METHOD 3640

GEL-PERMEATION CLEANUP

1.0 SCOPE AND APPLICATION

1.1 Gel-permeation chromatography (GPC) is a size exclusion procedure using organic solvents and hydrophobic gels in the separation of synthetic macromolecules (Gordon and Ford). The packing gel is porous and is characterized by the range or uniformity (exclusion range) of that pore size. In the choice of gels, the exclusion range must be greater than those of the molecules to be separated (Shugar, et al.).

1.1 a cross linked divinyl benzene-styrene copolymer (SX-3 Bio Beads or equivalent) is specified for this method.

1.2 General cleanup application - GPC is recommended for the elimination from the sample of lipids, polymers, copolymers, proteins, natural resins and polymers, cellular components, viruses, steroids, and dispersed high-molecular-weight compounds (Shugar, et al.). GPC is appropriate for both polar and non-polar analytes, therefore, it should be used when cleaning up extracts with a broad range of analytes.

1.3 Specific application - This method includes guidance for cleanup of sample extracts containing the analytes listed in Table 1. These analytes are from the RCRA Appendix VIII and Appendix IX lists. The data presented in the table represents average percent recovery for three replicate analyses, the % RSD of the three data points and the retention volumes of each analyte on a single GPC system. Retention volumes vary from column to column. Figure 2 provides additional information on retention volumes for certain classes of compounds. The data from the semivolatiles was determined by GC/MS, whereas, the pesticide data was determined by GC/ECD or GC/FPD. Compounds not amenable to GC were determined by HPLC. Other analytes may also be appropriate for this cleanup technique, however, recovery through the GPC should be > 70%.

1.4 Normally this method is most efficient for removing high boiling materials that condense in the injection port area of a gas chromatograph (GC) or the front of the GC column. This residue will ultimately reduce the chromatographic separation efficiency or column capacity because of adsorption of the target analytes on the active sites. Pentachlorophenol is especially susceptable to this problem. GPC, operating on the principal of size exclusion, will not usually remove interference peaks that appear in the chromatogram since the molecular size of these compounds is relatively similar to the target analytes. Separation cleanup techniques , based on other molecular characteristics (i.e. polarity), must be used to eliminate this type of interference.

2.0 SUMMARY OF METHOD

2.1 The column is packed with the required amount of preswelled absorbent and is flushed with solvent for an extended period. The column is calibrated and then loaded with the sample extract to be cleaned up. Elution is effected with a suitable solvent(s) and the product is then concentrated.

3.0 INTERFERENCES

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

3.2 More extensive procedures than those outlined in this method may be necessary for reagent purification.

4.0 APPARATUS

4.1 Gel-permeation chromatography system - GPC Autoprep Model 1002 A or B or equivalent, Analytical Biochemical Laboratories, Inc.. Systems that perform very satisfactorily have also been assembled from the following components - an HPLC pump, an auto sampler or a valving system with sample loops, and a fraction collector. All systems, whether automated or manual must meet the calibration requirements of 7.2.2.

4.1.1 Chromatographic column - 700 mm x 25 mm i.d. glass column. Flow ~~can be~~ upward~~, or downward, however, downward flow minimizes bubble formation~~ and ~~subsequent noise (on the UV scan baseline)~~.

(Optional) To simplify switching from the UV detector during calibration to the GPC collection device during extract cleanup, attach a double 3-way valve (Rheodyne Type 50 Teflon Rotary Valve #10-262 or equivalent) so that the column exit flow can be shunted either to the UV flow through cell or to the GPC collection device.

4.1.2 Guard column - (Optional) 5 cm, with appropriate fittings to connect to the inlet side of the analytical column (Supelco 5-8319 or equivalent).

4.1.3 Bio Beads (S-X3) - 200-400 mesh, 70 gm (Bio-Rad Laboratories, Richmond, CA Catalog 152-2750 or equivalent). An additional 5 gm of Bio Beads is required if the optional guard column is employed.

The quality of Bio Beads may vary from lot to lot because of excessive fines in some lots. The UV chromatogram of the Semivolatile Calibration solution should be very similar to that in Figure 1 and the backpressure should be within 6-10 psi. Also the gel swell ratio in methylene chloride should be in the range of 4.4-4.8 mL/gm. In addition to fines having a detrimental effect on chromatography, they can also pass through the column screens and damage the valve.

4.1.4 Ultraviolet detector - Fixed wavelength (254 nm) with a semi-prep flow-through cell.

4.1.5 Strip chart recorder, recording integrator or laboratory data system.

4.1.6 Syringe - 10-mL with Luerlok fitting.

4.1.7 Syringe filter assembly, disposable - Bio-Rad "Prep Disc" sample filter assembly #343-0005, 25 mm, and 5 micron filter discs or equivalent. Check each batch for contaminants. Rinse each filter assembly (prior to use) with methylene chloride if necessary.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all inorganics reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 ASTM Type II Water (ASTM D1193-77 (1983)). All references to water in the method refer to ASTM Type II unless otherwise specified. Water must be free of interferents at the method detection limit (MDL) of the analytes of interest. ASTM Type II water is further purified by the following technique:

5.2.1 A water purification system (Millipore Milli-Q Plus with the Organex-Q cartridge or equivalent) may be used to generate water.

5.3 Methylene chloride, CH_2Cl_2. Pesticide quality or equivalent.

5.3.1 Some brands of methylene chloride may contain unacceptably high levels of acid (HCl). Check the pH by shaking equal portions of methylene chloride and water, then check the pH of the water layer.

5.3.2 If the pH of the ~~methylene chloride~~ water layer is ≤ 5, filter the entire supply of solvent through a 2 in. x 15 in. glass column containing activated basic alumina. This column should be sufficient for processing approximately 20-30 liters of solvent. Alternatively a different ~~source~~ supply of methylene chloride should be found.

5.4 Cyclohexane, C_6H_{12}. Pesticide quality or equivalent.

5.5 n-Butyl chloride, $CH_3CH_2CH_2CH_2Cl$. Pesticide quality or equivalent.

~~5.6 GPC Calibration Solutions~~

5.6 GPC Calibration Solution. Prepare a calibration solution in methylene chloride containing the following analytes (in elution order):

Compound	mg/mL
corn oil	50.0
bis (2-ethylhexyl)phthalate	2.0
methoxychlor	0.4
perylene	0.02
sulfur	0.08

~~5.6.3~~ Store the calibration solution in an amber glass bottle with a Teflon lined screw-cap at 4°C, and protect from light. (Refrigeration may cause the corn oil to precipitate. Allow the calibration solution to stand at room temperature until the corn oil dissolves.) Replace the calibration standard solution ~~as necessary or a minimum of every 6 months.~~

6.0 SAMPLE COLLECTION, PRESERVATION, ~~AND HANDLING~~

6.1 See the introductory material to this chapter, Organic Analytes, Step 4.1.

7.0 PROCEDURE

7.1 It is very important to have consistent laboratory temperatures during an entire GPC run, which could be 24 hours or more. If temperatures are not consistent, retention times will shift and the dump and collect times determined by the calibration standard will no longer be appropriate. The ideal laboratory temperature to prevent outgassing of the methylene chloride is 72°F.

7.2 GPC Setup and Calibration

7.2.1 Column Preparation

7.2.1.1 Weigh out 70 gm of Bio Beads (SX-3). Transfer them to a quart bottle with a Teflon lined cap and add approximately 300 mL of methylene chloride. Swirl the funnel to ensure the wetting of all beads. Allow the beads to swell for a minimum of 2 hours. Maintain enough solvent to sufficiently cover the beads at all times. If a guard column is to be used, repeat the above with 5 gm of Bio Beads in a 125 mL bottle or a beaker, using 25 mL of methylene chloride.

7.2.1.2 Turn the column upside down from its normal position, and remove the inlet bed support plunger (the inlet plunger is longer than the outlet plunger). Position and tighten the outlet bed support plunger as near the end as possible, but no closer than 5 cm (measured from the gel packing to the collar).

7.2.1.3 Close the column outlet stopcock. Place a small amount of solvent in the column to minimize the formation of air bubbles at the base of poured column packing. Place the tubing from the column outlet into a waste beaker below the column.

7.2.1.4 Swirl the bead/solvent slurry to get a homogeneous mixture and quickly transfer it to a 500 mL separatory funnel with a large bore stopcock. Drain the excess methylene chloride directly into the waste beaker and then start draining the slurry into the column by placing the separatory funnel tip against the column wall. This will help to minimize bubble formation. Swirl occasionally to keep the slurry homogeneous. Drain enough to fill the column. Open the stopcock and allow the excess solvent to drain. Close the stopcock when the top of the gel begins to look dry. Add additional methylene chloride to just rewet the gel.

7.2.1.5 Wipe any remaining beads and solvent from the inner walls of the top of the column with a laboratory tissue. Loosen the seal slightly on the other plunger assembly (long plunger) and insert it into the column. Make the seal just tight enough so that any beads on the glass surface will be pushed forward, but loose enough so that the plunger can be pushed forward.

CAUTION: Do not tighten the seal if beads are between the seal and the glass surface, because this can damage the seal and cause leakage.

7.2.1.6 Compress the column as much as possible without applying excessive force. Loosen the seal

To Cal rewrite

and gradually pull out the plunger. Rinse and wipe off the plunger. Slurry any remaining beads and transfer them into the column. Repeat step 7.2.1.5 and reinsert the plunger. If the plunger cannot be inserted and pushed in without allowing beads to escape around the seal, continue compression without tightening the seal, and loosen and remove the plunger as described. Repeat this procedure until the plunger is successfully inserted.

7.2.1.7 Push the plunger until it meets the gel, then compress the column bed about four centimeters.

7.2.1.8 Pack the optional 5 cm column with approximately 5 gm of preswelled beads (different guard columns may require different amounts). Connect the guard column to the inlet of the analytical column.

7.2.1.9 Connect the column inlet to the solvent reservoir (reservoir should be placed higher than the top of the the column) and place the column outlet tube in a waste container. Placing a restrictor in the outlet tube will force air out of the column more quickly. A restrictor can be made from a piece of capillary stainless steel tubing of 1/16" OD x 10/1000" ID x 2". Pump methylene chloride through the column at a rate of 5 mL/min for 1 hour.

7.2.1.10 After washing the column for at least one hour, connect the column outlet tube, without the restrictor, to the inlet side of the UV detector. Connect the system outlet to the outlet side of the UV detector. A restrictor (same size as in 7.2.1.9) in the outlet tube from the UV detector will prevent bubble formation which causes a noisey UV baseline. The restrictor will not effect flow rate. After pumping methylene chloride through the column for an additional 1-2 hours, adjust the inlet bed support plunger until approximately 6-10 psi backpressure is achieved. Push the plunger in to increase pressure or slowly pull outward to reduce pressure.

Change last sentence to:

Cap the bottle to prevent.....

7.2.1.10 Add to the end of the paragraph.

Always recalibrate after column drying has occurred to verify retention volumes have not changed.

7.2.1.10 When the GPC column is not used for several days, ~~evaporation of methylene chloride may cause column drying, which can cause stratification or channeling in the gel. To prevent this drying,~~ connect the column outlet line to the column inlet. ~~This will allow a continuous recycling of the solvent when the system is not in use~~. If channeling occurs, the gel must be removed from the column, reswelled, and repoured as described above. If drying occurs, methylene chloride should be pumped through the column until the observed column pressure is constant and the ~~same~~ as when the column was new.

Always recalibrate after column drying has occurred to verify retention volumes have not changed.

7.2.2 Calibration of the GPC column

7.2.2.1 Using a 10 mL syringe, load sample loop #1 with calibration solution (Step 5.6). With the ABC automated system, the 5 mL sample loop requires a minimum of 8 mL of the calibration solution. Use a firm, continuous pressure to push the sample onto the loop.

Switch the valve so that GPC flow is through the UV flow-through cell.

7.2.2.2 Inject the calibration solution and obtain a UV trace showing a discrete peak for each component. Adjust the detector and/or recorder sensitivity to produce a UV trace similar to Figure 1 that meets the following requirements. Differences between manufacturer's cell volumes detector sensitivities may require a dilution of the calibration solution to achieve similar results. An analytical flow-through detector cell will require a much less concentrated solution than the semi-prep cell, and therefore the analytical cell is not acceptable for use.

7.2.2.3 Following are criteria for evaluating the UV chromatogram for column condition.

UV Trace Requirements:

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

o Corn oil and phthalate peaks must exhibit > 85% resolution.

o Phthalate and methoxychlor peaks must exhibit > 85% resolution.

o Methoxychlor and perylene must exhibit > 85% resolution.

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

o Perylene and sulfur peaks must not be saturated and must exhibit 90% baseline resolution. (Semivolatile and Pesticide)

7.2.2.4 Calibration for Semivolatiles - Using the information from the UV trace, establish appropriate

collect and dump time periods to ensure collection of all target analytes. Initiate column eluate collection just before elution of bis(2-ethylhexyl)phthalate (approximately 30 minutes) and after the elution of the corn oil (approximately 20 minutes). Stop eluate collection shortly after the elution of perylene (approximately 50 minutes). Collection should be stopped before sulfur elutes (approximately 55 minutes). Each laboratory is required to establish its specific time sequences. ~~The times provided are for general guidance only.~~ Figure 3 illustrates retention volumes for different classes of compounds.

Use a "Wash" time of 10 minutes.

[See Figures 1-2 for general guidance on retention times.]

7.2.2.5 Calibration for Organochlorine pesticides/PCBs - Determine the elution times for the phthalate, methoxychlor, perylene, and sulfur. Choose a dump time which removes > 85% of the phthalate. Choose a collect time so that > 95% of the methoxychlor is collected. Stop collection after the elution of perylene but before sulfur elutes.

7.2.2.6 Verify the flow rate by collecting column eluate for 10 minutes in a graduated cylinder and measure the volume, which should be 45-55 mL (4.5-5.5 mL/min). If the flow rate is outside of this range, corrective action must be taken, as described above. Once the flow rate is within the range of 4.5-5.5 mL/min, record the column pressure (should be 6-10 psi) and room temperature. Changes in pressure, solvent flow rate, and temperature conditions can affect analyte retention times and must be monitored. If the flow rate and/or column pressure do not fall within the above ranges, a new column should be prepared.

A UV trace that does not meet the criteria in 7.2.2.3 would also indicate that a new column should be prepared. It may be necessary to obtain a new lot of Bio Beads if the column fails all the criteria.

7.2.2.7 Reinject the calibration solution after appropriate collect and dump cycles have been set and the solvent flow and column pressure have been established.

7.2.2.6.1 Measure and record the volume of collected GPC eluate in a graduated cylinder. The volume of GPC eluate collected for each sample extract processed may be used to indicate problems with the system during sample processing.

7.2.2.6.2 The retention times for bis(2-ethylhexyl)phthalate and perylene must not vary more than ± 5% between calibrations. If the retention time shift is > 5%, take corrective action.

action. Excessive retention time shifts are caused by:

o Poor laboratory temperature control

o An unstablilized column that requires pumping methylene chloride through for several more hours or overnight.

o Excessive laboratory temperatures causing outgassing of the methylene chloride.

7.2.2.8 Analyze a GPC blank by loading 5 mL of methylene chloride into the GPC. Concentrate the methylene chloride by Kuderna-Danish (KD) evaporator that passes through the system during the collect cycle. Analyze it by whatever detectors will be used for the analysis of future samples. Exchange the solvent if necessary. If the blank exceeds the practical quantitation limit of the analytes, pump additional methylene chloride through the system for 1-2 hours. Analyze another GPC blank to ensure the system is sufficiently clean. Repeat the methylene chloride pumping if necessary.
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7.3 Extract Preparation

7.3.1 Adjust the extract volume to 10.0 mL. The solvent extract must be primarily methylene chloride. All other solvents, e.g. 1:1 methylene chloride/acetone, must be concentrated to 1 mL (or as low as possible if a precipitate forms) and diluted to 10.0 mL with methylene chloride. Thoroughly mix the sample before proceeding.

7.3.2 Filter the extract through a 5 micron filter disc by attaching a syringe filter assembly containing the filter disc to a 10 mL syringe. Draw the sample extract through the filter assembly and into the 10 mL syringe. Disconnect the filter assembly before transferring the sample extract into a small glass container, e.g. a 15 mL culture tube with a Teflon lined screw cap. Alternatively, draw the extract into the syringe without the filter assembly. Attach the filter assembly and force the extract thru the filter and into the glass container. The latter is the preferred technique for viscous extracts or extracts with alot of solids. Particulate greater than 5 micron may scratch the valve, which causes a leak and cross contamination of sample extracts in the sample loops. Repair of the damaged valve is quite expensive.

<u>NOTE</u>: Viscosity of a sample extract should not exceed the viscosity of 1:1 water/glycerol. Dilute samples that exceed this viscosity.

7.4 Screening the Extract

7.4.1 Screen the extract to determine the concentration of dissolved residue by evaporating a 100 uL aliquot to dryness and weighing the residue. The concentration of dissolved residue loaded on the GPC column cannot exceed 500 mg. Concentrations exceeding 500 mg will very likely result in incomplete extract cleanup and, contamination of the GPC switching valve (which results in cross contamination of sample extracts).

7.4.1.1 Transfer 100 uL of the filtered extract from 7.3.2 to a tared aluminum weighing dish.

7.4.1.2 A suggested evaporation technique is to use a heat lamp. Setup a 250 watt heat lamp in a hood so that it is 8 ± 0.5 cm from a surface covered with a clean sheet of aluminum foil. Surface temperature should be 80-100° C (check temperature by placing a thermometer on the foil and under the lamp). Place the weighing dish under the lamp using tongs. Allow it to stay under the lamp for 1 min. Transfer the weighing dish to an analytical balance or a micro balance and weigh to the nearest 0.1 mg. Determine if constant weight has been achieved by placing the weighing dish and residue back under the heat lamp for 2 or more additional 0.5 min intervals. Reweigh after each interval. Constant weight is achieved when three weights agree within ± 10%.

7.4.1.3 Repeat the above residue analysis on a blank and a spike. Add 100 uL of the same methylene chloride used for the sample extraction, to a weighing boat and determine residue as above. Add 100 uL of a corn oil spike (5 mg/100 uL) to another weighing boat and repeat the residue determination.

7.4.2 A residue weight of 10 mg/100 uL of extract represents 500 mg in 5 mL of extract. Any sample extracts that exceed the 10 mg/100 uL residue weight, must be diluted so that the 5 mL loaded on the GPC column does not exceed 500 mg. When making the dilution, keep in mind that a minimum volume of 8 mL is required when loading the ABC GPC unit. Following is a calculation that may be used to determine what dilution is necessary, if the residue exceeds 10 mg.

$$\frac{\text{mg of residue}}{\text{10 mg maximum}} \times \text{mL taken for dilution} = \text{10 mL final volume}$$

Example:

$$\frac{\text{15 mg residue}}{\text{10 mg maximum}} \times \text{Y mL taken for dilution} = \text{10 mL final volume}$$

Y mL taken for dilution = 6.7 mL

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Therefore, taking 6.7 mL of sample extract from 7.3.2 and diluting to 10 mL with methylene chloride, will result in 5 mL of diluted extract loaded on the GPC column that contains 500 mg of residue.

NOTE: This dilution factor must be included in the final calculation of analyte concentrations. In the above example, the dilution factor is 1.5.

7.5 GPC Cleanup

7.5.1 Calibrate the GPC at least once per week following the procedure outlined in 7.2.2 through 7.2.2.6. Ensure that UV trace requirements, flow rate and column pressure criteria are acceptable. Also, the retention time shift should be < 5% when compared to retention times in the last calibration UV trace.

7.5.1.1 If these criteria are not met, try cleaning the column by loading one or more 5 mL portions of butyl chloride and running it through the column. Butyl chloride removes the discoloration and particulate that may have precipitated out of the methylene chloride extracts. If a guard column is being used, replace it with a new one. This may correct the problem. If column maintenance does not restore the performance to acceptable criteria, the column must be repacked and calibrated.

7.5.2 Draw a minimum of 8 mL of extract (diluted if necessary and filtered) into a 10 mL syringe.

7.3.4 Attach the syringe to the turn lock on the injection port. Use firm, continuous pressure to push the sample onto the 5-mL sample loop. If the sample is difficult to load, some part of the system may be blocked. Take appropriate corrective action. If the back pressure is normal (6-10 psi) the blockage is probably in the valve. Reverse the inlet and outlet tubes, pump solvent through the tubes which should flush the blockage out of the valve. (This should be done before sample loading.)

NOTE: Approximately 2 mL of the extract remains in the lines between the injection port and the sample loop; excess sample also passes through the sample loop to waste.

7.3.5 After loading a loop, and before removing the syringe from the injection port index the GPC to the next loop. This will prevent loss of sample caused by unequal pressure in the loops.

7.3.7 After loading each sample loop, wash the loading port with methylene chloride in a PTFE wash bottle to minimize cross contamination. Inject approximately 10 mL of methylene chloride to rinse the common tubes.

7.3.8 Column overloading

7.3.8.1 Column overloading can occur when too much high boiling material is loaded in a sample loop.

7.3.8.1.1 For highly contaminated samples, dilute the extract and process in more than one sample loop. An example dilution procedure is to mix 10 mL of sample extract with 10 mL of methylene chloride or 1:1 butyl chloride/methylene chloride, shake well to thoroughly mix, and load into two sample loops.

7.3.8.1.2 After GPC cleanup, combine the collected fractions and treat as a single sample. Therefore, no additional dilution factor will be required when a sample extract is diluted and divided for GPC cleanup.

7.3.9 After loading all the sample loops, index the GPC to the 00 position, switch to the "RUN" mode and start the automated sequence.

7.3.10 Process each sample using the collect and dump cycle times established in Step 7.2.2. Process calibration standards, GPC blank of methylene chloride, samples and method blanks in the following order:

Loop 1 Calibration Standard
Loop 2 GPC Blank
Loop 3-23 Samples

7.3.10 Collect each sample in a 250-mL Erlenmeyer flask, covered with aluminum foil to reduce solvent evaporation, or directly into a Kuderna-Danish evaporator. Monitor sample volumes collected. Do not concentrate the processed GPC extract at this point. Changes in sample volumes collected may indicate one or more of the following problems:

- Change in solvent flow rate, caused by channeling in the column or changes in column pressure.
- Increase in column operating pressure due to the absorption of particles or gel fines onto either the guard column or the analytical column gel, if a guard column is not used.
- Leaks in the system or significant variances in room temperature.

7.3.11 Evaluation of calibration standards

7.3.11.1 Evaluate the retention times for bis(2-ethylhexyl)phthalate and perylene in each daily calibration standard UV trace. The retention time for either compound cannot exceed a 5% retention time shift when compared to the retention time of the previous calibration standard.

7.3.11.2 Corrective action must be taken before sample cleanup can proceed.

7.3.11.3 Acceptable GPC performance is demonstrated by the successful analysis of the calibration standard solution. Corrective actions may include those listed in Steps 7.2.1 and 7.2.2 above or may require repacking and recalibration of the column.

7.4 Concentrate the extract by the standard K-D technique (see any of the extraction methods, Step 4.2 of this chapter). The final volume is based on the results of the GC/FID screening in Step 7.3.2. If no peaks were detected or all peaks were < 10% of full scale, see the determinative methods (Chapter Four, Step 4.3) for the final volume.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 3600 for specific quality control procedures.

8.2 The analyst should demonstrate that the compound(s) of interest are being quantitatively recovered before applying this method to actual samples.

8.3 For sample extracts that are cleaned up using this method, the associated quality control samples must also be processed through this cleanup method.

9.0 METHOD PERFORMANCE

9.1 Refer to Table 1 for single laboratory performance data.

10.0 REFERENCES

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TABLE 1.
GPC RECOVERY AND RETENTION VOLUMES FOR RCRA
APPENDIX VIII ANALYTES

Compound	% Rec [1]	% RSD [2]	Ret. Vol. (mL) [3]
Acenaphthene	97	2	196-235
Acenaphthylene	72	10	196-235
Acetophenone	94	7	176-215
2-Acetylaminofluorene	97	2	156-195
Aldrin	99	9	196-215
4-Aminobiphenyl	96	7	176-215
Aniline	93	4	196-235
Anthracene	89	2	196-235
Benomyl	131	8	146-195
Benzenethiol	92	11	196-235
Benzidine	95	5	176-215
Benz(a)anthracene	100	3	196-235
Benzo(b)fluoranthene	93	5	196-235
Benzo(a)pyrene	93	3	196-235
Benzo(ghi)perylene	90	6	196-235
Benzo(k)fluoranthene	91	4	196-235
Benzoic acid	66	7	176-195
Benzotrichloride	93	7	176-215
Benzyl alcohol	95	17	176-215
Benzyl chloride	99	4	176-215
gamma-BHC	93	4	196-215
alpha-BHC	84	13	196-215
beta-BHC	94	9	196-215
delta-BHC	102	7	216-255
4-Bromophenyl phenyl ether	93	1	176-215
Butyl benzyl phthalate	104	3	136-175
2-sec-butyl-4,6-dinitrophenol (Dinoseb)	103	18	176-195
Carbazole	99	5	196-255
Carbendizim	131	8	146-195
alpha-Chlordane	97	2	196-235
gamma-Chlordane	93	2	196-215
4-Chloro-3-methylphenol	87	1	196-255
4-Chloroaniline	88	3	196-235
Chlorobenzilate	92	5	176-235
Bis(2-chloroethoxy)methane	89	1	156-195
Bis(2-chloroethyl)ether	76	2	156-215
Bis(2-chloroisopropyl)ether	83	2	156-195
2-Chloronaphthalene	89	1	196-235
2-Chlorophenol	90	1	196-215
4-Chlorophenol	87	2	196-215
3-Chlorophenol	86	3	196-215

TABLE 1.
(continued)

Compound	% Rec	% RSD	Ret. Vol. (mL)
4-Chorophenyl phenyl ether	98	2	176-215
3-Chloropropionitrile	80	5	176-215
Chrysene	102	1	196-235
2-Cresol	91	1	196-215
4-Cresol	88	2	196-215
3-Cresol	70	3	196-215
Cyclophosphamide	114	10	146-185
DDD	94	4	196-235
DDE	94	2	196-235
DDT	96	6	176-215
Di-n-butyl phthalate	104	3	136-175
Diallate	97	6	156-175
Dibenzo(a,e)pyrene	94	10	216-235
Dibenzo(a,i)pyrene	99	8	216-235
Dibenz(a,j)acridine	117	9	176-195
Dibenz(a,h)anthracene	92	5	196-235
Dibenzofuran	94	1	176-235
Dibenzothiophene	94	3	196-235
1,2-Dibromo-3-chloropropane	83	2	176-215
1,2-Dibromoethane	121	8	196-215
trans-1,4-Dichloro-2-butene	107	6	176-195
cis-1,4-Dichloro-2-butene	106	6	176-215
1,2-Dichlorobenzene	81	1	196-235
1,4-Dichlorobenzene	81	1	196-235
1,3-Dichlorobenzene	81	1	196-235
3,3'-Dichlorobenzidine	98	3	176-215
2,6-Dichlorophenol	86	3	196-215
2,4-Dichlorophenoxy acid (2,4-D)	80	NA	176-215
2,4-Dichlorophenol	87	2	196-215
α,α-Dichlorotoluene	70	9	196-235
1,3-Dichloro-2-propanol	73	13	176-215
Dieldrin	100	5	196-215
Diethyl phthalate	103	3	136-195
Dimethoate	79	15	146-185
3,3'-Dimethoxybenzidine[a]	15	11	156-195
Dimethyl phthalate	100	1	156-195
p-Dimethylaminoazobenzene	96	1	176-215
7,12-Dimethylbenz(a)anthracene	77	1	176-215
3,3-Dimethylbenzidine	93	2	156-215
2,4-Dimethylphenol	93	2	176-215
4,6-Dinitro-o-cresol	100	1	156-195
1,3-Dinitrobenzene	99	2	156-195
2,4-Dinitrophenol	118	7	176-195

TABLE 1.
(continued)

Compound	% Rec	% RSD	Ret. Vol. (mL)
2,4-Dinitrotoluene	93	4	156-195
2,6-Dinitrotoluene	101	2	156-175
Diphenylamine	95	6	176-235
Diphenyl ether	67	12	196-215
1,2-Diphenylhydrazine	92	1	176-215
Disulfoton	81	15	146-165
Endosulfan sulfate	94	2	176-195
Endosulfan I	99	8	176-215
Endosulfan II	92	6	196-215
Endrin	95	6	196-215
Endrin aldehyde	97	1	176-215
Endrin ketone	94	4	176-215
Ethyl methane sulfonate	62	7	176-235
Ethyl methacrylate	126	7	176-195
Bis(2-ethylhexyl)phthalate	101	1	120-145
Famphur	99	NA	126-165
Fluorene	95	1	176-235
Fluoranthene	94	1	196-235
Heptachlor	85	2	195-215
Heptachlor epoxide	91	11	156-195
Hexachlorobenzene	108	2	196-235
Hexachlorobutadiene	86	2	176-215
Hexachlorocyclopentadiene	89	3	176-215
Hexachloroethane	85	1	196-235
Hexachloropropene	91	2	196-235
Indeno(1,2,3-cd)pyrene	79	13	216-255
Isodrin	98	5	196-235
Isophorone	68	7	156-195
Isosafrole, isomer 1	90	4	176-215
Isosafrole, isomer 2	88	16	156-195
Kepone	102	NA	196-235
Malononitrile	111	9	156-195
Merphos	93	12	126-165
Methoxychlor	94	6	156-195
3-Methylcholanthrene	74	12	176-195
2-Methylnaphthalene	67	6	196-215
Methyl parathion	84	13	146-185
4,4'-Methylene-bis(2-chloroaniline)	96	1	176-215
Naphthalene	95	7	196-215
1,4-Naphthoquinone	73	7	176-215
2-Naphthylamine	94	8	196-235
1-Naphthylamine	96	6	196-235

TABLE 1.
(continued)

Compound	% Rec	% RSD	Ret. Vol. (mL)
5-Nitro-o-toluidine	77	2	176-195
2-Nitroaniline	96	8	176-215
3-Nitroaniline	96	2	176-215
4-Nitroaniline	103	8	176-215
Nitrobenzene	86	2	176-195
2-Nitrophenol	95	3	176-195
4-Nitrophenol	77	3	196-215
N-Nitrosodi-n-butylamine	89	4	156-175
N-Nitrosodiethanolamine	104	3	146-185
N-Nitrosodiethylamine	94	2	156-175
N-Nitrosodimethylamine	86	13	156-195
N-Nitrosodiphenylamine	99	2	156-195
N-Nitrosodi-n-propylamine	85	4	156-175
N-Nitrosomethylethylamine	83	7	156-175
N-Nitrosomorpholine	86	4	156-195
N-Nitrosopiperidine	84	4	156-195
N-Nitrosopyrolidine	92	1	156-175
Di-n-octylphthalate	883	4	120-156
Parathion	109	14	146-170
Pentachlorobenzene	95	2	196-235
Pentachloroethane	74	1	196-235
Pentachloronitrobenzene (PCNB)	91	8	156-195
Pentachlorophenol	102	1	196-215
Phenacetin	100	3	156-195
Phenanthrene	94	2	196-235
Phenol	83	2	156-195
1,2-Phenylenediamine	91	1	196-215
Phorate	74	NA	116-135
2-Picoline	99	14	156-215
Pronamide	105	15	156-195
Pyrene	98	2	215-235
Resorcinol	70	6	196-215
Pronamide	100	3	156-195
Safrole	93	1	176-215
Streptozotocin[a]	6	48	225-245
1,2,4,5-Tetrachlorobenzene	96	2	196-235
2,3,5,6-Tetrachloronitrobenzene	85	9	176-215
2,3,5,6-Tetrachlorophenol	96	7	196-215
2,3,4,6-Tetrachlorophenol	95	1	196-215
Tetraethyldithiopyrophosphate (Sulfotep)	89	14	116-135
Thiosemicarbazide	74	3	146-185

TABLE 1.
(continued)

Compound	% Rec	% RSD	Ret. Vol. (mL)
4-Toluidine	87	8	176-235
2-Toluidine	92	3	176-235
Thiourea, 1-(o-chlorophenyl)	75	11	166-185
Toluene-2,4-diamine	69	7	176-215
1,2,3-Trichlorobenzene	87	1	196-235
1,2,4-Trichlorobenzene	89	1	196-235
2,4,6-Trichlorophenol	95	1	216-235
2,4,5-Trichlorophenol	77	1	216-235
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	71	23	156-235
2,4,5-Trichlorophenoxypropionic acid	67	NA	216-215
Warfarin	94	2	166-185

NA = Not applicable, recovery presented as the average of two determinations.

[a] Not an appropriate analyte for this method.

[1] The percent recovery is based on an average of three recovery values.

[2] The % relative standard deviation is determined from three recovery values.

[3] These Retention Volumes are for guidance only as they will differ from column to column and from system to system.

FIGURE 1.
GPC CHROMATOGRAM OF A GPC CALIBRATION STANDARD CONTAINING 1 mg/mL POLY-STYRENE (~15 MIN), 48 mg/mL CORN OIL (~20 MIN), 1.6 mg/mL BIS(2-ETHYLHEXYL)PHTHALATE (~28 MIN), 0.028 mg/mL 2,4-DINITROPHENOL (~36 MIN), 0.17 mg/mL 4-NITROPHENOL (~41 MIN), 0.020 mg/mL PERYLENE (~45 MIN), AND 0.081 mg/mL SULFUR (~51 MIN). RUN CONDITIONS: COLUMN: 70 GRAM S-X3; MOBILE PHASE: DICHLOROMETHANE; FLOW RATE: 5 mL/min.

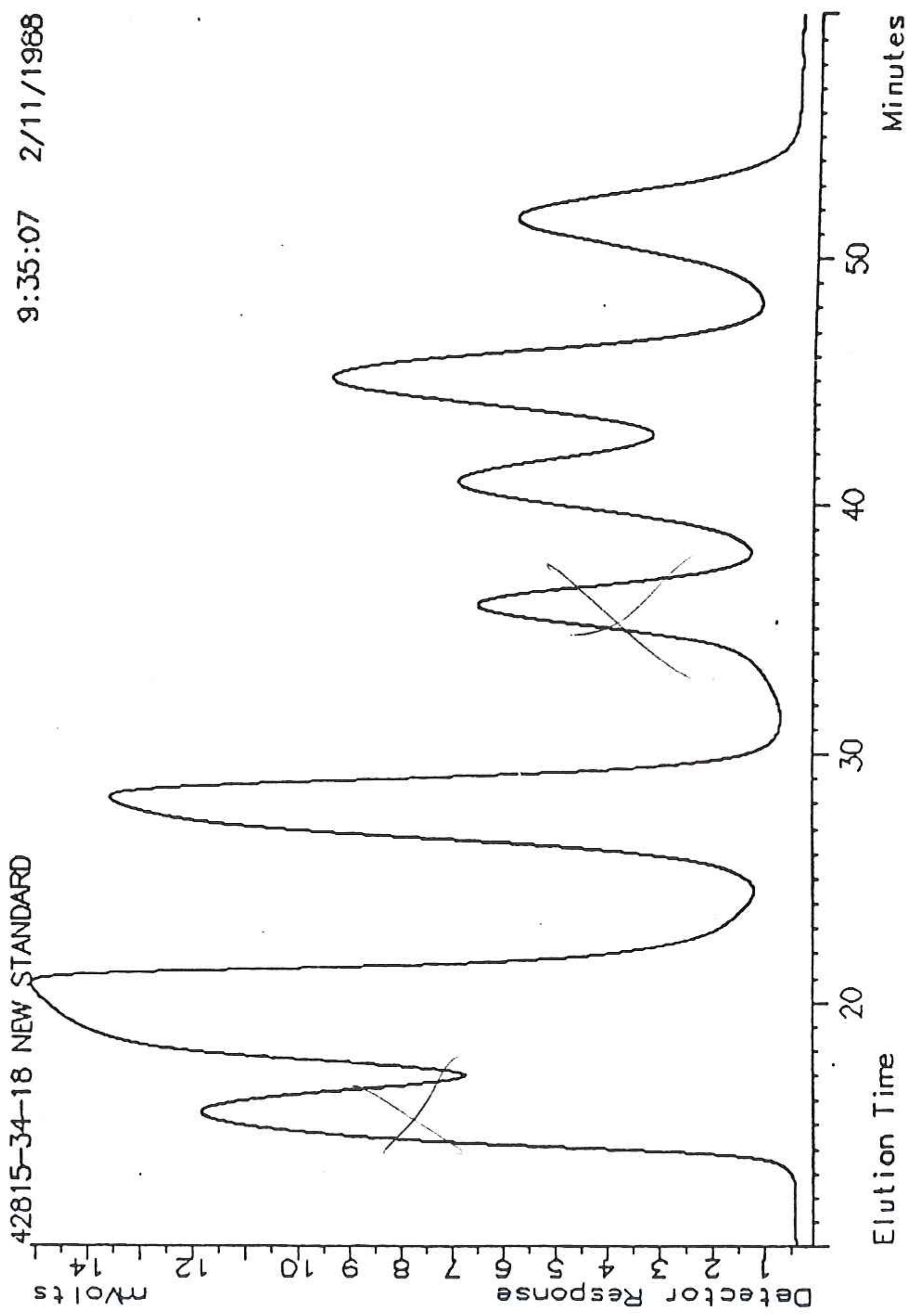
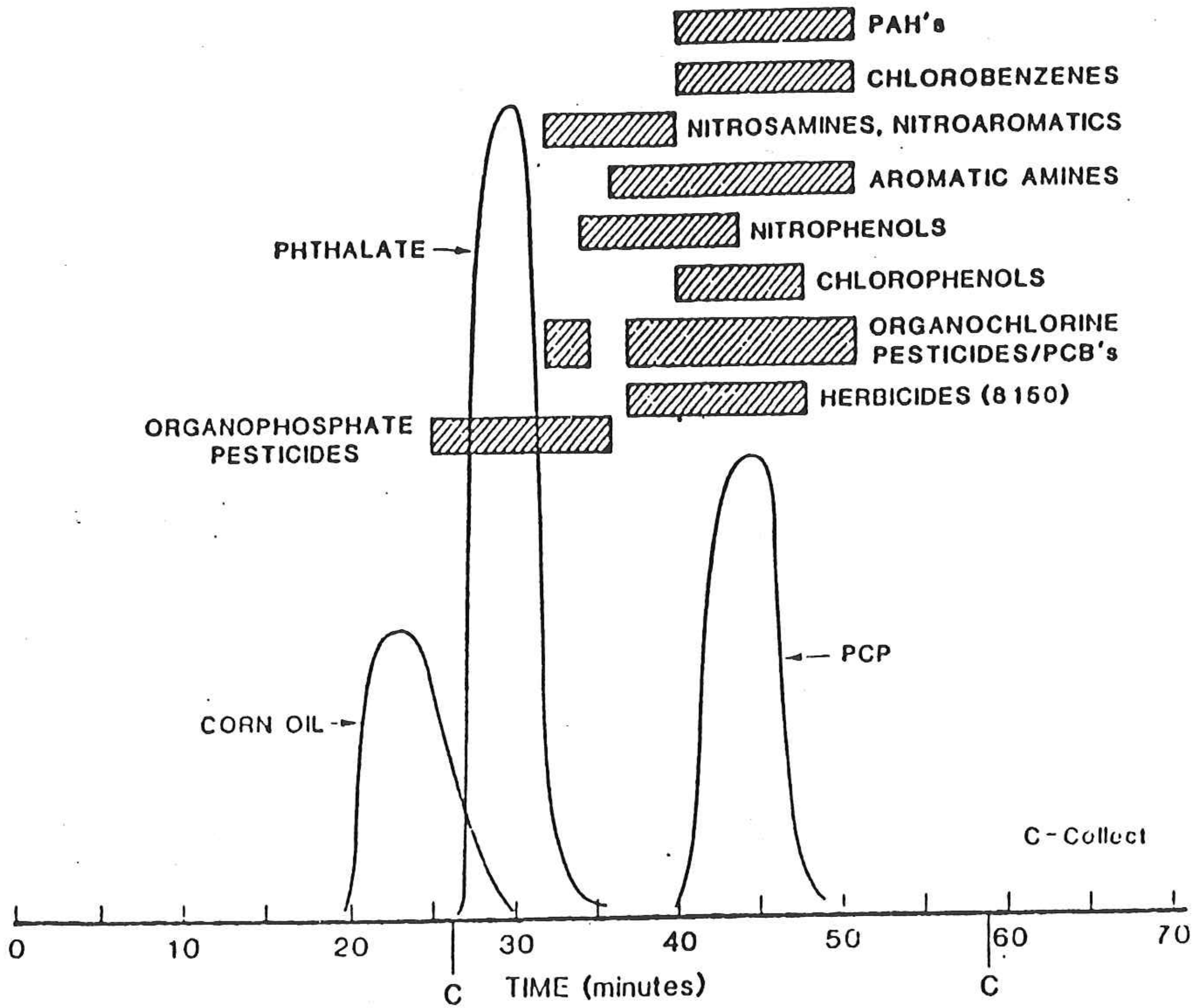
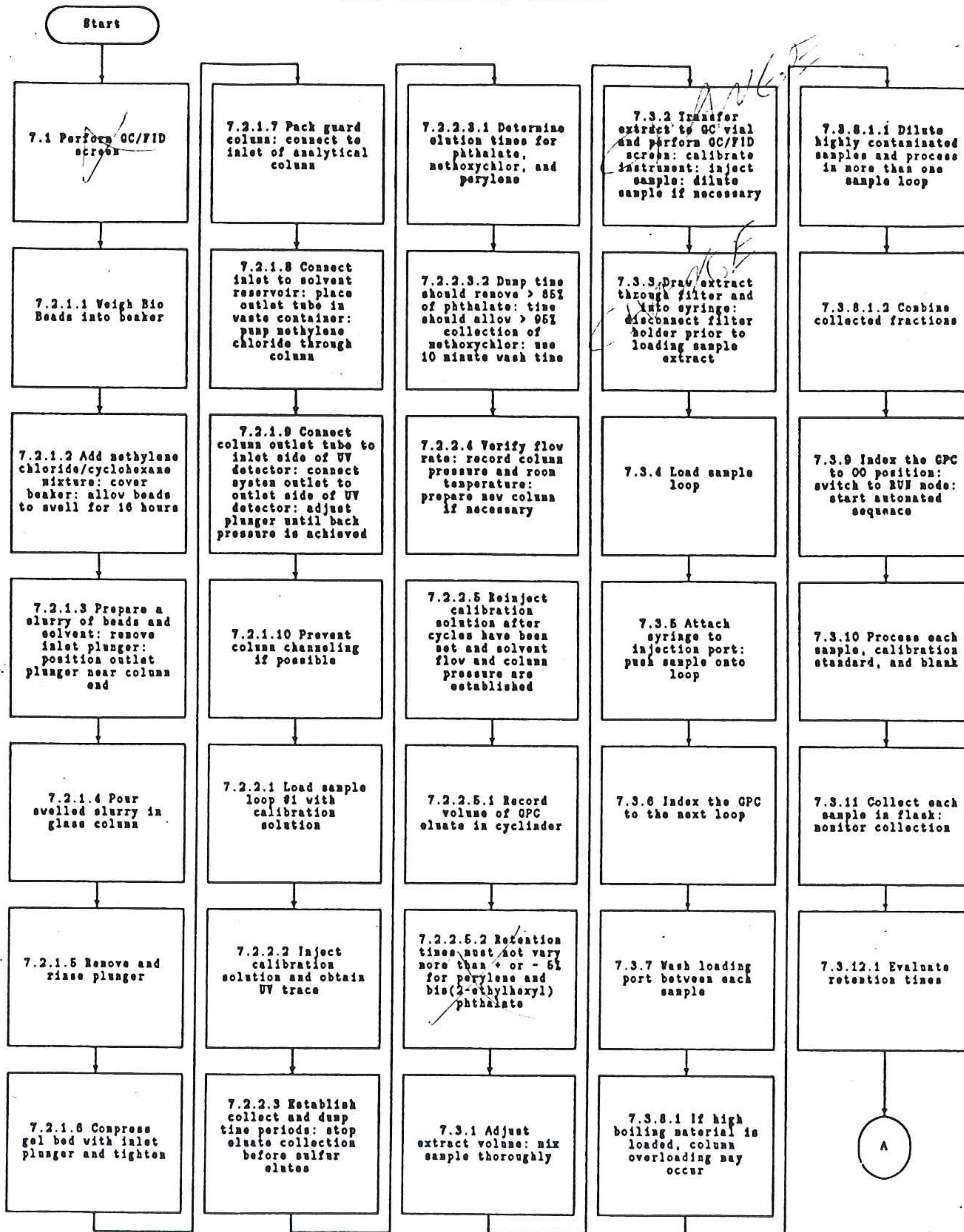
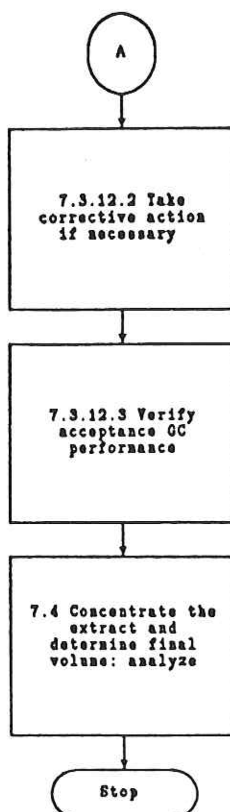


FIGURE 2.
GPC RETENTION VOLUME OF CLASSES OF ANALYTES



METHOD 3640
GEL-PERMEATION CLEANUP




A
7.3.12.2 Take corrective action if necessary
7.3.12.3 Verify acceptance QC performance
7.4 Concentrate the extract and determine final volume: analyze
Stop