Evaluation of Several Columns and Solvents for Post-Extraction Gel Permeation Chromatography (GPC) Clean-up of Fish Tissue Prior to PCB Analysis

Keywords: Automated GX-271 GPC Clean-up System, Contaminants, EnviroSep-ABC™ GPC Sample Clean-up Column, Fish, GC/ECD, Gel Permeation Chromatography (GPC), PCBs

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Introduction

The determination of PCBs, as well as other environmental contaminants, in fish tissue requires extensive sample cleanup prior to analysis by gas chromatography with an electron capture detector (GC-ECD). The high fat content of fish tissue can cause buildup of nonvolatile materials on the GC injection port and the analytical column resulting in poor analytical results and high instrument maintenance costs.

Gel Permeation Chromatography (GPC) is a size-exclusion clean-up procedure that uses organic solvents and a hydrophobic gel (primarily a cross-linked divinylbenzene-styrene copolymer) to separate macromolecules. It is a highly effective method for the removal of high molecular interferences such as lipids, proteins, pigments and cellular components from animal tissue.

GPC clean-up is often used for the clean-up of fish tissue extracts prior to analysis for halogenated compounds such as PCBs and chlorinated pesticides. The most common GPC clean-up procedures for fish extract cleanup utilize a 700 mm x 25 mm glass column containing 70 g of Bio-Rad Envirobeads® SX-3 resin with a mobile phase of 100% dichloromethane or 60 g of Envirobeads SX-3 resin with a mobile phase of 1:1 dichloromethane/cyclohexane at a flow rate of 5 mL/min. Although both columns are highly efficient in removing lipids from fish extracts, sample throughput is limited to approximately one hour per sample extract and both clean-up procedures utilize large amounts of solvent per sample.

The purpose of this study was to use a Gilson GX-271 Automated GPC Clean-up System (Figure 1) to evaluate several GPC clean-up columns and mobile phases that would give faster throughput and use less solvent for the post-extraction clean-up of fish tissue prior to PCB analysis via GC-ECD.

Figure 1. Gilson Automated GX-271 GPC Clean-up System with UV Detector (part number 21110000)

Experimental Conditions

Materials

All solvents were distilled in glass suitable for GC, HPLC, pesticide residues analysis and spectrophotometry. All reagents were ACS grade quality or better. GPC standards were prepared according to USEPA Method 3640A and contained corn oil, bis(2-ethylhexyl)phthalate, methoxychlor, perylene and sulfur. Stock solutions of PCBs included Congeners BZ #14, BZ #65 and BZ #166 obtained from Ultra Scientific.

Extraction

Weigh 10 g of ground fish tissue in a beaker. Fortify with 1g corn oil and add 60 g anhydrous sodium sulphate and 230 mL dichloromethane. Spike with appropriate surrogate PCB standards. Pour the mixture through a column containing Florisil® topped with a 1 mL layer of anhydrous sodium sulphate. Collect eluent and evaporate to near dryness using a gentle stream of nitrogen. Reconstitute in 5 mL dichloromethane.

GPC Clean-up

The following columns and conditions were employed for GPC clean-up:

Table 1. GPC Clean-up Protocols

Column	Mobile Phase	Flow Rate	Injection Volume
OI Analytical glass column with 60g Envirobeads SX-3	1:1 dichloromethane/cyclohexane	5 mL/min	5 mL
Phenomenex EnviroSep-ABC Column (21 x 350mm) with guard column	1:1 dichloromethane/cyclohexane	5 mL/min	5 mL
Phenomenex EnviroSep-ABC Column (21 x 350mm) with guard column	100% dichloromethane	5 mL/min	5 mL
Jordi Flash Gel Fluorinated DVB (10 X 250mm) with guard column	100% dichloromethane	2 mL/min	1 mL
OI Analytical Optima Column packed with 21g Envirobeads SX-3	1:1 ethyl acetate/cyclohexane	5 mL/min	1 mL

Column calibration used a GPC calibration standard (as described above), a Gilson 112 UV Detector set at 254 nm and Gilson TRILUTION® LC software with GPC Methods (See Figure 2). Based on the UV trace, column eluate was collected just after bis(2-ethlhexyl)phthalate elution and stopped after perylene elution. After GPC clean-up, collected fractions were further cleaned up with silica gel to remove any pesticides and then concentrated with a gentle stream of nitrogen and reconstituted in appropriate solvent for GC analysis.

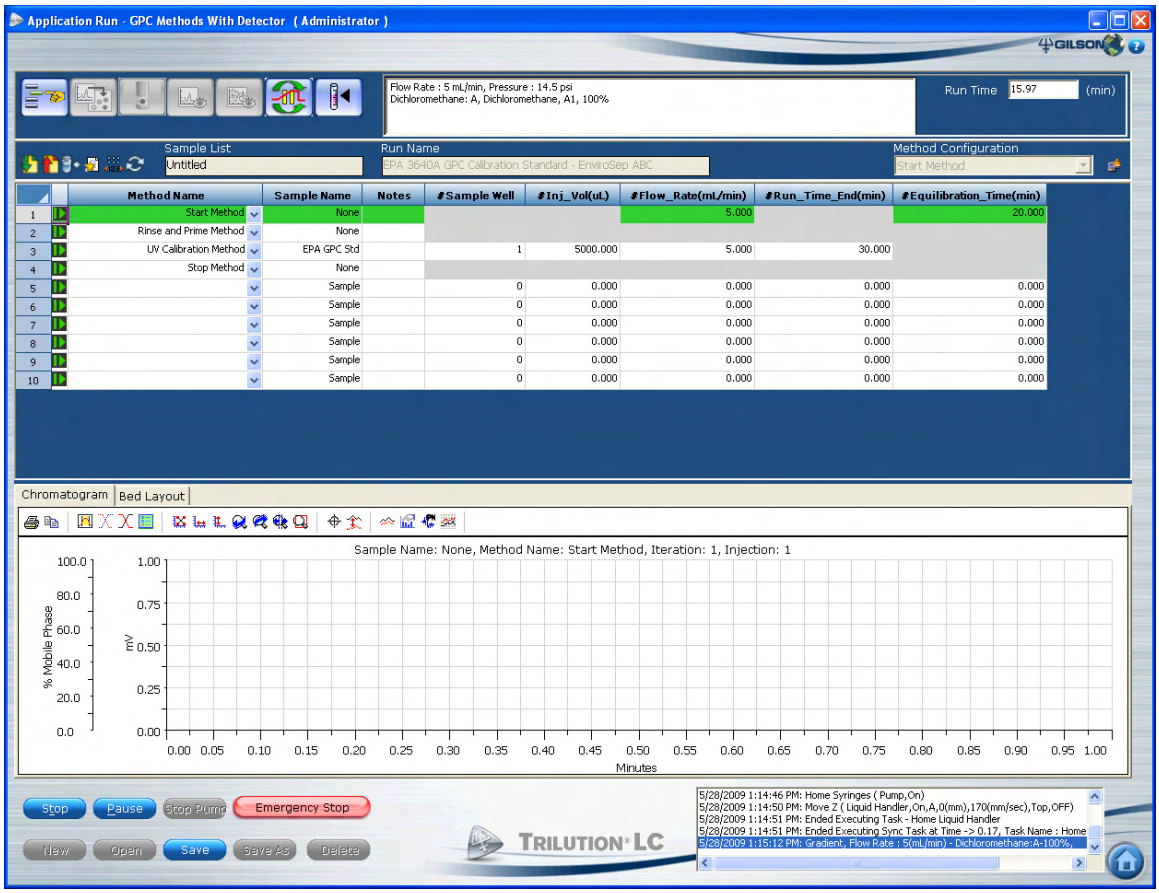

Figure 2. An example of a Sample List using GPC methods in TRILUTION LC v2.1

GC Analysis

PCBs were analyzed with an Agilent HP5890-II GC with a DB5 column (60 m x 0.25 mm ID, 0.1uM phase) configured with ECD.

Results

Table 2. GPC Column Parameters for Fish Extract Clean-up

Column	Dump Volume (mL)	Collect Volume (mL)	Total Run Time	Column Lipid Loading Capacity
OI Analytical Glass, 1:1 DCM/CYX	100	110	60 min	1 g
EnviroSep-ABC, 1:1 DCM/CYX	75	48	32 min	0.5 g
EnviroSep-ABC, 100% DCM	75	43	30 min	0.5 g
Jordi Flash Fluorinated DVB, 100% DCM	18	22	30 min	0.05 g
Optima Column, 1:1 CYX:ethyl acetate	45	60	28 min	0.2 g

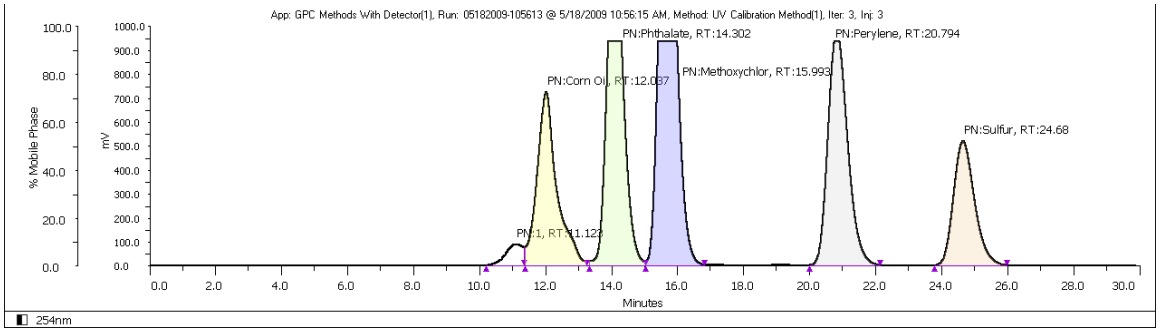

Figure 3. Chromatogram of a USEPA Method 3640A calibration standard using an EnviroSep-ABC column with a mobile phase of 100% dichloromethane. The flow rate is 5 mL/min with UV detection at 254 nm.

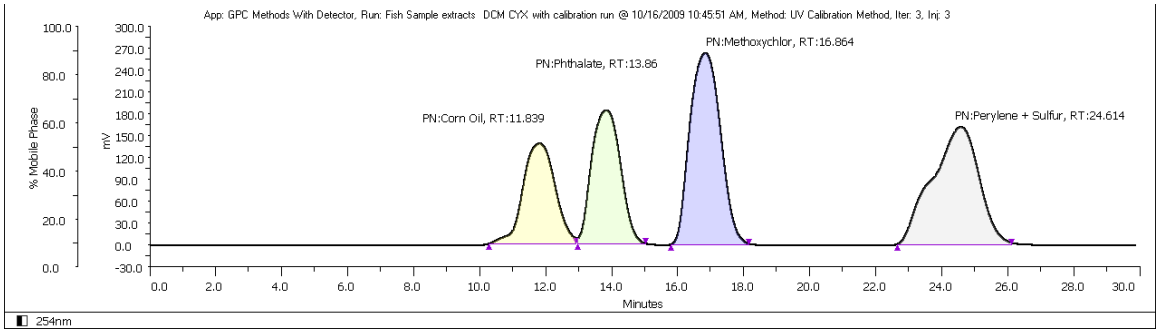

Figure 4. Chromatogram of a USEPA Method 3640A calibration standard using an EnviroSep-ABC column with a mobile phase of 1:1 dichloromethane/cyclohexane. The flow rate is 5 mL/min with UV detection at 254 nm.

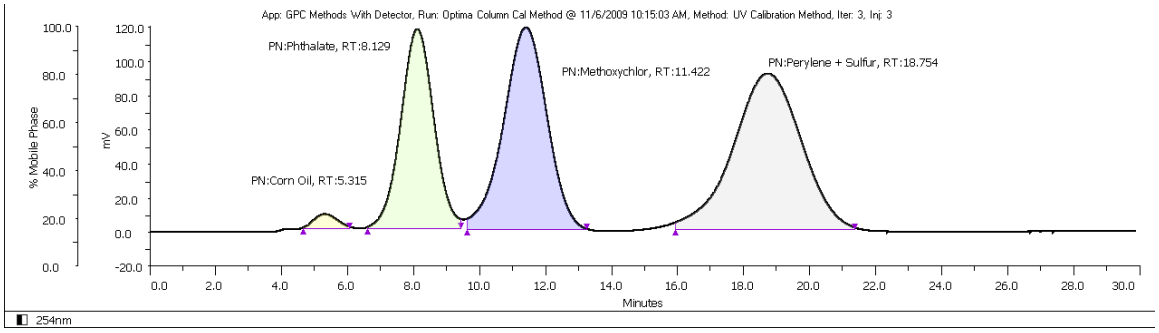

Figure 5. Chromatogram of a USEPA Method 3640A calibration standard using an OI Analytical Optima column with a mobile phase of 1:1 ethyl acetate/cyclohexane. The flow rate is 5 mL/min with UV detection at 254nm.

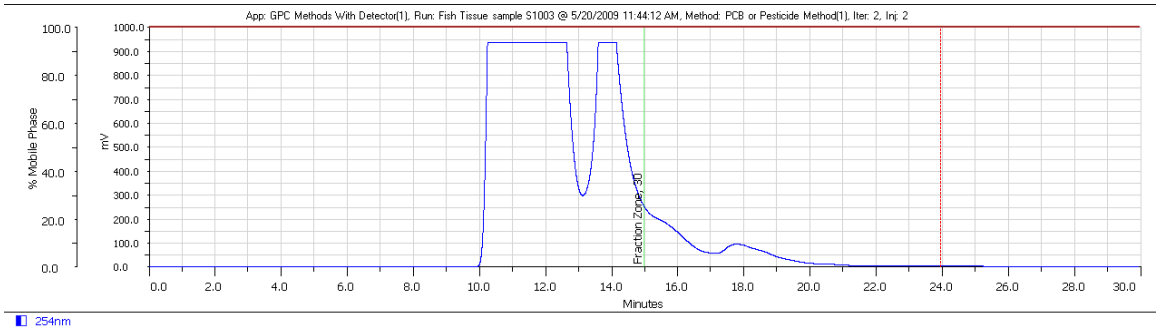

Figure 6. Chromatogram of a fish tissue extract during GPC clean-up using an EnviroSep-ABC column with a mobile phase of 100% dichloromethane at 5 mL/min. Fraction collection time marks are noted.

Table 3. PCB Recovery in Fish (n=3)

Column	Mobile Phase	PCB BZ #14	PCB BZ #65	PCB BZ #166
OI Analytical Glass, 60g Envirobeads SX-3	1:1 dichloromethane/cyclohexane	74.7	81.6	81.5
EnviroSep-ABC	1:1 dichloromethane/cyclohexane	80.5	86.8	92.9
EnviroSep-ABC	100% dichloromethane	87.9	86.7	91
Jordi Flash Fluorinated DVB	100% dichloromethane	61.1	58.8	62.9
OI Optima Column, 1:1 CYX/EA	1:1 ethyl acetate/cyclohexane	90	89	117.5

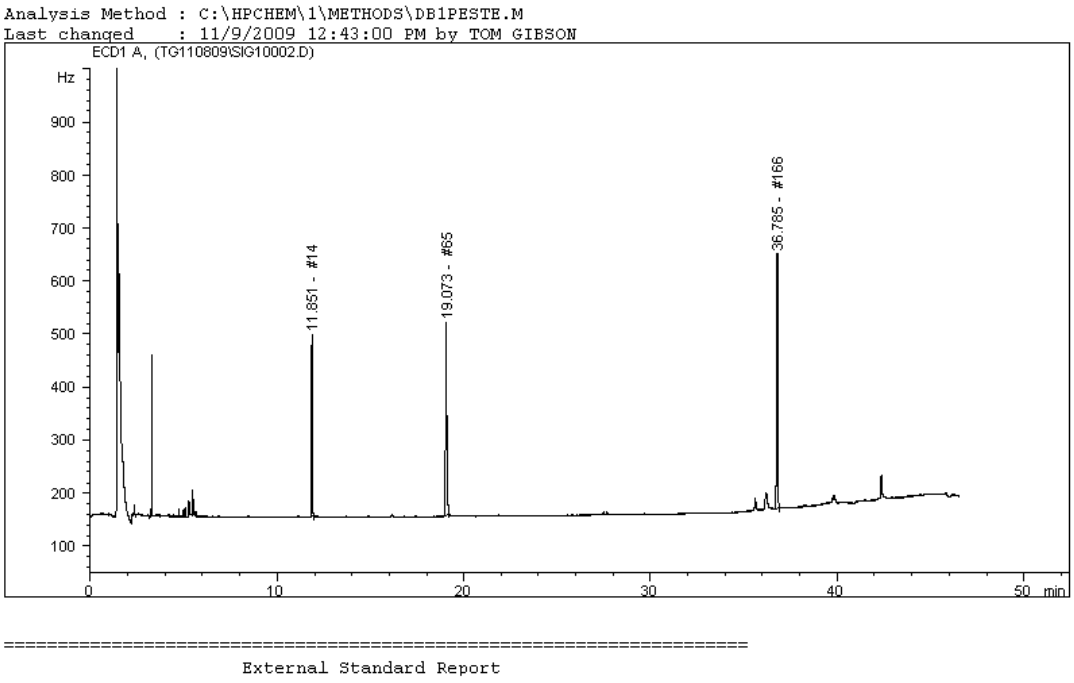

Figure 7. GC/ECD chromatogram of fish tissue extract after GPC clean-up showing PCB congeners.

Conclusion

GPC clean-up is a highly effective tool for removal of lipids and other high-molecular weight interferences prior to analysis for PCBs by GC/ECD. All tested columns were able to separate the lipids in fish extracts from the PCBs. The traditional glass column packed with 60 g Envirobeads SX-3, 1:1 dichloromethane:cyclohexane achieved excellent separation and had the highest lipid loading capacity. However, it utilized the most solvent and had the lowest throughput.

The Phenomenex stainless steel EnviroSep-ABC column, using either of the mobile phases tested, had twice the throughput of the traditional column and used half the amount of solvent. It provided very good recoveries (82.5 to 92.9%) of the PCBs tested. This column has the advantage of easily switching to a different mobile phase, if necessary. Lipid loading capacity is approximately one half that of the traditional column.

The OI Analytical Optima column with a 1:1 ethyl acetate/cyclohexane mobile phase was also effective, but demonstrated more recovery variability between extracts and had a lipid loading capacity of approximately 20% of the traditional glass column.

The Jordi Flash Gel Fluorinated DVB column utilized the least amount of solvent but had the lowest lipid loading capacities and the poorest recoveries (58.8 to 62.9%) compared to all the other columns tested. Throughput was the same as the Phenomenex column.

GPC clean-up readily lends itself to automation and helps reduce GC maintenance costs by preventing buildup on GC injection ports and columns.