APPLICATION

The Use of Phenogel GPC Columns for Environmental and Biomonitoring Applications

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In addition to chromatography, Brian also has a passion for ice cream-making, and enjoys experimenting with bold, new flavors.

This technical note provides examples and an application of preparative GPC (Gel Permeation Chromatography) columns for the automated purification of environmental samples in concordance to the 3640A EPA method. Results shown are the analysis and purification of PAHs by Phenogel™ GPC columns and the removal of biomaterial matrix interferences that are known to complicate the analysis of PAHs in biological tissues.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are contaminants resulting from incomplete combustion of organic materials. The Environmental Protection Agency has labeled 32 PAHs as priority pollutants because of their mutagenic and carcinogenic nature[1]. As such, PAHs are monitored in sediments, soils, wildlife, water, and air samples[2].

Since GC is used for PAH analysis and determination, a general cleanup step is recommended to remove biomaterial matrix interferences, such as lipids, proteins, and steroids[3]. GPC is the primary method since Krahn and colleagues implemented the methodology, showing it's superior over gravity flow[4]. This technique is known to be robust, efficient, and enables lower maintenance of GC instrumentation.

The Phenogel 100 Å preparative GPC column and guard were used to remove lipids and macromolecules efficiently from mussels and salmon samples prior to PAH analysis[5]. Those matrices were selected due to their difficulty for PAH analysis and for sufficient purification to provide quality results at low levels (pg/g and ng/g).

Materials and Methods

A mixture of 49 native PAHs, ^{13}C labeled and deuterated internal standards were purchased from Accustandard (New Haven, CT). Reference standard solution for GPC performance containing corn oil (250 mg/mL), bis(2-ethylhexyl)phthalate (5 mg/mL), methoxychlor (1 mg/mL), perylene (0.2 mg/mL), and sulfur (0.8 mg/mL) was purchased from Restek (Bellefonte, PA). The solution was diluted 50:1 in methylene chloride.

Fish and mussel tissue were prepared by homogenization in a blender and extracted by QuEChERS extraction with ethyl acetate to measure the efficiency of the matrix by the GPC preparative column.

Table 1 and **Table 2** list the internal standards and the 49 natural PAHs that were added in a blank to measure the recovery of the PAHs for the collected fraction.

Table 1.
Internal standards

D_{10}-2-Methylnaphthalene	D_{12}-Chrysene
D_{10}-Acenaphthene	$^{13}C_4$-Benzo[a]pyrene
$^{13}C_6$-Anthracene	D_{10}-Dibenz[a,h]anthracene
D_{10}-Pyrene	

Table 2.
Forty-nine natural PAHs

Naphthalene	Fluoranthene	Benzo[b]fluoranthene	Dibenz[a,h]anthracene
2-Methylnaphthalene	Pyrene	Benzo[k]fluoranthene	7H-Dibenzo[c,g]carbazole
1-Methylnaphthalene	2-Methylfluoranthene	Benzo[j]fluoranthene	Benzo[g,h,i]perylene
2-Chloronaphthalene	Benzo[c]phenanthrene	7,12-Dimethylbenz[a]anthracene	Anthanthrene
1-Chloronaphthalene	Benzo[c]acridine	Benzo[e]pyrene	Dibenzo[a,e]fluoranthene
1,3-Dimethylnaphthalene	Benz[a]anthracene	Benzo[a]pyrene	Dibenzo[a,l]pyrene
Acenaphthylene	Chrysene	Perylene	Coronene
Acenaphthene	3-Methylchrysene	3-Methylcholanthrene	Dibenzo[a,e]pyrene
2,3,5-Trimethylnaphthalene	2-Methylchrysene	Dibenzo[a,h]acridine	Dibenzo[a,i]pyrene
Fluorene	6-Methylchrysene	Dibenzo[a,j]acridine	Dibenzo[a,h]pyrene
Phenanthrene	5-Methylchrysene	Dibenz[a,j]anthracene	
Anthracene	4-Methylchrysene	Indeno[1,2,3-cd]pyrene	
Carbazole	1-Nitropyrene	Dibenz[a,c]anthracene	

HPLC analysis was performed using an Agilent® 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 400 bar, equipped with a binary pump, autosampler, UV-Vis detector and fraction collector. Phenogel™ 5 µm 100 Å 300 x 21.2 mm preparative column and 50 x 21.2 mm guard column (Phenomenex, USA) was used. The flow rate was 5 mL/min and the solvent used was methylene chloride. For both reference standards and samples, 1 mL was injected for each. Run times were 30 min per injection. Injections were monitored at 254 nm.

The eluate was then concentrated to 0.5 mL in isooctane and internal injection (D_8-Naphthalene, D_8-Acenaphthylene, D_{10}-Phenanthrene, D_{10}-Fluoranthene, D_{12}-Benz[a]anthracene, D_{12}-Benzo[e]pyrene, D_{12}-Benzo[g,h,i]perylene) standards were added prior to injection in GC-HRMS.

Results and Discussion

The system suitability was determined using the EPA standard solution. **Figure 1** shows 5 major compounds as the expected chromatogram by the US EPA Method 3640A, but with less solvent consumption and faster chromatography than previously reported methods. Compound retention times showed good repeatability (**Table 3**), with less than 0.2 % of RSD for the retention time for the calculated area that corresponds to the injected volume.

Figure 1.
Representative chromatogram for EPA 3640A test mix

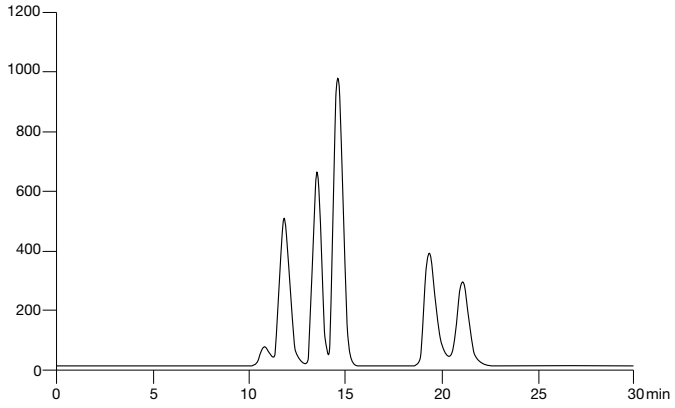


Table 3.
Retention times for EPA 3640A test mix

Analyte	Retention Time (min)	Standard Deviation	%RSD
Corn Oil	11.779	0.019	0.16
Bis(2-ethylhexyl) phthalate	13.466	0.022	0.16
Methoxychlor	14.55	0.048	0.33
Perylene	19.362	0.083	0.43
Sulphur compounds	21.022	0.031	0.15

The lipid removal efficiency for fish and mussel samples was evaluated by a 2.25 mL injection of extracts in the GPC system and the recording of the UV absorbance at 254 nm. Fraction collection analysis was performed to selectively separate lipids from PAHs and the optimal fraction collection range was determined to be 15.5 to 20 minutes. Specificity was determined by the % recovery of deuterated standards by GC/MS (**Table 4**).

Table 4.
Recovery of ^{13}C-labeled and deuterated internal standards

Compounds	% Recovery
D_{10}-Methylnaphthalene	106.56
D_{10}-Acenaphthene	105.76
$^{13}C_6$-Anthracene	106.54
D_{10}-Pyrene	99.15
D_{12}-Chrysene	94.02
$^{13}C_4$-Benzo[a]pyrene	106.23
D_{10}-Dibenz[a,h]anthracene	120.40

Figure 2 and **Figure 3** show representative chromatograms from mussel and salmon, respectively. The major peaks observed between 10 and 15 min are attributed to lipids and high matrix content of the fish liver extracts. All the native PAHs were adequately recovered.

Figure 2.
Representative chromatogram for 5 g mussel extract

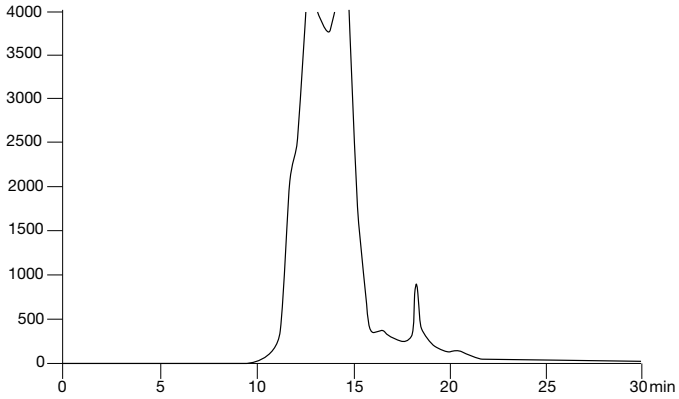
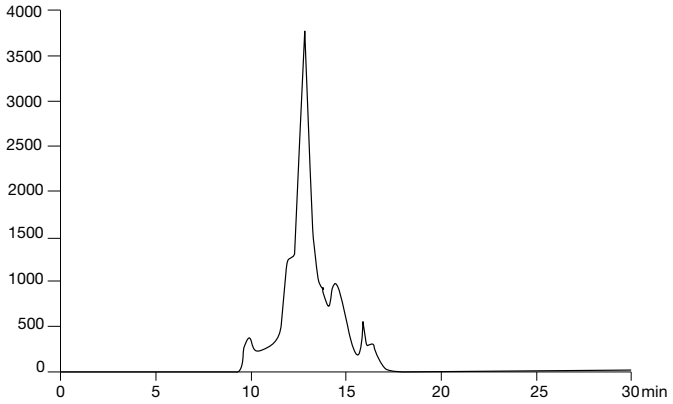


Figure 3.
Representative chromatogram for 2.5 g fish liver extract



Conclusion

This method showed that Phenogel™ 5 µm 100 Å preparative GPC columns efficiently removed lipids from salmon and mussel samples with high recovery of the targeted 49 PAHs. The method showed good repeatability and could easily be automated for overnight injection.

Future studies will include an integrated method for the complete extraction and purification of different complex biological matrices. Additionally, PCBs, PBDEs, PCDD/Fs, and polychlorinated naphthalene have also shown interesting preliminary results and further development will be performed to analyze all persistent organic pollutants and PAHs simultaneously. Sediments and soils have also shown intriguing results and further validation will be performed for the chemical contaminants enumerated before.

References

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4. Krahn, M. M., C. A. Wigren, R. W. Pearce, L. K. Moore, R. G. Bogar, W. D. MacLeod, Jr., S-L. Chan, and D. W. Brown. 1988b. Standard analytical procedures of the NOAA National Analytical Facility, 1988: New HPLC cleanup and revised extraction procedures for organic contaminants. U.S. Dep. Commer. Tech. Memo. NMFS F/NWC-153, 52 p.
5. Cloutier, P.-L., Fortin, F., Fournier, M., Brousseau, P., Groleau, P.-E., Desrosiers, M. 2014. Method development for the determination of PAHs in biological samples. Journal of Xenobiotics 4 (4897) : 70-72.

Ordering Information

Phenogel SEC/GPC Columns

5 µm Analytical Columns (mm)			SecurityGuard™ Cartridges (mm)
		300 x 7.8	4 x 3.0*
Pore Size	**MW Range**		
50 Å	100-3 K	00H-0441-K0	AJ0-9292
100 Å	500-6 K	00H-0442-K0	AJ0-9292
500 Å	1 K-15 K	00H-0443-K0	AJ0-9292
10^3 Å	1 K-75 K	00H-0444-K0	AJ0-9292
10^4 Å	5 K-500 K	00H-0445-K0	AJ0-9292
10^5 Å	10 K-1,000 K	00H-0446-K0	AJ0-9292
10^6 Å	60 K-10,000 K	00H-0447-K0	AJ0-9292
		300 x 7.8	4 x 3.0*
Mixed Beds			
Linear(2)	100-10,000 K	00H-3259-K0	AJ0-9292

for 3.2-8.0 mm ID

5 µm Narrow Bore (NB) Columns (mm)			SecurityGuard™ Cartridges (mm)
		300 x 4.6	4 x 3.0*
Pore Size	**MW Range**		
50 Å	100-3 K	00H-0441-E0	AJ0-9292
100 Å	500-6 K	00H-0442-E0	AJ0-9292
500 Å	1 K-15 K	00H-0443-E0	AJ0-9292
10^3 Å	1 K-75 K	00H-0444-E0	AJ0-9292
10^4 Å	5 K-500 K	00H-0445-E0	AJ0-9292
10^5 Å	10 K-1,000 K	00H-0446-E0	AJ0-9292
10^6 Å	60 K-10,000 K	00H-0447-E0	AJ0-9292
		300 x 4.6	4 x 3.0*
Mixed Beds			
Linear(2)	100-10,000 K	00H-3259-E0	AJ0-9292

for 3.2-8.0 mm ID

10 µm Analytical Columns (mm)			SecurityGuard™ Cartridges (mm)
		300 x 7.8	4 x 3.0*
Pore Size	**MW Range**		
50 Å	100-3 K	00H-0641-K0	AJ0-9292
100 Å	500-6 K	00H-0642-K0	AJ0-9292
500 Å	1 K-15 K	00H-0643-K0	AJ0-9292
10^3 Å	1 K-75 K	00H-0644-K0	AJ0-9292
10^4 Å	5 K-500 K	00H-0645-K0	AJ0-9292
10^5 Å	10 K-1,000 K	00H-0646-K0	AJ0-9292
10^6 Å	60 K-10,000 K	00H-0647-K0	AJ0-9292
		300 x 7.8	4 x 3.0*
Mixed Beds			
Linear(2)	100-10,000 K	00H-3260-K0	AJ0-9292

for 3.2-8.0 mm ID

5 µm Preparative Columns (mm)			Guards
		300 x 21.2	50 x 21.2
Pore Size	**MW Rasnge**		
100 Å	500-6 K	00H-0442-P0	03B-0642-P0

10 µm Preparative Columns (mm)			Guards
		300 x 21.2	50 x 21.2
Pore Size	**MW Range**		
100 Å	500-6 K	00H-0642-P0	03B-0642-P0

* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

Guard Cartridge Holder

Part No.	Description
KJ0-4282	Reusable Holder (SecurityGuard Kit)

Note: SecurityGuard cartridges for Non-Aqueous Polymer GPC columns are not compatible with HFIP solvent.

Column Union

Part No.	Description
AQ0-8507	Zero Dead Volume Union, SS, with 10-32 fittings

Note: Additional union (AQ0-8507) may be necessary for SecurityGuard to fit in column oven with less than 30 cm length capacity.

Phenogel columns are routinely shipped in THF. Please contact your Phenomenex representative for information about other shipping solvents.

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