# Gel Permeation Chromatography

References: **Method 3640A,** Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Final Update III, December 1996 (USEPA, Office of Solid Waste and Emergency Response, Washington, DC).

NOAA 1993. Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects, 1984-1992. NOAA Technical Memorandum NOS ORCA 71.

"USEPA Contract Laboratory Program Statement of Work for Organics Analysis; Multi-Media, Multi-Concentration" August 1991. Document Number OLM03.1.

## 1. Scope and Application

**Matrices:** This method is applicable to the cleanup of solid samples (generally tissues and sediments) for lipids, polymers, proteins, natural resins, cellular components, and dispersed high molecular weight compounds.

**Definitions:** Refer to Alpha Analytical Quality Manual.

This method is recommended for the cleanup of samples from lipids, polymers, proteins, natural resins, cellular components, and dispersed high molecular weight compounds.

This procedure is a size exclusion cleanup using methylene chloride and hydrophobic synthetic gels on a HPLC. In the choice of gels the exclusion range must be larger than the molecular size of the molecules to be separated.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of analysts. Each analyst must demonstrate the ability to generate acceptable results with this method by performing an initial demonstration of capability, analyzing a proficiency test sample and completing the record of training.

After initial demonstration, ongoing demonstration is based on acceptable laboratory performance of at least a quarterly laboratory control sample or acceptable performance from an annual proficiency test sample. A major modification to this procedure requires demonstration of performance. The identification of major method modification requiring performance demonstration is directed by the Quality Assurance Officer and/or Laboratory Director on a case-by-case basis.

## 2. Summary of Method

Samples are extracted using the appropriate extraction protocol based on the matrix and required analytical parameters.

The sample extract is concentrated to a 4mL final volume in methylene chloride and vialed accordingly.

Between 1mL and 2mL of the sample extract are injected on the HPLC system and 2mL to 3mL are left in the vial for archive. The injection is carried by a mobile phase of methylene chloride through a calibrated GPC column. A fraction of the sample extract is collected for a designated time window (based on analysis parameters). The solvent before and after that time frame is discarded.

The extract collected during the specified time window is concentrated to an appropriate final volume, and submitted for the analysis determined based on project specifics.

### 2.1 Method Modifications from Reference

None.

## 3. Reporting Limits

Not Applicable

## 4. Interferences

**4.1** Solvents, reagents and glassware may introduce interferences. These must be demonstrated to be free of interferences by the analysis of a method blank. See the *Reagent, Solvent and Standard Control* (G-008) SOP and *Laboratory Glassware Cleaning* (G-002) SOP, for additional details.

**4.2** Many interferences can be removed by sample cleanup. Other cleanup methods performed include the following: *Alumina Column Cleanup of Organic Extracts* (OP-009), *Sulfur Cleanup* (OP-007), Sulfuric *Acid* (OP-010), *Silica Gel Cleanup Procedure* (OP-014 or NF02-001). Only appropriate cleanup techniques must be performed based on the suspected interference and the compounds of interest. For example, sulfuric acid cleanup is not applicable to samples requiring pesticide analysis because this rigorous cleanup will destroy the majority of pesticides.

**4.3** Soapy residue may result in basic conditions on glassware and may cause degradation of the pesticides Aldrin and Heptachlor, and some organophosphorous pesticides. All glassware must be rinsed thoroughly with deionized water and solvent to remove soapy residue. See SOP (G-002) *Laboratory Glassware Cleaning,* for additional details.

**4.4** Phthalate esters can be a major source of contamination if any material containing plasticizers (phthalates) comes in contact with the sample during the extraction process. Use of plastic or any material containing plasticizers (phthalates) should be avoided during extraction, cleanup, or analysis.

## 5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; 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. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

# 6. Sample Collection, Preservation, Shipping and Handling

## 6.1 Sample Collection

Refer to applicable extraction SOPs.

## 6.2 Sample Preservation

Refer to applicable extraction SOPs.

## 6.3 Sample Shipping

Refer to SOP/01-01 Sample Receipt and Login for sample shipping requirements

## 6.4 Sample Handling

Refer to applicable extraction SOPs.

# 7. Equipment and Supplies

**7.1 Column:** Waters Envirogel GPC 19x150mm Guard Column (WAT036555) and Envirogel GPC 19x300mm Cleanup Column (WAT036554) attached.

**7.2 Column:** J2 21.2 x 300mm Cleanup Column (CO100-1049-1.1)

**7.3 Syringe:** Luer Lock Gastight; 5mL, 10 mL or 20mL size.

**7.4 J2 Scientific AccuPrep MPS**

**7.5 J2 Scientific Prep Linc GPC**

**7.6 Computer:** Dell Optiplex GX 260

**7.7 Vials:** 4mL, caps, and PTFE Septa replacement cap inserts (WAT073005)

**7.8 Vials:** 60mL glass collection and 180 ml glass collection vails by J2 Scientific

# 8. Reagents and Standards

**8.1 Methylene Chloride:** Pesticide grade

**8.2 Hexane:** Pesticide grade

**8.3 Methanol:** Optima grade for HPLC

**8.4 Toluene:** Optima grade for HPLC

**8.5 Xylene:** Lab Grade.

**8.6 Tetrahydrafuran (THF):** HPLC Grade

**8.7 Sodium Sulfate:** granular, anhydrous. Purified by heating to 400°C for 4 hours.

**8.8 Glass wool:** purified by heating to 400°C for 4 hours

**8.9 GPC Calibration STD:** This Standard is prepared by diluting 5mL of the RESTEK Revised GPC Calibration Mix with 195mL of methylene chloride. Expires 6 months from the date of preparation. The Revised GPC Calibration Mix contains:

Corn Oil 250mg/mL
Bis (2-ethylhexyl) phthalate 5.0mg/mL
Methoxychlor 1.0mg/mL
Perylene 0.2mg/mL
Sulfur 0.8mg/mL

**8.10 Spiking solutions:** See the appropriate analytical SOP for details on the correct spike concentrations and spike volumes.

**8.10.1 Surrogate spiking solution**

**8.10.2 Laboratory Control Sample (LCS) solution**

# 9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

## 9.1 Blank

**9.1.1 DCM Blank – Typically required for environmental forensic programs.**

**9.1.1.1** A DCM (methylene chloride blank) is run through the GPC and collected at the proper window depending on which test is being run.

**9.1.1.2** The DCM will be concentrated down to 1mL and transferred for analysis.

**9.1.1.3** When the results of the DCM check blank are evaluated as “clean” and acceptable for the determinative analytical analysis, samples then can be run on the GPC.

## 9.2 Laboratory Control Sample (LCS)

Refer to the appropriate extraction/preparation and determinative method SOPs.

## 9.3 Initial Calibration Verification (ICV)

Not applicable to this method.

## 9.4 Continuing Calibration Verification (CCV)

Not applicable to this method.

## 9.5 Matrix Spike

Refer to the appropriate extraction/preparation and determinative method SOPs.

## 9.6 Laboratory Duplicate

Refer to the appropriate extraction/preparation and determinative method SOPs.

## 9.7 Method-specific Quality Control Samples

### 9.7.1 GPC Calibration Standard

**9.7.1.1** A GPC calibration standard must be run on the HPLC prior to running any samples for cleanup The frequency is once weekly, or sooner if there has been system maintenance. The GPC calibration standard acts as a retention time marker to check the condition of the GPC Column. The elution order for the GPC calibration standard compounds is: Corn Oil, bis(2-ethylhexyl)phthalate, Methoxychlor, Perylene, and Sulfur. The peaks should be resolved to at least 85% and 95% for the Perylene and Sulfur compounds. Two GPC calibration standards should be run sequentially to be sure there is no shift in retention time between the two. The GPC calibration standard can also be used to determine if there is air in the HPLC system. Air in the system would cause either a shift in the run or a loss of resolution.

**9.7.1.2** Corrective Action: If the GPC calibration standard does not produce the same retention time on two consecutive standard runs the GPC Column must undergo maintenance. Likewise if the Perylene and Sulfur peaks do not produce resolution at 85% and 95%, the GPC Column must undergo maintenance. Refer to section 10.5 for a description of column maintenance.

### 9.7.2 GPC Standard Review

**9.7.2.1** GPC standards need to be reviewed for 4 peak to valley resolution ratios to pass QC. Bis(2)ethylhexylphthalate is related to both it's preceeding and following valley and Methoxychlor just it's preceeding valley. All three need to pass for resolution criteria by at least 85%.

**9.7.2.2** Perylene is related to its following valley and needs to have 95% resolution to be viable.

**9.7.2.3 J2 Scientific GPC**

**9.7.2.3.1** To produce these percentages in the J2 Scientific GPC, open up the J2 AccuChrom program on the desktop. Click on "File", then "Import". Click on the run name that you are looking to analyze. After a minute wait, click on the button right below "Edit" in the menu bar. Then click on the box to the left of it, and a list of data sets appear. Click on the proper date, then the sequence name drops down, then the GPC STD, Method, and finally Data Plot. A chromatogram will appear. Click "Find Peaks". If the resolution check passes, print the chromatogram and place it into the GPC run log binder with the run sequence. If the resolution checks do not pass, column maintenance may be required. From this chromatogram you can calculate the collection times. Refer to section 16 Diagram 2 to see collection time windows for specific test.

**9.7.2.4** To set a collection window go to Prep Linc program. Go to the GPC tab and go under Methods and pick the correct analysis that is being performed (i.e. 8270, PCB, PAH). Set the collection start time in Fraction 1, length of collection time in Fraction 2, and the remaining time in Fraction 3. Total time should add up to 27 minutes on GPC 3 with the

Waters Column, and 56 minutes on GPC 4 and 5 (J2 Cleanup Column). Make sure Fraction 2 box is checked. Click Save.

**9.7.3 8270 Standard – Evaluated for 8270, 8081 and 8082 analyses**

**9.7.3.1** The 8270 standard is prepped by combining 100uL of 8270 LCS and 3900ul of Methylene chloride. 2mL of the standard are run on the instrument and collected via an 8270 window. The elution is then concentrated, transferred, and analyzed by GC/MS to ensure the compounds of interest are found in the collection window and to ensure there is no contamination from the HPLC itself.

**9.7.3.2** The 8270 STD is run once a week, or sooner if system maintenance has been performed. This check is also required after the instrument has been shut down for an extended period of time. If for some reason a GPC column has gone dry an 8270 Standard must be run and checked before samples can be cleaned again.

**9.7.3.3** Corrective Action: Recovery of the LCS solution is 70%-130% recovery. 10%, or up to seven compounds may be 60%-140%. Typical poor performing compounds are Benzaldehyde, Benzoic acid and Benzidine which historically recover at 40%-60%. If all compounds of importance are not recovered in the 8270 standard, either the collection windows on the fraction collector need to be adjusted or the GPC Column must undergo maintenance. Refer to section 10.5 for a description of column maintenance.

**9.7.4 PAH Standard – Evaluated for environmental forensic programs**

**9.7.4.1** The PAH standard is prepped by combining 100uL of PAH LCS and 3900uL of Methylene chloride. 2mL of the standard are run on the instrument and collected via a PAH window. The elution is then concentrated, transferred, and analyzed by GC to ensure the compounds of interest are found in the collection window and to ensure there is no contamination from the HPLC itself.

**9.7.4.2** The PAH STD should be run once a week or after the instrument has been shut down for an extended period of time. If for some reason a GPC column has gone dry a PAH Standard must be run and checked before samples can be cleaned again.

Corrective Action: If all compounds of importance are not recovered in the PAH standard, either the collection windows on the fraction collector need to be adjusted or the GPC Column must undergo maintenance. Refer to section 10.5 for a description of column maintenance.

## 9.8 Method Sequence

**9.8.1 GPC Preparation**

**9.8.2 Sample cleanup**

**9.8.2.1** Load sample onto GPC

**9.8.2.2** Ensure correct collection times, flow rate sample position, collection vial position, computer sequence, and enough eluent and process samples.

**9.8.2.3** Concentrate samples

**9.8.2.4** Final volume

# 10. Procedure

## 10.1 Equipment Set-up

### 10.1.1 J2 Scientific Setup

**10.1.1.1** First make sure that the J2 Scientific instrument is turned on. There are 2 switches, one on the front and another located on the back.

**10.1.1.2** Make sure that the Dell Optiplex GX 260 computer is turned on.

**10.1.1.3 GPC Startup Procedure**

**10.1.1.3.1** On the computer desktop open the Prep Linc program. The program will open the program 1.00.25 and will bring up the start up page.

**10.1.1.3.2** Make sure that there is enough methylene chloride in the HPLC solvent reservoir and enough room in the waste container.

**10.1.1.3.3** Click the “Prime” button located in the AS4 tab.Make sure to put A:DCM in the line under the Prime option. Press Resey Syringe Pump and press Set. The instrument will now pull 15mL of DCM into the syringe and then expel it to the waste. If there is air in the syringe repeat until no air is present.

**10.1.1.3.4** Next check the flow rate of the instrument. Using a 10mL graduated cylinder, pull the thinnest waste line that runs to the waste container. Put the waste line into the graduated cylinder and time for 1 minute. You should get a measurement of 5.0ml of flow.

**10.1.1.3.5** Once the flow rate is established, it is time to run a GPC Standard. Fill one 4mL vial with the GPC Calibration Solution and cap with PTFE septa and place the standards in sample tray positions 2 . Fill a $^{2nd}$ and $4^{th}$ 4mL vial with DCM and place in positions 1 and 3 .

**10.1.1.3.6** Place a 60mL VOA vials or the J2 collection vials in the collection rack in the corresponding positions.

**10.1.1.3.7** Under the GPC tab go under the sequence editor and type the sequence in the correct window.

**10.1.1.3.8** Start the sequence by clicking the start button and saving the sequence as GPCxmmddyyz where z= the Run of the day and x = the GPC number.

**10.1.1.3.9** 2mL of the GPC calibration mix will be run through the column in replicate and the chromatograms are evaluated to ensure proper separation as described in Section 9.7.2.

**10.1.1.3.10** Client Specified: If the GPC standard passes criteria, (refer to Sections 9.7.2) save the collection times and run a DCM Blank. Concentrate the collected DCM Blank and submit for the appropriate determinative analysis. If the DCM meets the analytical method for cleanliness, the rest of the sequence can be setup to run.

**10.1.1.3.11** If either the GPC Calibration Standard or the DCM Blank (client specified) does not meet the criteria specified by the determinative analytical method for cleanliness or separation/resolution, refer to Section 10.5 for GPC column maintenance.

**10.1.1.3.12** Double check that the samples in the sample tray, match the labels on the 60mL vial or the J2 collection vialsin the collection tray, and match the sequence written in the computer. Also make sure that the proper method is loaded for each sample.

**10.1.1.3.13** After the sequence has been double checked, start the run.

**10.1.1.3.14** All collection vials should be capped promptly after the cleanup is complete. Extraction vials must also be re-capped. Since the HPLC only injects 2mL of the 4mL extract there will 2mL remaining in the re-capped vial. The 2mL archive is placed in the archive freezer in case of future need.

## 10.2 Initial Calibration

Not Applicable

## 10.3 Equipment Operation and Sample Processing

### 10.3.1 Extraction

**10.3.1.1** Samples are extracted based on the client/project specifications. Sediment samples are extracted by Shaker Table (SOP OP-013) or MSE (SOP OP-016) and tissue samples are extracted by Tissuemizer Extraction (SOP OP-018) or MSE (SOP OP-016).

**10.3.1.2** Extracts are generally concentrated to a Final Volume of 4mL in Methylene chloride. Extracts are placed into a 4mL vial for GPC cleanup. Some special projects require a greater final volume. In such cases, the sample extract is concentrated in Methylene chloride to the necessary volume and 4mL are removed and placed in a 4mL vial to be GPC cleaned. Any remaining pre-GPC extract is saved as an archive.

### 10.3.2 Cleanup

**10.3.2.1** Replace the existing caps on the 4mL extract vials with PTFE septum replacement cap inserts. Place in the vials in the order to be cleaned starting with the lightest colored to the darkest colored sample on the HPLC vial tray. Refer to the procedures in Section 10.1 to begin the sample cleanup.

**10.3.2.2** All collection vials must be capped promptly after the cleanup is complete. Extraction vials must also be re-capped. Since the HPLC only injects 2mL of the 4mL extract there will be 2mL remaining in the re-capped vial. The 2mL archive is placed in the archive freezer in case of future need.

## 10.4 Continuing Calibration

Not Applicable

## 10.5 Preventive Maintenance

**10.5.1** If the GPC calibration standard does not produce the same retention time on two consecutive standard runs, the GPC Column must undergo maintenance. Likewise if the Perylene and Sulfur peaks do not produce resolution of 95% and Methoxychlor and Bis(2)ethylhexylphthalate do not produce resolution of 85% then the GPC column must undergo maintenance.

**10.5.2** The first step is to reverse the flow of the column by disconnecting the lines coming into the column and reversing them.

**10.5.3** Turn the flow on the instrument to 5mL/minute.

**10.5.4** Next setup the sample tray with fifteen 4mL vials. Fill the vials 1 – 5 with Xylene. Fill vials 6 - 10 with Tetrahydrofuran. Fill vials 11 – 15 with Toluene.

**10.5.5** Program the HPLC instrument to run the 15 samples. (Refer to Section 10.1 for instructions to setup and program the HPLC).

**10.5.6** When the maintenance run is completed, stop the flow and reverse the column, (refer to Section 10.5.2). Restart the flow at 5mL/minute. It usually takes approximately 3 hours of flushing the newly cleaned column to flush out any residual cleanup solvent and to restore the baseline.

**10.5.7** Setup GPC Calibration Standards and check for resolution issues. (Refer to section 9.7.1 and 9.7.2). Follow this with a DCM Blank check.

**10.5.8** If this process does correct the issue, consult with the Preparation Team Leader, as outside service may be warranted to resolve difficult HPLC issues.

# 11. Data Evaluation, Calculations and Reporting

**11.1** Procedures for data and record management for organic extraction must adhere to the Quality Systems Manual, other subordinate documents covering record keeping, and the *Document Control* SOP (08-01). All records must be stored in such a manner as to be safe and accessible for at least 10 years.

**11.2** The extraction bench sheets and other relevant laboratory notebooks must follow the specifications in the *Laboratory Notebook Usage* Work Instruction (WI 108-01), and all record keeping and document control practices.

**11.3** See the appropriate analytical SOPs for details on sample analysis, data evaluation, calculations and data reporting.

# 12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Section 9.0, Quality Control, defines the corrective actions that must be taken in instances where QC outliers exist.

# 13. Method Performance

## 13.1 Method Detection Limit Study (MDL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

The laboratory follows the procedure to determine the MDL, LOD, and/or LOQ as outlined in Alpha SOP/08-05. These studies performed by the laboratory are maintained on file for review.

## 13.2 Demonstration of Capability Studies

Refer to Alpha SOP/08-12 for further information regarding IDC/DOC Generation.

### 13.2.1 Initial (IDC)

The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

#### 13.2.2 Continuing (DOC)

The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

## 14. Pollution Prevention and Waste Management

Refer to Alpha's Chemical Hygiene Plan and Waste Management and Disposal SOP for further pollution prevention and waste management information.

## 15. Referenced Documents

Chemical Hygiene Plan
SOP/01-01 Sample Receipt and Login
SOP/08-01 Document Control.
SOP/08-05 MDL/LOD/LOQ Generation
SOP/08-12 IDC/DOC Generation
SOP/G-006 Hazardous Waste and Sample Disposal
SOP/G-008 Reagent, Solvent and Standard Control
SOP/G-002 Laboratory Glassware Cleaning
SOP/OP-007 Sulfur Cleanup
SOP/OP-009 Alumina Column Cleanup of Organic Extracts
SOP/OP-010 Sulfuric Acid
SOP/OP-013 Shaker Table Extraction
SOP/OP-014 Silica Fractionation Cleanup by HPLC
SOP/OP-016 Microscale Solvent Extraction (MSE)
SOP/OP-018 Tissue Extraction
SOP/NF02-001 NewFields Silica Gel Cleanup Preparation (Manual)

## 16. Attachments

Diagram 1: Determination of GPC Standard Resolution

Diagram 2: Determination of GPC Collection Window

**Diagram 1 - Determination of GPC Standard Resolution**

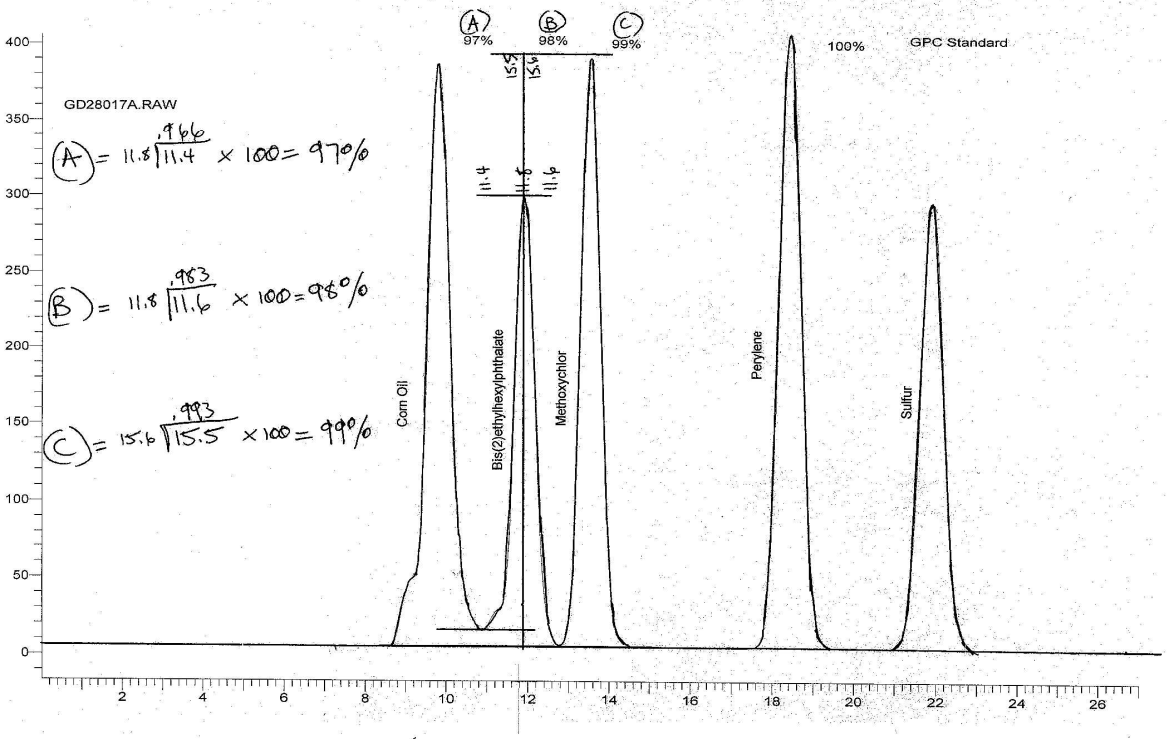
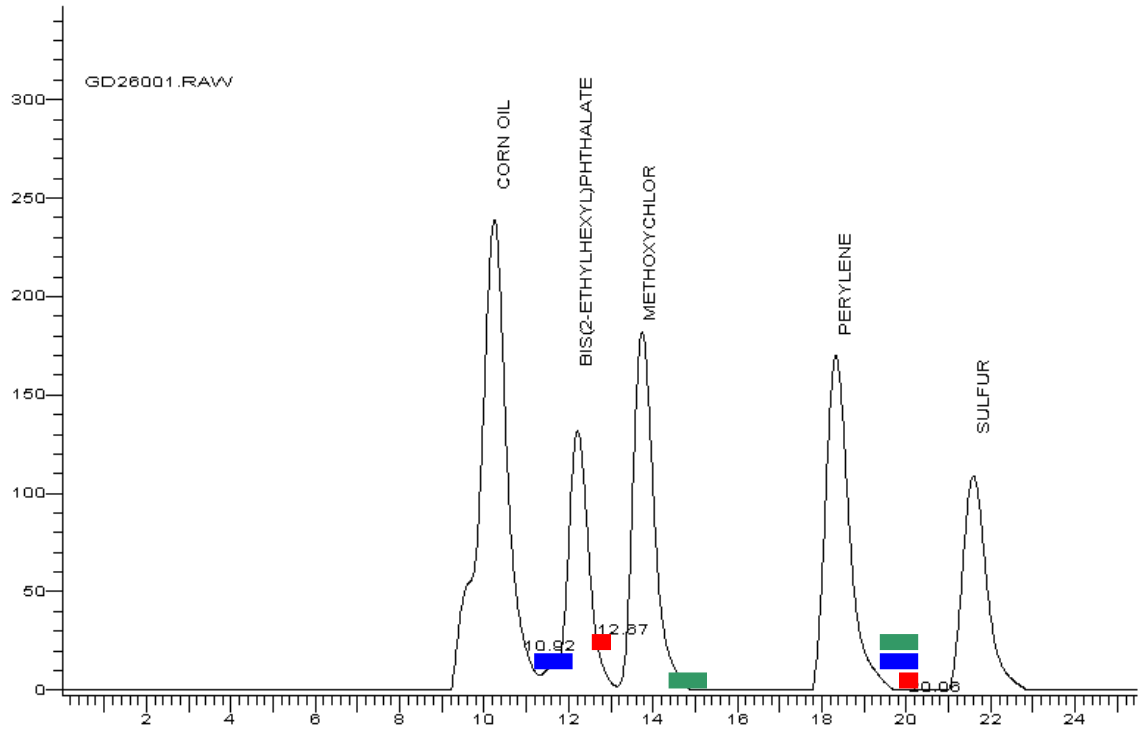


**Diagram 2 - Determination of GPC Collection Window**

PEST WINDOW (BASED ON GPC STD CHROMATOGRAM)
COLLECT APPROXIMATELY 12.6 MINUTES TO 20.5 MINUTES (THIS WILL VARY SLIGHTLY BASED ON THE RETENTION TIMES OF THE GPC STD CHROMATOGRAM). COLLECT APRROXIMATELY FROM THE END OF THE BIS(2-ETHYLHEXYL)PHTHALATE PEAK TILL BEFORE THE SULFUR PEAK.

8270 WINDOW (BASED ON GPC STD CHROMATOGRAM)
COLLECT APPROXIMATELY 11.0 MINUTES TO 20.0 MINUTES.
THIS COLLECTS FROM THE BEGINNING OF THE BIS(2-ETHYLHEXYL)PHTHALATE TO JUST BEFORE THE SULFUR PEAK

FOR EITHER PCB, DDT SERIES ISOMERS OR PAH (BASED ON GPC STD)
COLLECT APPROXIMATELY 15.0-20.0