fraction, expected to consist mostly of lipid material, was determined by air drying at room temperature (21±2°C) a portion of the extract for 18 h and until constant mass. Four 1-ml aliquots of the test matrix containing 170 mg each of high-molecular-mass material were fortified to a concentration of 16 μg/ml of the compounds listed in Table 1.

2.3. Size-exclusion chromatography (SEC)

SEC of the fortified test matrix aliquots was performed at room temperature using one 10 cm×22 mm and two 50 cm×22 mm, 100 Å Jordi-Gel DVB columns in series (Jordi Associates, Bellingham, MA, USA). 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 at 254 nm was used in series with a Waters 410 differential refractive index (RI) detector (Milford, MA, USA) to detect the eluting compounds. Upchurch Scientific stainless steel tubing of 1 m×1/16 in. O.D.×0.010 in. I.D. (Oak Harbor, WA, USA) was used for all column connections (1 in.=2.54 cm). A Perkin-Elmer Turbochrome 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 GPC calibration mixture, Ultra Scientific catalog No. CLP-340, diluted to concentrations of 25 mg/ml corn oil, 1 mg/ml bis(2-ethylhexyl)phthalate (BEHP), 0.2 mg/ml methoxychlor, 0.02 mg/ml perylene, and 0.08 mg/ml sulfur in methylene chloride. Fig. 1 compares the RI detector chromatogram of a 200 mg load of high-molecular-mass material from a fish extract to the UV detector chromatogram of the GPC calibration solution to demonstrate typical performance. 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 in methylene chloride, without the fish extract matrix present, of the compounds listed in Table 1. Collections of fractions at 10–15 min intervals from 105 to 205 min and GC–MS analyses of each fraction after concentrating determined a collection end time of 190 min from the time of injection.

Each aliquot of the fortified test matrix was injected manually. The collected fractions of the fortified test matrix aliquots were concentrated to 1.0 ml using a 250-ml Kuderna–Danish apparatus with a three-ball Snyder column and steambaths followed

Table 1
Percentage recoveries and RSD values ($n=4$) of 69 semivolatile organic compounds from a fortified fish extract

Compound	Average recovery[a] (%)	RSD (%)
Polynuclear aromatic hydrocarbons		
Naphthalene	88.8 (91.2)	8.2
2-Methylnaphthalene	86.3 (95.2)	10.8
2-Chloronaphthalene	95.7 (86.8)	6.6
Acenaphthylene	93.3 (92.5)	8.1
Acenaphthene	93.0 (93.1)	7.1
Fluorene	96.5 (102.9)	7.0
Phenanthrene	91.5 (93.0)	5.1
Anthracene	92.0 (97.7)	6.0
Fluoranthene	86.3 (92.3)	3.5
Pyrene	97.0 (93.7)	4.7
Benz(*a*)anthracene	100.9 (97.1)	5.5
Chrysene	98.8 (100.4)	4.9
Benzo(*b*)fluoranthene	106.7 (102.8)	6.3
Benzo(*k*)fluoranthene	96.3 (92.5)	5.5
Benzo(*a*)pyrene	99.3 (93.1)	5.8
Indeno(1,2,3-*cd*)pyrene	112.6 (96.3)	5.5
Dibenz(*a*,*h*)anthracene	123.0 (97.4)	6.2
Benzo(*g*,*h*,*i*)perylene	101.9 (98.3)	4.4
Other neutral organic compounds		
Bis(2-chloroethyl)ether	79.8 (86.8)	10.6
1,3-Dichlorobenzene	85.0 (87.2)	6.9
1,4-Dichlorobenzene	86.4 (92.1)	7.4
1,2-Dichlorobenzene	85.1 (84.8)	7.5
Hexachloroethane	85.2 (86.9)	5.5
Nitrobenzene	83.1 (100.6)	6.5
Isophorone	85.4 (89.1)	12.3
Bis(2-chloroethoxy)methane	83.5 (85.8)	10.5
1,2,4-Trichlorobenzene	87.5 (88.6)	7.7
Hexachlorobutadiene	90.3 (96.9)	8.7
Hexachlorocyclopentadiene	105.7 (100.5)	12.8
Dimethylphthalate	89.9 (98.1)	11.4
2,6-Dinitrotoluene	90.8 (104.7)	12.9
Dibenzofuran	90.7 (94.6)	8.7
2,4-Dinitrotoluene	90.5 (94.9)	12.8
Diethylphthalate	81.1 (101.3)	19.8
4-Chlorophenyl-phenylether	96.1 (97.2)	8.3
4-Bromophenyl-phenylether	94.5 (87.6)	6.3
Hexachlorobenzene	93.2 (93.2)	8.4
Di-*n*-butylphthalate	73.6 (91.0)	24.7
Butylbenzylphthalate	84.7 (97.4)	28.6
Bis(2-ethylhexyl)phthalate	96.1 (105.4)	27.8
Di-*n*-octylphthalate	97.8 (101.9)	27.7
Acidic organic compounds		
Phenol	89.2 (81.2)	5.0
2-Chlorophenol	90.9 (89.8)	7.5
Benzyl alcohol	91.2 (77.8)	9.7

(*Continued on next page*)

Table 1 (*continued*)

Compound	Average recovery[a] (%)	RSD (%)
Acidic organic compounds		
2-Methylphenol	90.3 (85.7)	7.8
4-Methylphenol	89.2 (85.7)	9.8
2-Nitrophenol	103.3 (89.0)	12.8
2,4-Dimethylphenol	58.6 (81.2)	15.9
Benzoic acid	61.5 (0.0)	13.3
2,4-Dichlorophenol	97.0 (90.3)	10.8
4-Chloro-3-methylphenol	6.8 (71.8)	13.7
2,4,6-Trichlorophenol	98.6 (94.1)	9.0
2,4,5-Trichlorophenol	94.9 (76.2)	7.5
4-Nitrophenol	5.3 (0.0)	107
Pentachlorophenol	134.6 (78.1)	4.0
Basic organic compounds		
N-Nitroso-di-*n*-propylamine	83.5 (98.1)	15.0
4-Chloroaniline	13.8 (0.0)	101
2-Nitroaniline	0.0 (84.8)	NA[b]
3-Nitroaniline	0.0 (0.0)	NA
4-Nitroaniline	0.0 (20.0)	NA
N-Nitroso-diphenylamine	78.7 (82.0)	7.9
Semivolatile organic surrogate compounds		
2-Fluorophenol	87.5 (82.1)	6.7
Phenol-d_5	89.4 (85.8)	7.0
2-Chlorophenol-d_4	89.7 (85.0)	8.3
1,2-Dichlorobenzene-d_4	83.6 (88.7)	9.1
Nitrobenzene-d_5	82.5 (92.7)	9.6
2-Fluorobiphenyl	86.6 (97.5)	9.3
Pyrene-d_{10}	85.4 (88.2)	14.2
Terphenyl-d_{14}	97.2 (109.0)	8.3

[a] Recoveries from the fractionation study without the fish extract matrix present are in parentheses with $n=1$.
[b] NA=Not applicable.

by final volume adjustments using a gentle flow of nitrogen.

2.4. GC–MS analysis

The collected fractions were analyzed by GC–MS as described in the US Environmental Protection Agency (EPA) Method 8270C [13]. The GC–MS instrument was a Hewlett-Packard 5890 series II+ gas chromatograph with a 5972 mass selective ion detector and 7673 auto-injector. A J&W Scientific DB-5MS column (Folsom, CA, USA) of 30 m× 0.25 mm I.D., 0.25 μm film thickness was used for analysis. The mass spectrometer scanned from 35 to 500 amu using 70 eV in the electron impact ionization mode. The injection port was used in the splitless mode at 280°C and the oven temperature program was 35°C for 5 min, ramping at 12°C/min to 320°C and then held for 5 min. The concentrated extracts were fortified with the internal standards 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} at 20 μg/ml before analysis. Recoveries of the fortified semivolatile compounds from the test matrix were calculated using the GC–MS responses for a standard at the same concentration.

3. Results and discussion

Near baseline resolution can be achieved with the

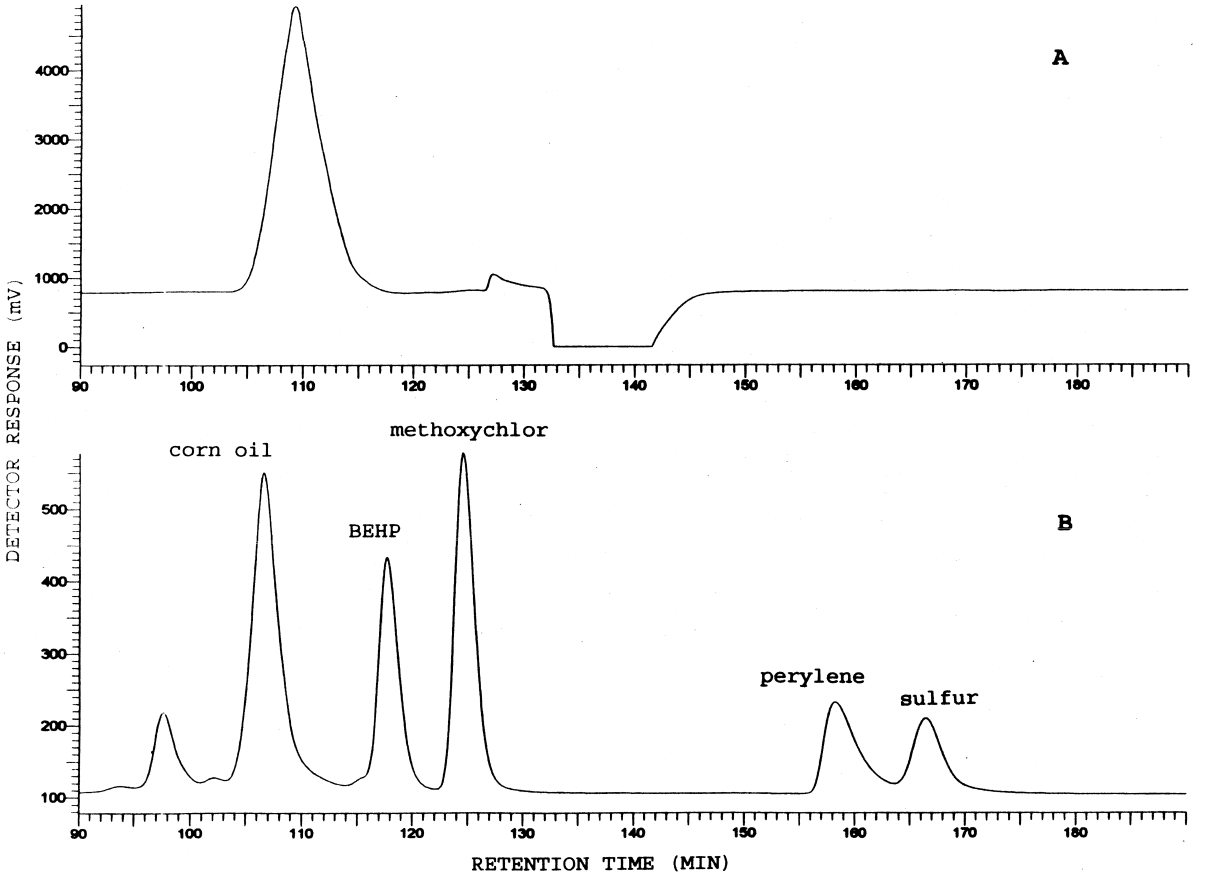

Fig. 1. Chromatograms of the RI detector response for a non-fortified fish extract (A) and of the UV detector response for a GPC calibration solution (B) injected onto the DVB column system. Chromatographic conditions as described in Section 2.3.

DVB columns between the high-molecular-mass material (peak maximum at 110 min) from fish extracts and BEHP as displayed in Fig. 1. With the collection period of 115 to 190 min from the time of injection for the semivolatile organic compounds, the high-molecular-mass fraction from fish lipids was demonstrated to be effectively removed for loads up to 200 mg. The compounds tested were not detected in fractions collected before 115 min and after 190 min during the fractionation study without the fish extract matrix present. Higher loads of lipid will result in more overlap into the collection period which may require follow-up measures depending on the ruggedness of the GC–MS instrumentation or analysis technique to be used. For large lipid loads, this method may be used after the low pressure GPC clean-up method described in Ref. [2].

The fraction between 132 and 145 min that resulted in a negative deflection in the RI chromatogram for the fish extract (see Fig. 1A) was isolated and determined to consist mainly of large fatty acids. These fatty acids were found to not significantly interfere with the GC–MS analyses performed for this study. Vortexing the concentrated extract with an aqueous saturated potassium carbonate solution effectively removed these fatty acids but, as expected, also reduced recoveries of the acidic organic compounds tested.

The semivolatile organic compound recoveries measured for the fortified test matrix aliquots after clean-up using the DVB columns are summarized in Table 1. Recoveries from the fractionation study without the fish extract matrix present are in parentheses. The recoveries for the PAHs and other neutral

organic compounds fortified were quantitative. Benzoic acid along with several of the phenolics and anilines were not recovered well (<70%) with the fish extract matrix present. Recoveries for 4-chloro-3-methylphenol and 2-nitroaniline were significantly higher without the fish extract which suggests a matrix effect. The low recoveries may be due to adsorption onto the DVB material [14]. Retention of aromatic hydrocarbons are affected by π–π interactions with the polystyrene–divinylbenzene material [8]. Similar interactions may be occurring with the DVB material. This effect could be neutralized by the addition of a modifier to the mobile phase, however, this would complicate the concentration steps of the final extract. Experiments with solvent mixtures as the mobile phase were not done.

Over a long period of use of the DVB columns for fish extract clean-ups, it was determined that the recoveries for *N*-nitrosodiphenylamine had deteriorated. This problem was resolved by flushing the columns with the mobile phase for several hours.

After more than 240 fish extract injections and one guard column change, the performance of the DVB columns was found to be stable with regard to the elution time and resolution of the lipid material from the semivolatile organic compounds tested. Recoveries of the neutral organic compounds remained quantitative.

Use of a third 50 cm×22 cm DVB column in series was also tested which resulted in a few more minutes of separation between the lipid fraction and BEHP. However, this additional column increased the run time by 100 min.

4. Conclusions

The DVB columns were effective for the clean-up of fish extracts for the analysis of PAHs and some other semivolatile organic compounds. The method developed here used methylene chloride as the mobile phase which allowed simple concentration steps to be used without the need for vacuum conditions. However, low recoveries for some acidic and basic organic semivolatile compounds indicate that further work is needed to optimize for these compounds.

5. Note

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

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