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

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TABLE OF CONTENTS

1 SCOPE AND APPLICATION
2 SUMMARY OF THE METHOD
3 INTERFERENCES
4 APPARATUS AND EQUIPMENT
5 REAGENTS AND CHEMICALS
6 SAMPLE COLLECTION, PRESERVATION AND HANDLING
7 GPC CLEANUP PROCEDURE
8 MAINTENANCE
9 CALIBRATION OF GPC COLUMN
10 QUALITY CONTROL
11 SAFETY/ HAZARDOUS WASTE MANAGEMENT
12 APPENDIX 1. GPC CALIBRATION SOLUTION PREPARATION
13 REFERENCES

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1. SCOPE AND APPLICATION

1.1. This SOP describes the procedure for the cleanup of sample extracts for subsequent analysis for chlorinated and nitrogen-phosphorus pesticides, as well as PCBs, 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. For Nitrogen Phosphorus pesticides the elution solvent is 3% methanol in methylene chloride.

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 (pump) LC-20AD or LC-40D pump.

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 UV detector working at fixed -fixed wavelength (254 nm).

4.1.6. Fraction collector Shimadzu FRC-40.

4.1.7. 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 Agilent PL EnviroPrep (300 mmx21.2) mmwith PLgel Prep guard column(25x 25mm)

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

4.4. Helium compressed gas Ultra-Pure Carrier Grade

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

4.6. Thermo Scientific 2 mL Target DP high recovery vials (conical bottoms)

4.7. Thermo Scientific 9 mm clear glass screw thread caps (pre-slit, must be used on GPC-1).

4.8. 50 mL “Pyrex” glass tubes

4.9. Waste liquid 4 L glass bottle

4.10. 3 cc disposable syringe

4.11. Whatman 0.45um disposable filters. Prior to use, if necessary, rinse each filter assembly with methylene chloride.

4.12. 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.

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).

7. GPC CLEANUP PROCEDURE

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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 three methods; for example, for GPC1: GPC1_method_191028 and GPC1_Shutdown, and GPC1_method_injvoltest.

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., It is highly recommended to run the pump, purge the autosampler and rinse the syringe before each run. The "auto purge" program does all those three things automatically. For best instrument performance run the "auto purge" program twice! 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:

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

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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. Auto Purge the instrument twice to remove the gas bubbles.

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

7.3.3.3. Run the "auto purge" program.

7.3.3.4. Make sure no bubbles are in the flow line and the solvent is flowing to the waste container.

7.3.3.5 Close the drain valve before running the samples!

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 Test the GPC injection volume by running the "injvoltest" method. It is a short, 5 minute run that does not involve the fraction collector. Several injection volume test samples should be run to properly assess the injection consistency. **No samples can be run if the correct injection volume is not assured.**

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. One can use the first two (or, preferably, more than two) solvent vials to check the GPC injection volume**.**

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

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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.

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 the GPC run is completed, 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 undertaken, 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 line to the column is not clogged. If so, cut or replace, until proper flow is restored.

8.2.2 Methylene chloride forms bubbles easily which affects check valves. Therefore, prime the pump using fresh CH_2Cl_2 (filtered, tightly capped). Open purge valve and make solvent follow at 5 mL/min. Prime for 5-10 minutes. Watch smooth flow and make sure there are no bubbles. Then, close the purge and test at low flow.

8.2.3 Clean check Valves: Methylene chloride corrodes polymer and forms sticky residues which might be the reason valves do not operate properly in the GPC, leading to rising pressure. Therefore, to address these issues, first tun off the pump. Then remove inlet and outlet check valves. Clean these

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valves using CH_2Cl_2, then soak with isopropyl alcohol. Sonicate briefly, dry and reinstall. Prime again as mentioned earlier.

8.2.4 Remove the sample loop and the needle and flush manually to clear out the debris.

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.

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 the GPC Calibration Solution (5.2) for calibration.

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.

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

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

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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 Never overtighten the cap on the solvent bottle. Excessive pressure may build up inside!

11.12 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

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.

APPENDIX OF CHANGES

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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.

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.

06/09/2023 Updates to chapters 4, 7.2 and 7.3. other editorial changes throughout the text.

05/21/2025 Updated chapter 11 regarding safety. References to PAHs removed.

05/26/2026. Updated chapter 8.

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