GEL–PERMEATION CHROMATOGRAPHY (GPC) CLEANUP PROCEDURE FOR PESTICIDES, POLYCHLORINATED BIPHENYLS (PCBs) AND OTHER ORGANIC ANALYSES

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1. SCOPE AND APPLICATION

1.1. This SOP describes the procedure for the cleaning up of sample extracts for subsequent analysis for chlorinated and nitrogen-phosphorus pesticides, PCBs and PAHs, following the extraction from water, soil/sediment/tissue and wastes.

1.2. This SOP is based on EPA Method 3640A (see 17.1).

1.3. Gel-permeation chromatography (GPC) is a size exclusion procedure using organic solvents and hydrophobic gels in the separation of macromolecules. The packing gel is porous and is characterized by the exclusion range of that pore size. The exclusion range must be greater than those of the molecules to be separated.

1.4. GPC is recommended for the elimination of lipids, polymers, copolymers, proteins, natural resins, cellular components, viruses, steroids, other high-molecular-weight compounds and sulfur from the sample.

2. SUMMARY OF THE METHOD

2.1. The GPC column, packed with the required amount of pre-swelled absorbent is flashed with solvent for an extended period of time. The column is calibrated and then loaded with the sample extract to be cleaned. Elution is effected with methylene chloride and the collected sample extract is then concentrated.

3. INTERFERENCES

3.1. A reagent blank should be analyzed for the compounds of interest prior to the use of this method. The level of interferences must be below the method detection limits before this method is performed on actual samples.

4. APPARATUS AND EQUIPMENT

4.1. Gel-permeation chromatography system consists of a Shimadzu HPLC SYSTEM consisting of the following modules:

- 4.1.1. Solvent delivery module LC-20AD
- 4.1.2. Degasser DGU 14A or DGU-20A3R
- 4.1.3. Automatic sample injector SIL-10AP or SIL 20AHT
- 4.1.4. System controller SCL-10AVP or CBM 20A
- 4.1.5. UV Detector Shimadzu SPD-20A or Shimadzu SPD-10AVP UV detector-fixed wavelength (254 nm). Fraction collector ISCO FOXY 200 or ISCO FOXY R2
- 4.1.6. For detailed descriptions of the modules see the manufacturer instruction manuals.

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4.2. GPC column: Phenomenex Phenogel 5um, 100A (350mm x 21.2 mm) with Guard column 1 um, 100A(60mm x 21.2mm) or Waters Envirogel GPC cleanup column 15 um (19mmX300mm; 100-10K) with Waters guard column (4.6mmX30mm)

4.3. Nitrogen blower for sample concentration, N-Evap 116 (Organomation Associates, Inc.)

4.4. Helium compressed gas cylinder Ultra-Pure Carrier Grade

4.5. National Scientific Company Target I-D Vials (2 mLs GC glass vials)

4.6. Chromacol LTD 500 x 1.1-CTVG Vials (1 mL LC glass vials)

4.7. 50 mL “Pyrex” glass tubes

4.8. Waste liquid 4 L glass bottle

4.9. Becton Dickinson & Co, 5 cc disposable syringe

4.10. Gelman Nylon Acrodisc 13, 0.45um disposable filters. Prior to use, if necessary, rinse each filter assembly with methylene chloride

5. REAGENTS AND CHEMICALS

5.1. Solvents:

5.1.1. Methylene chloride – Fisher Scientific, Pesticide Grade of HPLC Grade

5.1.2. Iso-Octane Fisher Scientific, Pesticide Grade

5.1.3. Methanol, Fisher Scientific, Pesticide Grade

5.2. GPC calibration solution (for pesticides and PCBs): Prepare a solution in methylene chloride that contains the following compounds in the concentrations listed below using neat individual standards:

5.2.1.	Component	mg/mL
5.2.2.	Corn Oil	12.5
5.2.3.	Bis-2-ethylhexyl phthalate	0.5
5.2.4.	Perylene	0.01
5.2.5.	Sulfur	0.04

5.2.6. For GPC calibration solution preparation see Appendix 1.

5.3. GPC calibration solution (for PAHs): Prepare a solution in methylene chloride that contains the following compounds in the concentrations listed below using neat individual standards:

5.3.1 Component	mg/mL
5.3.2 Biphenyl	0.01
5.3.3 Perylene	0.01

5.3.4 For GPC calibration solution preparation see appendix 1.

6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1. See SOPs GC-011-5 (see 17.2) and GC-013-2 (see 17.3).

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7. SAMPLE PREPARATION PROCEDURE

7.1. GPC Cleanup procedures are applied to all sediment samples, water samples for GC-ECD analysis with final volume of 2 ml or less, tissue samples, and may be used for any sample if needed.

7.2. Setup a run sequence in EzChrom to collect data:

7.2.1. Open EZChrom by selecting the EZChrom Elite icon on server desktop or from the Start menu under Programs/Chromatography. Select the EZserver GPC Lab 428 EZServer 6 or GC Lab 303 EZServer 3. Check the status of the instrument to make sure it is available and not in use. Open the instrument "online" by selecting the instrument and entering your password. There are currently 3 instruments designated for GPC analyses: GPC_1, GPC_2 on EZServer 6 and GPC_3 on EZServer 3.

7.2.2. Load the acquisition method using the drop-down menu: File/Method/Open and chose the appropriate method. The method name will appear at the top of the screen if properly loaded.

7.2.3. Load the sequence using the drop-down menu: Select File/Sequence/Open and chose the latest GPC sequence for GPC_1 or GPC_2 or GPC_3 instrument existing in the data base. (ex. 080812GPC_1AD.seq, 080812 is the run date, and GPC_1 is the instrument name.

7.2.4. Edit the sequence. Type in the sample names under sample ID. Select the correct method (e.g. GPC_1.met or GPC_2.met) for each instrument in the Method column. Next enter the filenames using the current date. Enter the filenames for the first sample as for ex. 0808121<01>. To increment the filenames through the entire sequence, right click on the first filename and select "fill down" and enter.

7.2.5. Fill down all other columns by right click on the column and select "Fill Down" in the drop-down menu.

7.2.6. Enter the GPC calibration standard tracker serial number in the comment line. Also enter prep batch ID and collection window used for the current run.

7.2.7. Save the sequence as ex. 0801207GPC_1 or GPC_2.seq or GPC_2 and start the run in EZChrom by pressing the start button on the toolbar. This step initializes acquisition within EZchrom but does not initialize the GPC Chromatograph. EZChrom Data Acquisition is waiting for injection in the GPC instrument.

7.2.8. When the acquisition is completed the data are automatically stored in the EZChrom directory for that GPC instrument and can be reached using File/Data/Open.

7.3. Prepare the GPC Shimadzu System to run samples:

7.3.1. Fill the reservoir bottle with methylene chloride pesticide grade. For NP pesticides in soil and waste by tests S-PSNP-TQ, WS-PSNP-TQ elution is performed with 3% MeOH in methylene chloride.

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7.3.1.1. Take off the reservoir cap assembly

7.3.1.2. Fill up to 80% of the volume reservoir

7.3.1.3. Put the filter element assembly into the reservoir and then firmly close the cap

7.3.2. Start degassing methylene chloride by opening the valve on the helium line leading to the GPC system for at least 2 minutes as follows:

7.3.3. Purge the line to remove the gas bubbles as follows:

7.3.3.1. On solvent delivery unit (LC-20AD) press the button on to start the pump

7.3.3.2. Press POWER ON at SCL-10AVP System Controller

7.3.3.3. Press Menu

7.3.3.4. Press 0 to get Analysis file parameters

7.3.3.5. Enter for “T. Flow” 4 mL/min

7.3.3.6. Turn the drain valve 180^0 counterclockwise to open

7.3.3.7. Purge the flow line with the mobile phase for about 5 minute.

7.3.3.8. Make sure no bubbles are in the flow line

7.3.3.9. Fill the cleaning liquid reservoir for the Syringe Unit, with degassed methylene chloride

7.3.3.10. Check the “time program file 0” on system controller by pressing Menu / 1 (see “time program file 0” in appendix 2).

7.3.4. Set up fraction collector with proper time collection window. To edit the collection window:

7.3.4.1. Press POWER ON at FOXY 200 (or FOXY R2) fraction collector. (For Foxy R2 please see the manual.

7.3.4.2. Press EDIT A (for method A, for Foxy 200)

7.3.4.3. Press PEAK in the EDIT MENU

7.3.4.4. Press A to select Peak Detection mode: MODE: time windows only

7.3.4.5. Press ENTER

7.3.4.6. WINDOW 1: enter four digits for blinking start collection time (ex: 1536 for 15min: 36sec)

7.3.4.7. Press ENTER key

7.3.4.8. Enter 4 digits for stop collection time (ex: 25:20 for 25min and 20sec)

7.3.4.9. Press ENTER

7.3.4.10. Press EXIT

7.3.4.11. Press BACK STEP key 2 times

7.3.4.12. Press “SAVE AS A full”

7.3.5. Put 50 mL tubes with proper labels in the collector rack to collect samples

7.3.6. For GPC_2 and GPC_3 set up the run sequence in the System Controller SCL-10AVP(see Appendix 3 as example):

7.3.6.1. Press key 0 in the “main menu” and edit the Analytical Sequence screen:

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7.3.6.1.1. Enter vial numbers to be used as range of numbers xx-xx (example 0 – 22, always first vial number starts with 0)

7.3.6.1.2. Enter 1 for number of injections per vial

7.3.6.1.3. Enter the injection volume expressed in microliters (ex. 1000 uL for 1 mL)

7.3.6.1.4. Enter the TIME PROGRAM FILE 0 for all samples (see APPENDIX 2)

7.3.6.1.5. Enter 0 for "prep file" to execute the standard injection mode

7.3.6.1.6. Enter a new instruction row in the sequence to stop the run. Specify a vial number bigger than all sample numbers and specify TIME PROGRAM FILE 1 (because time program file 1 is empty, this will stop the pump, see APPENDIX 2). Put injection volume 100 uL. Put in the auto-injector tray a vial in the position specified filled with methylene chloride.

7.3.6.1.7. Enter "–" in FRAC FILE# column to show that it is not used a Shimadzu fraction collector

7.3.7 For GPC_1 the run sequence is setup in the computer attached to the CBM-20A controller (CBM-20A does not have a direct display and it does not communicate well with LC-20AD without software). To run samples in GPC_1;

7.3.7.1 Start all the instrument modules first and wait until they are ready.

7.3.7.2 Login into the laptop using ID name Lab_GCUser and the current password.

7.3.7.3 Launch Internet Explorer from the desktop shortcut. This will immediately open the controller software since the home page is set to the IP address 192.168.200.99 (CBM-20A controller).

7.3.7.4 To login click in the system name box using "admin" for both the user ID and password. The last sequence used will now be displayed.

7.3.7.5 Click the "pump" button to start the pump.

7.3.7.6 Modify the sequence; put vial# from Vial # to, injection volume and run time. The flow rate is setup to be 4 mL/min.

7.3.7.7 Click the "save" button to save the sequence.

7.3.7.8 Click the "run" button to run the sequence. The run will be automatically terminated 2 minutes after the last sample is completed. The pump will stop.

7.3.8 Pour the samples in the appropriate vials: Pre-slit vials crew caps have to be used for GPC-1 since it does not have a pressure equilibrating needle.

7.3.9 Load the sample vials into automatic injector sample rack

7.3.10 Turn the detector ON

7.3.11 Equilibrate system for 30 min.

7.3.12 Start the run sequence with at least two solvent vials, followed by a GPC calibration standard, and followed by another solvent vial as the first GPC blank, followed by samples in the set. End the GPC run by injecting methylene chloride as the second GPC blank. **Use the first two solvent vials to check the GPC**

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injection volume. No samples can be run if the correct injection volume is not assured.

7.3.13 Dirty, dark colored and viscous or tissue samples which are difficult to cleanup need multiple injections of diluted 1mL extract (2 to 4 times dilution).

7.3.14 Make sure to enter appropriate information in GPC logbook.

7.3.15 For tissue, sediment and regular water samples inject 1mL of sample extract in methylene chloride. For low level water samples inject 0.5 mL (W-PC-AA-SF). Refer to the appropriate SOPs for the injection volume for the GC/MS/MS and GC/MS-NCI tests

7.3.16 Start the GPC Chromatograph by pressing run button on the system controller keyboard

7.3.17 Collect the sample extract.

7.3.18 Concentrate the sample extract using N-blower.

7.3.19 Exchange the solvent to iso-octane for pesticide samples. After GPC clean-up all the pesticide samples are subjected to a 1 g Florisil clean-up.

7.3.20 Make up the final volume equal to the injected volume (1mL or 0.5 mL).

7.3.21 If more than one injection was made for the same sample (tissue or dirty samples), then concentrate by N-blower and solvent exchange in the concentrator tube and make up the same volume as the other samples in the extraction batch (1mL or 0.5 mL).

7.3.22 After GPC run is completed turn off the degasser on/off valve to avoid flooding back the degasser with methylene chloride.

Record the preparation in the electronic prep worksheet by entering the GPC sequence name in the header prep comments field. Enter other pertinent information in the grid comment lines (volume injected, dilution, volume submitted after Florisil clean-up.

7.3.23 Proceed with ONE-GRAM FLORISIL CARTRIDGE CLEANUP if needed.

8 MAINTENANCE

8.1 The flow rate shall be verified in the beginning of each run by collecting the column eluate for 1 minute in a graduated cylinder and measuring the volume. The volume should correspond to 4.0 mL/min. If the flow rate is outside this limit, corrective action must be taken and the dump and collection times must be re-checked by column re-calibration. Once the flow rate is in the range, column pressure and room temperature should be recorded.

8.2 Check the effective pressure on the GPC_1 or GPC_2 or GPC_3 screen to be in the range of 50-100 Kg/cm^2. (maximum pressure is set to 250 Kg/cm^2). If the pressure is higher:

8.2.1 Check if the pipe to the column is not clogged. If so, cut or replace,

8.2.2 If pipes are freely flowing the line filter in the pre-mixer is clogged and must be replaced

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8.2.2.1 Remove the male nut and loosen the screw to replace the filter (P/N 228-18872-91)

8.3 The column should be back-flushed between large sample sets (35-50 samples) or after a set of "dirty" samples as follows:

8.3.1 Reverse the flow of methylene chloride through the column and allow it to run at 4mL/min.

8.3.2 While the column is reversed, inject 1mL of tetrahydrofuran (THF) followed after five minutes by an injection of 1mL of toluene. Allow the column to back-flush for another 30 min.

8.4 Return the column to its normal configuration and let flow run through for 30 min.

9. CALIBRATION OF THE COLUMN

The column is calibrated using the chromatogram generated by the UV detector.

9.1 Use for calibration the GPC Calibration Solution (5.2)

9.2 Inject 1 mL of methylene chloride blank followed by 1 mL GPC calibration solution onto column.

9.3 The calibration standard chromatogram will gradually appear on the monitor. Use the following criteria for choosing dump time and collect time:

9.3.1 Peaks must be observed and should be symmetrical for all compounds in the calibration solution

9.3.2 Corn oil and phthalate peaks must exhibit > 85% resolution;

9.3.3 Perylene and sulfur peaks must not be saturated and must exhibit >90% baseline resolution.

9.3.4 The collection window should begin after 50% of the bis-2-ethylhexyl phthalate peak has eluted and should end after perylene peak has 100% eluted and before sulfur peak starts. The window usually extends further for Nitrogen-Phosphorus pesticides.

9.3.5 For PAHs the calibration mix contains only 2 peaks. The collection window starts about 0.2 min before the start of the biphenyl peak and ends about 0.5 minutes after the end of the perylene peak. The collection window has to be verified and readjusted by running the GPC PAHs spike, whenever LFB/MS/Surrogate recovery problems occur.

NOTE: collection window for PAHs is considerable shorter than for pesticides so smaller fraction volume will be collected!

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9.4 If collection window have changed enter the new collection window in the Sample Collector method as described in 7.3.5

9.5 Run GPC Calibration Solution in the beginning of every run sequence to check the collection range.

9.6 Run GPC Spike ECDA+C alternately with GPC Spike ECDB in the beginning of the run sequence whenever problems with LFBs and Matrix Spikes recoveries are noticed.

9.7 Pay attention to the recoveries of Cypermethrin, the first eluting, and Beta-BHC the last eluting chlorinated pesticide in the GPC column. In case of T-PAH analysis the recovery of terphenyl-d14 surrogate has to be monitored very closely. For Nitrogen Phosphorus pesticides the recovery of Atrazine and Simazine must be monitored. Usual it is necessary to extend the end window by about two minutes to collect those two analytes.

10 QUALITY CONTROL

10.1 For Quality Assurance/Quality Control see SOP GC-001-2 (see 17.4)

11 SAFETY/HAZARDOUS WASTE MANAGEMENT

11.1 Wear safety glasses, gloves and a lab coat at all times while performing sample preparation

11.2 Handle all the sample extraction, organic solvent transfer, concentration, and cleanups in a hood. Avoid breathing organic solvent vapors. When not working directly with samples, pull the hood sash down to the bottom. Do NOT lift the hood sash above the halfway point (marked with a red line) while extracting samples.

11.3 Discard all wastes in the appropriate Non-chlorinated/ Chlorinated Waste bottle.

11.4 Dispose leftover and expired neat standards into a sealed, labeled waste container.

11.5 Dispose all the stock standards, working standards and used sample extracts into a labeled waste container.

11.6 Dispose of all used GC vials containing standards or samples into a labeled waste container.

11.7 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated

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as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available.

11.8 A reference file of material data handling sheets is kept in the office area, across from the entrance to the tech room (room A316J). Refer to this file for any material you handle. Update this file upon receiving new chemicals or reagents. The MSDS for many chemicals can also be viewed on-line, at http://hazard.com/MSDS/

11.9 Primary standards of these toxic compounds should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when an analyst handles high concentrations of these toxic compounds.

11.10 Dispose of all unwanted, broken glassware into a broken glassware disposal box. Inspect every piece of glassware. Do not use any that are chipped, cracked, etched, or scratched. Glassware with minor damage should be stored for repair.

11.11 See Safety-Waste DEP SOP OG-001-3 for further details (see 17.5).

12 APPENDIX 1. GPC calibration solution preparation

12.1 Weigh following compounds with one decimal accuracy:

- Corn Oil 6.25 g
- Phthalate 250 mg
- Sulfur 20 mg
- Perylene 5 mg
- Biphenyl 5 mg

12.2 Transfer the ingredients into a 500 mL volumetric flask

12.3 Add 500 mL methylene chloride

12.4 Shake well to completely dissolve all ingredients

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13 APPENDIX 2. TIME PROGRAM FILE 0 & FILE 1

TIME PROGRAM
FILE 0

#	TIME	FUNC	VALUE
0	0.33	T.FLOW	4.00
1	30.01	STOP	

TIME PROGRAM
FILE 1

#	TIME	FUNC	VALUE
0			
1			
2			
3			
4			
5			
6			
7			
8			
9			

14 APPENDIX 3. ANALYTICAL SEQUENCE

	SAMPLE # XX-XX	# INJ	VOL (ul)	FILE# RUN T.	PREP FILE #	FRAC FILE #
	0-25	1	1000	F0	0	-
	40-40	1	100	F1	0	-

15 APPENDIX 4. OPERATION CHECK LIST

15.1 Setup a run sequence in EZChrom to collect data

15.2 Prepare the GPC Chromatograph Shimadzu 10 A to run samples

15.2.1 Fill reservoir bottles with methylene chloride

15.2.2 Degas methylene chloride in the reservoirs

15.2.3 Turn on the LC-10ADVP pump

15.2.4 Purge the line to remove gas bubbles

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15.2.5 Set up fraction collector with proper method and collection window

15.2.6 Put 50 ml tubes with proper labels in the collector rack

15.2.7 Set up the run sequence in the System Controller

15.2.8 Pour the samples in the appropriate vials

15.2.9 Load the sample vials into automatic injector sample rack

15.2.10 Turn the UV detector ON

15.2.11 Equilibrate system for 30 min

15.3 Start the GPC Chromatograph

15.4 Collect the sample extract

15.5 Concentrate the sample extract

15.6 Exchange the solvent to iso-octane

15.7 Make up the final sample volume equal to the injected volume

15.8 Proceed with ONE-GRAM FLORISIL CARTRIDGE CLEANUP

16 REFERENCES

16.1 EPA 3640A Gel-Permeation Cleanup: Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW-846; 3rd Edition, Update 4, January 3, 2008.

16.2 DEP SOP GC-011-5 Analysis of Organochlorine Pesticides and PCBs in Water, Soil/Sediment, and Wastes by Gas Chromatography with Electron Capture Detection – GC-ECD.

16.3 DEP SOP GC-013-2 Analysis of Organochlorine Pesticides and PCBs in fish tissue by Gas Chromatography with Electron Capture Detection (GC-ECD).

16.4 DEP SOP GC-001-2 Quality Assurance / Quality Control in the GC Pesticides Laboratory.

16.5 DEP SOP OG-001-3 Safety and Waste Management Practices in the Organic Analysis Group (Volatile, Semi-Volatile and Pesticides).

APPENDIX OF CHANGES

09/04/2013 Added procedure for GPC cleanup of T-PAH tissue extracts. Includes GPC calibration using biphenyl and perylene.

08/26/2015 GPC paper logbook has been removed. The GPC run information now entered in the electronic prep worksheet.

08/21/2017 Replacement of Phenomenex columns (no longer available) with Waters columns in some instruments.

08/21/2017 Addition of tests S-PSNP-TQ/WS-PSNP-TQ. Elution with 3% MeOH in methylene chloride.

12/14/2017 Requirement to check GPC injection volume before each run is added.

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12/05/2018 Updated GPC column information.

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