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Performing EPA Method 3640A on the PrepLinc™ GPC Cleanup System

Application Note 130

Introduction

Gel Permeation Chromatography (GPC) is a size exclusion cleanup procedure using organic solvents and hydrophobic gels in the separation of synthetic macromolecules. 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 larger than the molecular size of the molecules to be separated. A cross-linked divinylbenzene-styrene copolymer (SX-3 Bio Beads or equivalent) is specified for this method.

General cleanup - 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. GPC is appropriate for both polar and non-polar analytes; therefore, it can be effectively used to cleanup extracts containing a broad range of analytes.

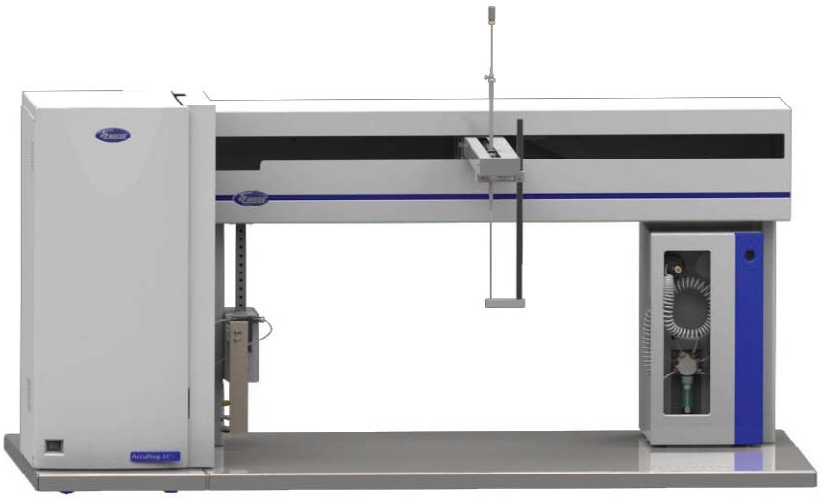
In this document we will demonstrate how the EPA Method 3640A for GPC Cleanup can easily be performed on the PrepLinc™ GPC Cleanup system.

Instrumentation

Instrumentation consists of a J2 Scientific PrepLinc™ system including:

- ✓ GPC Cleanup module with 5mL Sample Loop*
- ✓ AS4 Autosampler module
- ✓ 100% DCM Traditional Glass Column – J2 P/N CO100
- ✓ 254 nm UV Detector
- ✓ PrepLinc™ Operating Software.

*Other sizes available.

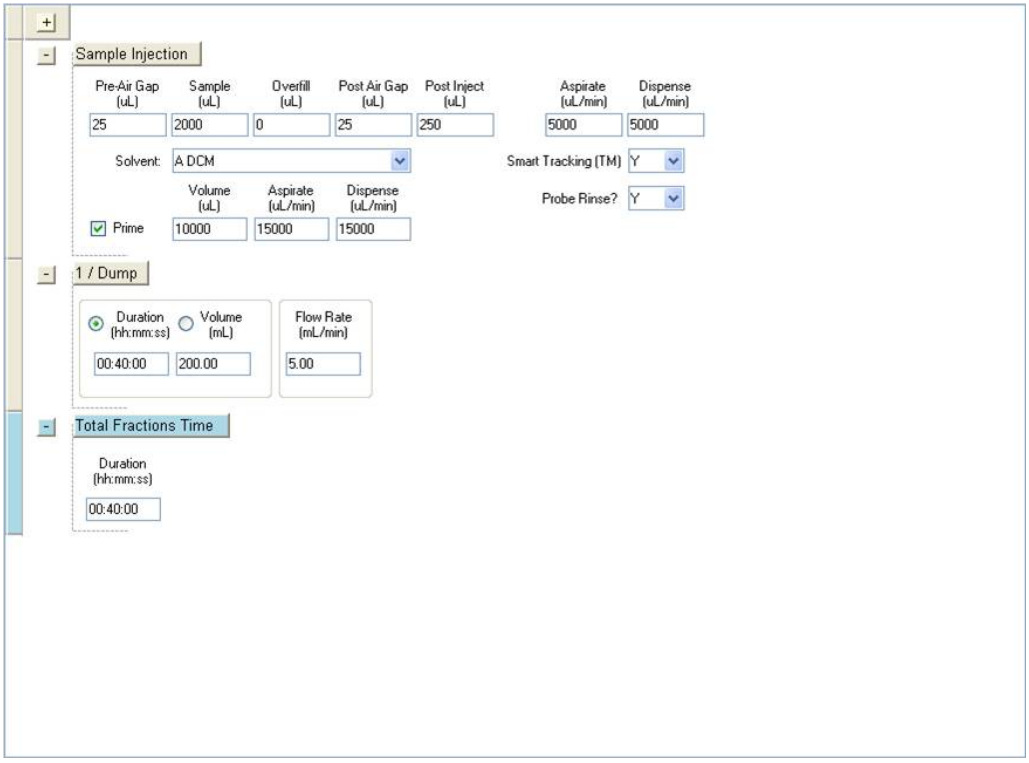


GPC Column Calibration with UV Detector

A UV detector is a useful tool in determining the elution profile for this application. Following the requirements for EPA 3640A, a Method was created using the PrepLinc™ Operating Software for the GPC cleanup system. The software method includes the following key parameters:

- ✓ 5 mL injection of EPA Calibration Solution onto the sample loop
- ✓ 5 ml/min flowrate through a DCM Column
- ✓ Total elution time through the column of 60 minutes
- ✓ Data collection using a 254 nm UV Detector and PrepLinc™ Software
- ✓ EPA Calibration Solution, J2 Scientific part # ST077-1

With this method, the calibration solution will flow through the column and UV detector to obtain a UV trace showing a discrete peak for each component.



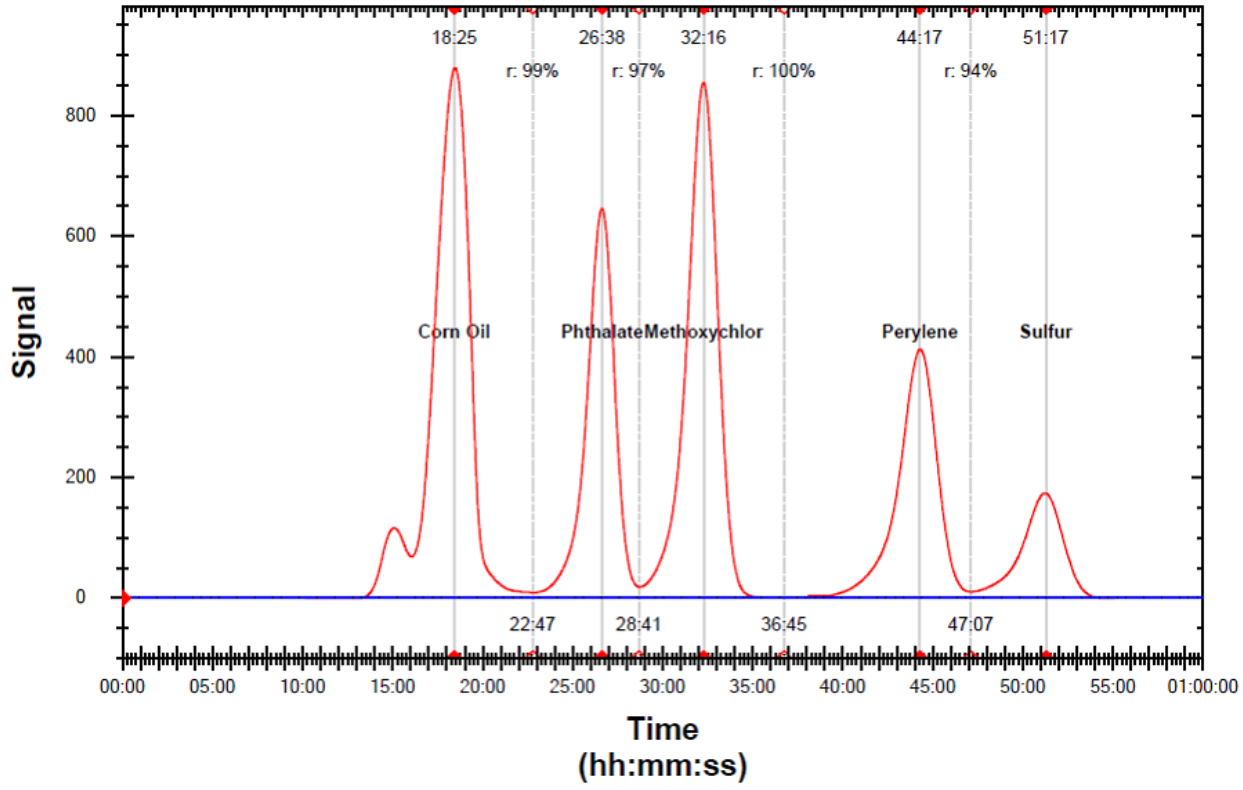
GPC Cleanup Method for EPA 3640A Column Calibration

Evaluation of UV Calibration Data

The method above was performed on the PrepLinc™ GPC Cleanup system, automatically injecting the Calibration Solution for processing through the GPC Cleanup column. The Calibration Solution contains the following analytes (in elution order):

Compound	**mg/L**
corn oil	*25,000*
bis(2-ethylhexyl) phthalate	*1,000*
methoxychlor	*200*
perylene	*20*
sulfur	*80*

Next it is necessary to evaluate the chromatogram to see if it meets or exceeds the EPA 3640 method requirements. The sample date can be opened for viewing in the software. The view of the chromatogram displays the compounds, retention times, and resolution of the compounds. The resolution and retention information is calculated using the EPA method requirements and is stored in a template.



The EPA method requires the following criteria for evaluating the UV chromatogram to determine column condition for the EPA Method.

- Peaks must be observed, and should be symmetrical, for all compounds in the calibration solution.
- Corn oil and phthalate peaks must exhibit >85% resolution.
- Phthalate and methoxychlor peaks must exhibit >85% resolution.
- Methoxychlor and perylene peaks must exhibit >85% resolution.
- Perylene and sulfur peaks must not be saturated and must exhibit >90% baseline resolution.

The calibration report below was generated from the PrepLinc™ reporting feature by selecting the appropriate sample from the stored data. The report automatically displays information required for the method and gives the operator pass fail criteria based on the method requirements (circled in red below). This Column Calibration easily passed the method requirements.

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================================================================================
CALIBRATION REPORT
================================================================================
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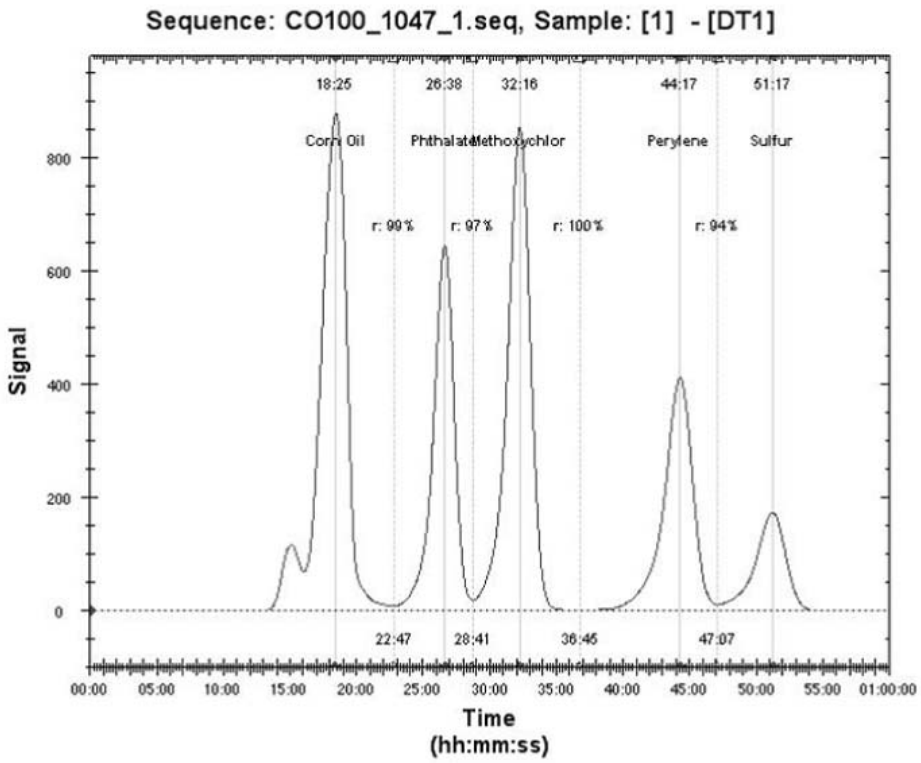


```
TEMPLATE NAME                    : CO100

PEAK  SAMPLE                     RET. TIME  SIGNAL

1     Corn Oil                   18:25      880
2     Phthalate                  26:38      647
3     Methoxychlor               32:16      856
4     Perylene                   44:17      413
5     Sulfur                     51:17      174

VALLEY  PEAKS                                RES.  LIMIT  P/F

1       Corn Oil/Phthalate                   99    85     P
2       Phthalate/Methoxychlor               97    85     P
3       Methoxychlor/Perylene                100   85     P
4       Perylene/Sulfur                      94    90     P
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Calculated resolution (**RES.**) is compared to the user-entered method criteria (**LIMIT**) and Pass/Fail is reported automatically

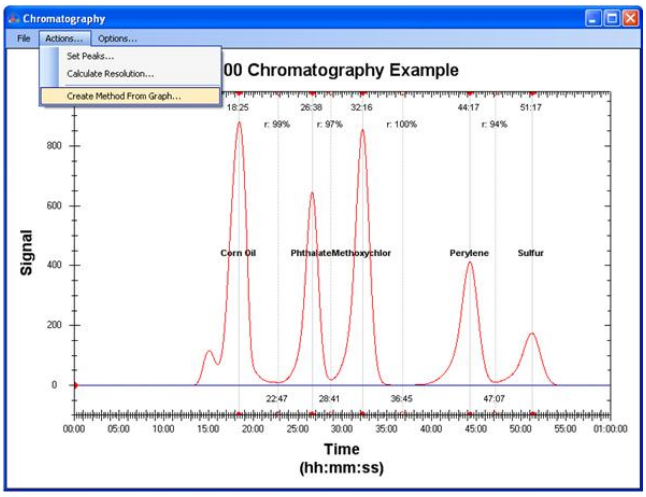
Using UV Calibration for Method Creation

After the column calibration data is reviewed and the column passes the requirements, there are 2 types of analyte groups that can be programmed for using the information: Semivolatiles and Pesticides/PCBs. The main difference between the two applications when performing GPC Cleanup is the whether to retain (Collect) or discard (Dump) the phthalate fraction following Corn Oil.

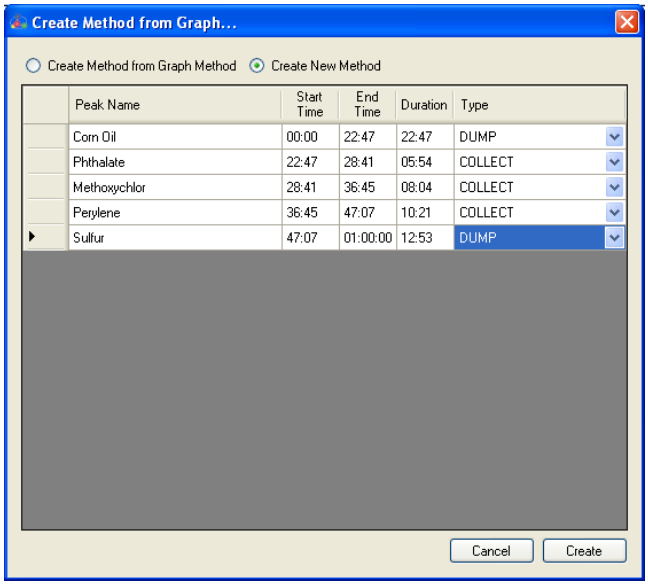
Calibration for Semivolatiles - Initiate column eluate collection just before elution of bis(2-ethylhexyl) phthalate and after the elution of the corn oil. Stop eluate collection shortly after the elution of perylene. Collection should be stopped before sulfur elutes. Use a "Wash Time" of 10 minutes after the elution of sulfur.

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, but collects >95% of the methoxychlor. Stop collection after the elution of perylene, but before sulfur elutes.

In the following example we will create a method for the Semivolatiles application.



Annotate the chromatogram, dragging the resolution lines to the desired cut points as described above for the desired analytes. From the chromatography window, select “Actions”, then “Create Method from Graph”.

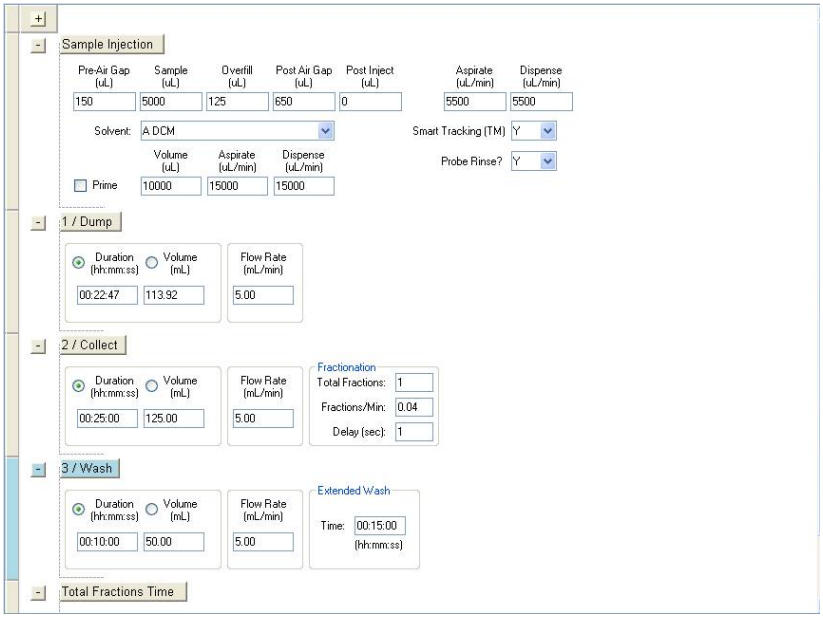


Select the compounds you wish to dump and those you wish to collect.

This will vary depending on whether you are running a calibration for Semivolatiles or for Organochlorine Pesticides/PCBs. Since we are building a method for Semivolatiles we will “Dump” the Corn Oil and Sulfur and “Collect” the Phthalate through Perylene fractions.

Then click “Create”.

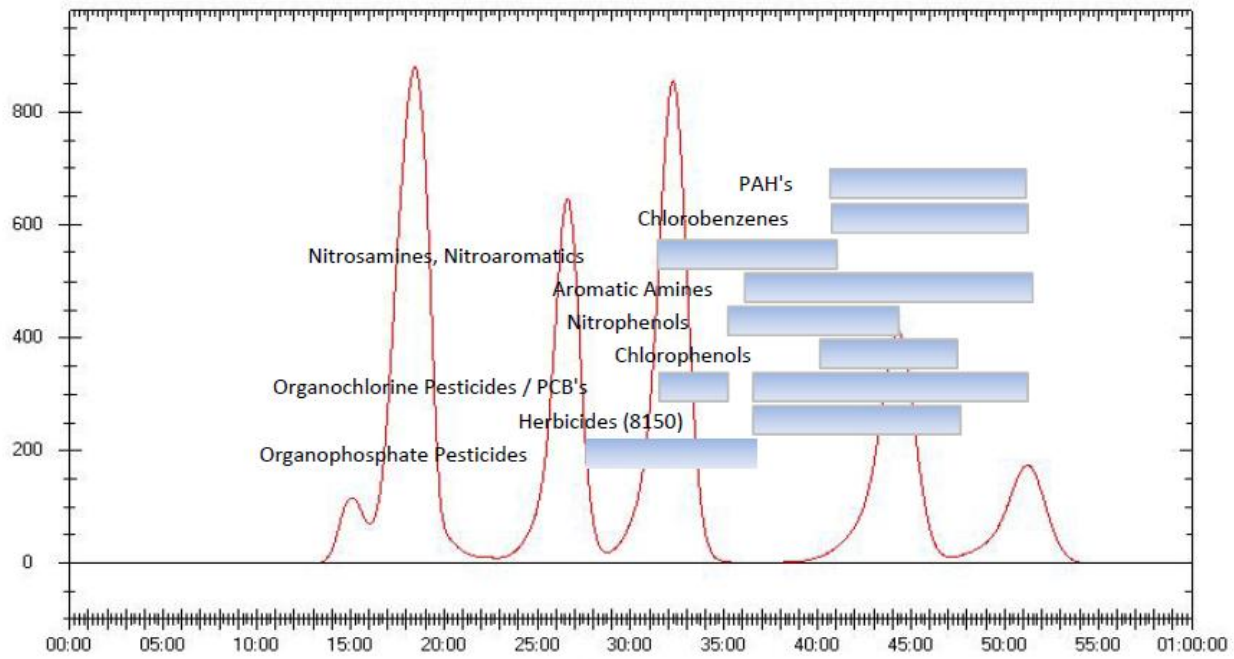
Note: The image shown is for Semivolatiles. For Pesticides/PCBs you would select “Dump” for Phthalate in place of “Collect”.



After you select “Create”, the GPC Method Editor is automatically opened with the times and fractions for “Dump” and “Collect” from the window above. Simply make any required adjustments for other parameters and “Save” your new method. You are now ready to use the method for sample processing.

GPC Column Retention Volumes for Classes of Analytes

We have included for reference the following chart showing approximate retention times for classes of analytes. It can be useful in creating methods for classes of analytes and troubleshooting chromatography problems. Actual retention times may vary by columns and other chromatography factors**.**



Conclusions

The J2 Scientific PrepLinc™ GPC cleanup system has standard features for easy GPC Cleanup column calibration and method programming per the specifications of US EPA Method 3640A. With the system you can easily calibrate the column and create methods for both analyte groups. The system includes reporting features specific to the application but also includes feature useful for a wide variety of GPC Cleanup applications.

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