



## Automated Gel Permeation Chromatography (GPC) Clean-up of Soil Extracts Prior to Analysis for Semivolatile Organic Compounds by GC/MS (USEPA Method 8270)

**Keywords:** Automated GX-271 GPC Clean-up System, EnviroSep-ABC™ GPC sample clean-up column, GC/MS, Gel Permeation Chromatography (GPC), Semivolatiles, Soil, TRILUTION® LC, USEPA Method 3545, USEPA Method 3640A, USEPA Method 8270

*This study was performed in collaboration with CT Laboratories of Baraboo, WI, USA.*

### **Introduction**

Gel permeation chromatography (GPC) is a size-exclusion clean-up procedure that readily separates high molecular weight interferents from sample extracts. The procedure uses organic solvents and a porous hydrophobic gel (primarily a cross-linked divinylbenzene-styrene copolymer) that readily separates large molecular weight molecules from the smaller molecular weight analytes of interest. GPC clean-up is recommended for the elimination of lipids, polymers, copolymers, proteins, natural resins, cellular components and other high molecular weight compounds from a sample extract.

GPC clean-up is used extensively for numerous environmental analysis, especially for preparing sample extracts prior to semivolatile, pesticide, PCB, polyaromatic hydrocarbons (PAHs), dioxins and dibenzofuran analysis by gas chromatography (GC) or GC/MS. GPC clean-up is efficient at removing high boiling point materials that can condense in the injection port area of a GC or in the front of a GC column. GPC clean-up protects GC columns, improves accuracy, reduces GC maintenance costs and allows for lower detection limits. Laboratories that participate in the USEPA Contract laboratory Program (CLP) or are following CLP protocols perform GPC clean-up according to USEPA Method 3640A.

The Gilson GX-271 GPC Clean-up System shown in Figure 1 will readily automate the GPC clean-up process. The system is modular in design and utilizes an autosampler for injecting a sample extract via the GX-Direct Inject Module onto a GPC clean-up column. The eluent of interest is then collected into the appropriate collection vessel via a collection valve mounted directly on the Z-arm of the autosampler. The system is controlled by PC using Gilson TRILUTION LC software with pre-programmed, easy-to-use GPC Methods that meet USEPA and many other regulatory guidelines.



Figure 1. Automated GX-271 GPC Clean-up System with 112 UV Detector

This application note evaluates the Gilson Automated GX-271 GPC Clean-up System to perform the post-extraction clean-up of soil extracts prior to analysis for semivolatile organic compounds. Semivolatile organic compounds or SVOCs is a general term for solvent extractable organic compounds that can be determined by GC/MS. It includes chemical classes such as polyaromatic hydrocarbons (PAHs), phthalates, nitrosamines, cyclic ketones and nitroaromatics. Semivolatiles were extracted from soil using pressurized fluid extraction (also called accelerated solvent extraction or ASE) via USEPA Method 3545. The extracts were then purified using GPC clean-up. Analysis was performed by GC/MS. Recoveries for a variety of semivolatiles were determined.

## **Experimental**

### *Materials*

All reagents were pesticide grade or higher. All chemicals were ACS grade quality. GPC calibration standards were prepared according to USEPA Method 3640A and contained corn oil, bis (2-ethylhexyl) phthalate, methoxychlor, perylene and sulfur. Commercial GPC calibration mix standards were obtained from Restek Corporation (Bellefonte, PA). Semivolatile stock standards and surrogate standards were obtained from Restek Corporation (Bellefonte, PA) or Perkin Elmer (Waltham, MA). Dilutions were prepared in dichloromethane or methanol.



#### *Soil Extraction*

Soil extraction was carried out utilizing pressurized solvent or accelerated solvent extraction (ASE) per USEPA Method 3545. Ten grams of soil was placed in a beaker and 2.5 mL of diatomaceous earth were added to the sample and then transferred to an extraction cell. Appropriate matrix spikes and matrix spike duplicate was added to each sample batch. The samples were then extracted using a Dionex ASE 200 with the following conditions:

Oven temperature = 100 degrees C; Pressure = 1500psi

Static Time = 5 minutes; Heat = 5 minutes

Flush Volume = 60%; Solvent A = 100%

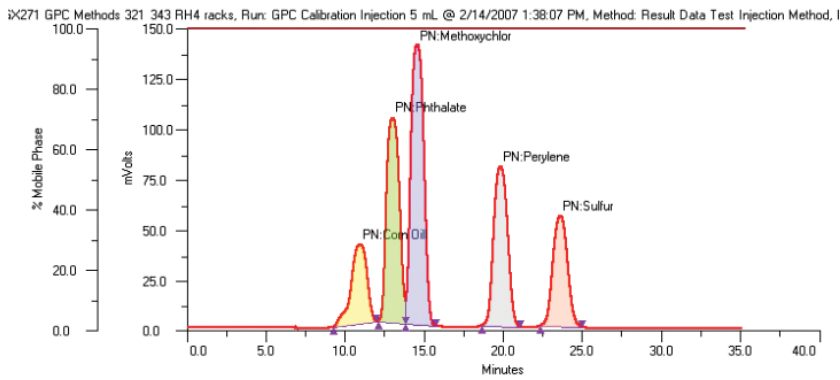
Nitrogen purge = 60 seconds at 150psi

Extraction fluid = 1:1 dichloromethane:acetone

The sample extracts were then concentrated using Kuderna-Danish (K-D) apparatus and then reconstituted in dichloromethane prior to GPC clean-up.

#### *GPC Clean-up*

GPC clean-up was achieved using a Gilson (Middleton, WI) Automated GX-271 GPC Clean-up System equipped with a Phenomenex (Torrance, CA) EnviroSep-ABC™ GPC Sample Clean-up column with guard column. The system used a 5 mL sample loop and a flow rate of 5 mL/min with dichloromethane as the mobile phase. Column calibration profiles were recorded using TRILUTION LC software and a Gilson Model 112 UV Detector set at 254nm. The GPC clean-up column was calibrated using the method outlined in USEPA Method 3640A. The column flow rate was verified by collected the eluate in a graduated cylinder for 10 minutes and measuring the volume. The elution times for the corn oil, bis (2-ethylhexyl) phthalate, methoxychlor, perylene and sulfur were determined (See Figure 2).



Sample Table

Injection Number	Sample Name	Sample Location	Fraction Site(s)	Peak Name	Retention Time (min)	Area (uVmin/100)	Height (mV)	Front Resolution %	Back Resolution %
1	Sample	Sample Zone,73,Reservoir,-1	<NONE>	Corn Oil	10.9771	4510346.6667	39.0269	0	93.54
1	Sample	Sample Zone,73,Reservoir,-1	<NONE>	Phthalate	13.0603	9494656.2716	101.6768	93.54	85.61
1	Sample	Sample Zone,73,Reservoir,-1	<NONE>	Methoxychlor	14.6066	13177787.6885	139.1887	85.61	245.64
1	Sample	Sample Zone,73,Reservoir,-1	<NONE>	Perylene	19.8885	7983740.0291	79.4326	245.64	150.05
1	Sample	Sample Zone,73,Reservoir,-1	<NONE>	Sulfur	23.6771	5779671.3132	54.9145	150.05	0

Figure 2. Chromatogram of USEPA Method 3640A Calibration Standard using a Phenomenex EnviroSep-ABC GPC sample Clean-up Column. This information allowed for the determination of appropriate collection times for the analytes of interest (See Figure 3). Column eluate collection was initiated just before the elution of bis (2-ethylhexyl) phthalate and after elution of the corn oil. Fraction collection was stopped after perylene elution but before sulfur elution. The collected fraction was then concentrated by K-D and reconstituted in appropriate solvent for GC/MS analysis.

Method Name	#Sample Well	#inj vol(uL)	#max tube collect vol(uL)	#fcstart1(min)	#fcstop1(min)	#run time end(min)	#End Equil Time(min)
1 Start Method							0.000
2 Rinse Method							
3 Semivolatle Method	2	5000.000	69000.000	12.000	22.000	0.000	
4 Semivolatle Method	3	5000.000	69000.000	12.000	22.000	0.000	
5 Stop Method							

Figure 3. TRILUTION LC GPC Sample List. The user can choose the method of interest and fraction collection variables for each sample.



### GC/MS Analysis

Semivolatile organic compounds were analyzed by GC/MS using an Agilent 6890 GC with 7683 Autosampler and 5973 MSD. Separation was achieved using a J&W Scientific DB 5.625, 30mm x 0.25mm x 0.25um column. The carrier gas was Helium at a flow rate of 1.2 mL/minute. 0.5 uL of sample was injected in pulsed splitless mode with an injector temperature of 250 degrees C. The MS conditions were as follows:

MS Interface = 280 degrees C

MS Source = 230 degrees C

Mass Range = 35 – 500 amu

Scan Time = .317 sec/scan

### Results

Table 1. Recoveries of semivolatiles from soil samples using pressurized fluid extraction and GPC clean-up. Samples were run on two separate days with n=7.

Compound	Recovery (%) 0.1 mg/kg	Acceptable Soil Recovery Limits (%)
N-Nitrosodimethylamine	47.2	20 - 115
Pyridine	4.7	1 - 114
Aniline	21.2	1 - 81
Bis (2-chloroethyl) ether	68.6	40 - 105
Phenol	73.2	40 - 100
2- Chlorophenol	70.2	45 - 105
1,3 - Dichlorobenzene	65.5	40 - 100
1,4 - Dichlorobenzene	66.3	35 - 105
1,2 - Dichlorobenzene	64.1	45 - 95
Benzyl alcohol	54.0	20 - 125
Bis (2-chloroisopropyl)ether	72.0	20 - 115
2-Methylphenol	72.4	40 - 105
N-Nitrosopyrrolidine	85.2	52 - 119
Acetophenone	82.9	43 - 127
Hexachloroethene	60.1	35 - 110
N-Nitroso-di-n-propylamine	66.5	40 - 115
3 & 4 - Methylphenol	71.0	40 - 105
Nitrobenzene	68.4	40 - 115
Isophorone	70.8	45 - 110
2 - Nitrophenol	67.6	40 - 110
2,4 - Dimethylphenol	53.4	30 - 105
Bis (2-chlorethoxy)methane	69.5	45 - 110
2,4 - Dichlorophenol	71.1	45 - 110

1,2,4 – Trichlorobenzene	63.3	45 – 110
Benzoic acid	14.4	0 – 110
Napthalene	65.0	40 – 105
4 – Chloroaniline	31.5	10 – 95
2, 6 – Dichlorophenol	72.1	40 – 131
Hexachloropropene	66.1	1 – 158
Hexachlorobutadiene	65.4	40 – 115
4- Chloro 3 – methylphenol	67.7	45 – 115
2 – Methylnapthalene	74.0	45 – 105
1- Methylnapthalene	73.0	70 – 130
Hexachlorocyclopentadiene	78.6	1 – 141
1,2,4,5 – Tetrachlorobenzene	66.3	51 – 117
2,4,6 – Trichlorophenol	63.9	45 – 110
2,4,5 – Trichlorophenol	75.4	50 – 110
2 – Chloronapthalene	70.9	45 – 105
2 – Nitroaniline	73.6	45 – 120
Acenaphthylene	69.8	45 – 105
Dimethylphthalate	72.4	50 – 110
2,6 – Dinitrotoluene	73.2	50 – 110
Acenaphthene	63.6	45 – 110
3 – Nitroaniline	47.5	25 – 110
2, 4 – Dinitrophenol	50.4	15 – 130
Dibenzofuran	64.2	50 – 105
2, 4 – Dinitrotoluene	76.4	50 – 115
4 – Nitrophenol	70.9	15 – 140
2,3,4,6 – Tetrachlorophenol	94.2	70 – 130
Fluorene	65.3	40 – 115
4-Chlorophenyl-phenyl ether	65.9	45 – 110
Diethyl phthalate	92.9	59 – 119
4-Nitroaniline	57.3	35 – 115
4,6 –Dinitro-2-methylphenol	70.2	30 – 135
N-Nitrosodiphenylamine & diphenylamine	65.2	50 – 115
Azobenzene & 1,2 – Diphenyl hydrazine	66.4	62 – 104
4 – Bromophenyl – phenyl ether	64.6	45 – 115
Hexachlorobenzene (HCB)	64.9	45 – 120
Pentachlorophenol	69.5	25 – 120
Phenanthrene	69.0	50 – 110
Anthracene	66.7	55 – 105
Carbazole	72.3	45 – 115



Di - n - butylphthalate	100.4	55 - 130
Fluoranthene	69.4	55 - 115
Benzidine	8.6	6 - 12
Pyrene	69.1	45 - 125
Butyl benzyl phthalate	80.8	50 - 125
3,3' - Dichlorobenzidine	74.6	10 - 130
Benzo (a) anthracene	72.5	50 - 110
Chrysene	69.0	55 - 110
Bis (2-ethylhexyl) phthalate	92.3	45 - 125
Di -n- octylphthalate	79.8	40 - 130
Benzo (b) fluoranthene	73.4	45 - 125
Benzo (k) fluoranthene	73.4	45 - 125
Benzo(a) pyrene	69.4	50 - 110
Indeno (1,2,3-cd) pyrene	59.4	25 - 135
Dibenzo (a,h) anthracene	59.6	40 - 125
Benzo (g,h,i) perylene	57.8	40 - 125

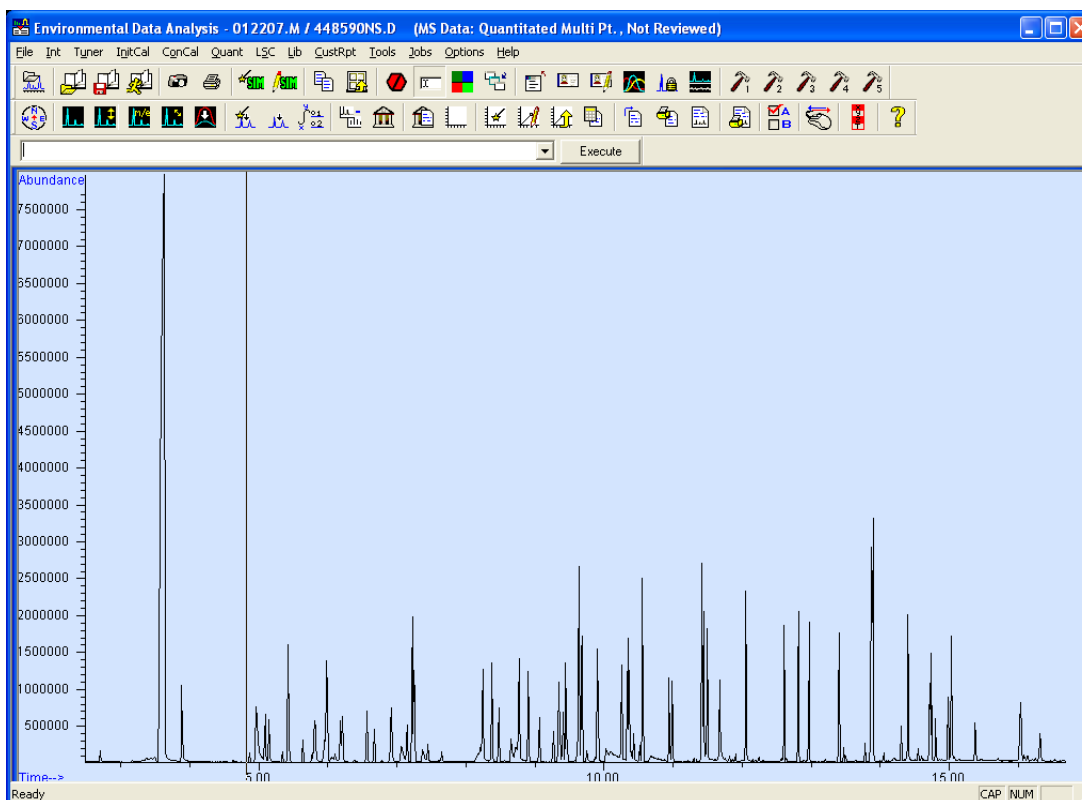


Figure 4. Chromatogram (GC/MSD) of a soil sample that has been spiked with the semivolatile organic compounds listed in Table 1. No GPC clean-up was performed. Note the fronting and tailing of peaks between 8 and 12 minutes.

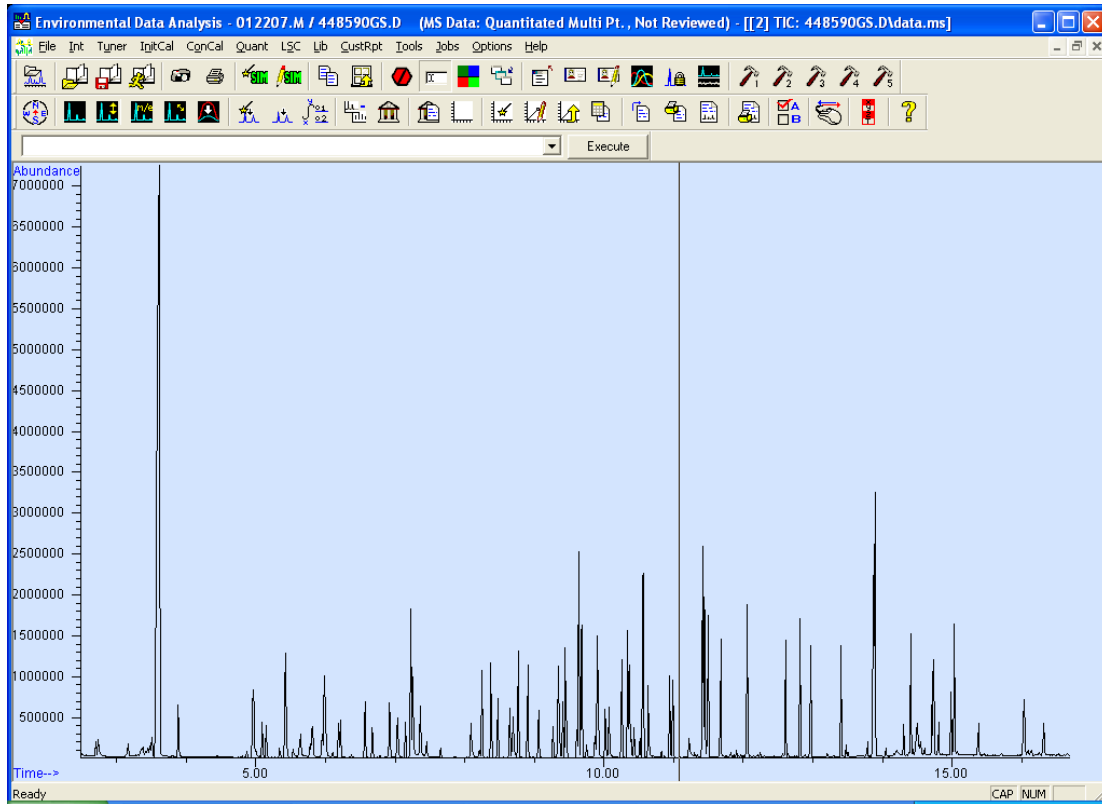


Figure 5. Chromatogram (GC/MSD) of a soil sample that has been spiked with semivolatile organic compounds listed in Table 1. GPC clean-up was performed. Note the improved resolution of peaks between 8 to 12 minutes compared to the chromatogram in which no GPC had been performed (Figure 4).

## **Conclusions**

The use of GPC post-extraction clean-up improved peak resolution and reduced the presence of fronting and tailing for the analysis of semivolatiles from soils. Recovery data was adequate for semivolatile analysis and all compounds were within acceptable recovery limits. The use of GPC clean-up also reduced maintenance costs for the GC/MS systems since less contaminants were able to accumulate in the injection port area or on the front end of the column.





### **References**

USEPA Method 3640A. Gel-Permeation Cleanup. Revision 1, September 1994.

Available at

<http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3640a.pdf>

USEPA Method 8270D. Semivolatile Organic Compounds by Gas

Chromatography/Mass Spectrometry (GC/MS). Revision 4, February 2007. Available at

<http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/8270d.pdf>