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

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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 soil/sediment/tissue and wastes.

1.2. This SOP is based on EPA Method 3640A (see 13.1).

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 performed 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 403 or DGU-20A3R
- 4.1.3. Automatic sample injector SIL-40 or SIL 20AHT
- 4.1.4. System controller SCL-40 or CBM 20A or CBM-40
- 4.1.5. UV Detector Shimadzu SPD-20A or SPD-40 or UV detector working at fixed - fixed wavelength (254 nm). Fraction collector Shimadzu FRC-40
- 4.1.6. For detailed descriptions of the modules see the manufacturer instruction manuals.

4.2. GPC column: Phenomenex Phenogel 5um, 100A (350mm x 21.2 mm) with Guard column 1 um, 100A (60mm x 21.2mm). .

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4.3. Nitrogen blowdown 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.

4.11. The GPC systems are controlled by Shimadzu Lab Solutions software.

5. REAGENTS AND CHEMICALS

5.1. Solvents:

5.1.1. Methylene chloride – Fisher Scientific, GC-Resolve Grade. Pesticide Grade or HPLC Grade may also be used.

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:

Component	**mg/mL**
Corn Oil	12.5
Bis-2-ethylhexyl phthalate	0.5
Perylene	0.01
Sulfur	0.04

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:

Component	**mg/mL**
Biphenyl	0.01
Perylene	0.01

For PAH GPC calibration solution preparation see Appendix 1.

6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1. See SOPs GC-033-1 (see 13.4), GC-034-1 (see 13.5) and SV-020-1 (see 13.6).

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7. GPC CLEANUP PROCEDURE

7.1. GPC Cleanup procedures are applied to all sediment and tissue samples.

7.2. Setup a run sequence in LabSolutions software to collect data:

7.2.1. Before opening the software make sure that all the modules of your instrument have been manually turned on. Click on the LabSolutions icon and select your instrument. The instrument window will open, and the software will now be controlling the instrument. If the instrument opens in the shutdown mode, load the analytical method and then run the startup function. This should wake the instrument and make it ready for the samples.

7.2.2. Use the left side panel to toggle between the "Main" and "Realtime Batch" views. Click on "Data Acquisition" in "Main" view to look at the method. The methods are stored at. Windows (C)\LabSolutions\Data\Methods. For each instrument there should be at least two methods; for example, for GPC1: GPC1_method_191028 and GPC1_Shutdown.

7.2.3. Data is stored at Windows (C)\LabSolutions\Data\GPCname in data folders named after the date of the run and the instrument (i.e. 220720GPC1). Those folders also contain batch files (sequences) named in the same way.

7.2.4. To run the samples, switch back to the "Realtime View" window. It will typically show the last batch run in that instrument. Before creating a new batch go to the folder window and to create a new folder for the current run. In the folder window click on the select project/folder icon. When the small window opens click on instrument name and click 'new folder". Enter the folder name as 220720GPC1 (current date and instrument name) and click Ok. Close the folder window.

7.2.5. To create a new batch file, go to "File" – "New batch file" and select a template file for your instrument. Save the batch under current name (like 220720GPC1 in folder with the same name). You can also open the most recent sequence and rename it. Edit the sequence by modifying the batch for the current run. Type in sample names, add/delete lines. Enter the GPC calibration standard tracker serial number next to the "GPC cal" name. Make sure a correct collection window is shown in the data comment column. Also verify injection volume. The file name column is set to "autofill". The file names will be created as Foldername_sample name_vial number; for example; 220720_LFB-1_007. Verify whether the line just above the batch table shows the correct folder. Save the batch.

7.2.6. If needed before the run, click the appropriate icon to run the pump, purge the autosampler or rinse the syringe. You can also run the autopurge program which does all those three things automatically. When everything is ready, click on the green arrow to start the run. Choose between running selected samples or the entire batch. Upon completion of the run, the shutdown method will be initiated automatically. The pump will cease running.

7.3. GPC Shimadzu System preparation to run samples:

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7.3.1. Fill the reservoir bottle with GC-HPLC grade methylene chloride. Place a label on the reservoir with the lot # of the solvent. For NP pesticides in soil and waste by tests S-PSNP-TQ, WS-PSNP-TQ elution is performed with 3% MeOH in methylene chloride. Since a mixed mobile phase is considered a reagent by TNI, a reagent serial number has to be generated in the Standard Tracker in LIMS and a label (printed from the Standard Tracker) placed on the mobile phase container.

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.

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

7.3.3.1. Turn on the pump from the software 7.3.3.2. Turn the drain valve 180^0 counterclockwise to open

7.3.3.3. Purge the flow line with the mobile phase for about 5 minutes.

7.3.3.4. Make sure no bubbles are in the flow line

7.3.4. After GPC calibration sample is run verify that the Fraction Collector's window is correct. . If necessary, adjust the collection window in the GPC LabSolutions software and save the acquisition method under a new name (date versioning).

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

7.3.6. Start the run from the LabSolutions software.

7.3.7. 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.

7.3.8 Load the sample vials into automatic injector sample rack

7.3.9 Equilibrate system for 30 min.

7.3.10 Begin the run sequence with at least two solvent vials, followed by a GPC calibration standard, and followed by another solvent vial as the GPC blank, followed by samples in the set. End the GPC run by injecting methylene chloride again, possibly several times to clean the system. **Use the first two solvent vials to check the GPC injection volume. No samples can be run if the correct injection volume is not assured.**

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

7.3.12. For soil samples vial 1 mL, inject 0.8 mL and submit 0.6 mL for NP pesticides (no Florisil), or 0.7 mL for chlorinated pesticides and PCBs (after Florisil cleanup). For tissue samples for chlorinated pesticides/PCBs vial 1 mL, inject 0.8 mL and submit 0.7 mL (after Florisil cleanup). Use conical-bottom vials for 0.8 mL injections.

7.4. Sample handling after the run.

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7.4.1 Collect the sample extract. Concentrate the sample extract using nitrogen blowdown.

7.4.2. Exchange the solvent to iso-octane for pesticide samples. After GPC clean-up all pesticides (except NP) samples are subjected to a 1 g Florisil clean-up.

7.4.3 Make up the final (submitted) volume.

7.4.4. If more than one injection was made for the same sample (tissue or dirty samples), then concentrate by nitrogen blowdown and solvent exchange in the concentrator tube and make up the same volume as the other samples in the extraction batch

7.4.5. After GPC run is completed turn off the degasser on/off valve to avoid flooding back the degasser with methylene chloride. Also turn off the helium flow. The instrument should go into the shutdown mode automatically.

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.

7.4.6. 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

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 backflushed 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 backflush for another 30 min.

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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 at 254 nm.

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. It may, however, by modified due to ongoing additions of new analytes like pyrethroids. The window usually extends further for Nitrogen-Phosphorus pesticides.
- 9.3.5 In order to document the GPC criteria, the chromatogram for the GPC calibration mix has to be printed out for each sequence run and included in the raw data package. The resolutions must be calculated for the two pairs of analytes (corn oil/phthalate and perylene/sulfur). The resolution calculations must be documented on the chromatogram along with collection time window.
- 9.3.6 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!

9.4 If collection window has changed enter the new collection window in the Sample Collector method as described in 7.3.4

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

9.6 Pay attention to the recoveries of the early eluting compounds like Cypermethrin and other Pyrethroids, as well as Beta-BHC, the last eluting chlorinated pesticide on 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 recoveries of polar triazine herbicides such as Atrazine, Atrazine Desethyl, Prometon, Metribuzin, Simazine and Ametryn must be monitored. It may be necessary to extend the end window to collect those analytes in some cases.

10 QUALITY CONTROL

10.1 For Quality Assurance/Quality Control see SOP GC-001-2 (see 13.2)

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 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/

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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

13 REFERENCES

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

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

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

13.4 DEP SOP GC-033-1 Analysis of Pesticides and PCBs in Water, Soil and Tissue Samples Using GC/MS/MS.

13.5 DEP SOP GC-034-1 Analysis of Chlordane and Toxaphene Using GC/MS with Negative Chemical Ionization.

13.6 DEP SOP SV-020-1 Analysis of polycyclic Aromatic Hydrocarbons (PAHs) in Fish Tissue using GC/MS/MS.

APPENDIX OF CHANGES

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

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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.
12/05/2018	Updated GPC column information.
11/21/2019	Changes throughout due to the replacement of EZchrom with Shimadzu's LabSolutions software.
10/28/2020	Added recent, generation "40" Shimadzu equipment. Removed references to GC-ECD methods, that are no longer in use.
12/14/2020	Reagent Serial Number and a label generated for the mixed mobile phase (3% MeOH in methylene chloride).
03/12/2021	Editorial changes to Section 1.2, Section 7.3.7, Section 6.1 and Section 10.1 as per CERP audit of 2/26/2021.
08/06/2021	Chapter 9.3.5 (GPC chromatograms) was added as per CERP audit on-site assessment report of 7/28/2021. Other updates resulting from recent upgrades to the GPC equipment. Reference to water ECD samples removed.
07/22/2022	Update to instrument models. Updates resulting from software upgrade. Other editorial updates throughout the text.

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