

ENVIRONMENTAL ANALYSIS

AN AUTOMATED SYSTEM FOR THE ROUTINE CLEANUP OF ENVIRONMENTAL SAMPLES PRIOR TO INSTRUMENT ANALYSIS



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Introduction

The Clean Seas Environmental Monitoring program (CSEMP [1]) is an initiative designed to monitor the levels of chemical contamination of sediments and shellfish in inshore waters. The program specifies 16 organo-chlorine compounds (OCPs), 28 polycyclic aromatic hydrocarbons (PAHs) and 7 polychlorinated biphenyl ethers (PCBs).

Due to the varying nature of the samples submitted for analysis, and the presence of interfering compounds, extensive yet consistent sample clean-up is essential. Samples are extracted using Accelerated Solvent Extraction (ASE).

Experimental

Mussel tissue samples submitted to the laboratory were depurated and homogenised before being extracted using ASE. The resulting extracts were then prepared for GPC analysis. The fraction collected from the GPC was further cleaned-up using Solid Phase Extraction before final analysis by GC/MS.

Typical Agilent GPC Clean-Up Conditions

GPC System: Agilent 1260 Infinity Series Pump, Auto-sampler, VWD and Fraction Collector (with cooling)
Column: Agilent EnviroPrep 300 x 25 mm (p/n PL1210-6120EPA)
Guard Column: Agilent PLgel Prep Guard 25 x 25 mm (p/n PL1210-1120)
Mobile Phase: 100% Dichloromethane
Flow Rate: 5 mL/min
Run time: 35 min
Injection Volume: 3600 µL (900 µL x 4)
Fraction Collection Time: 17.70 min – 27.20 min
Fraction Collection Volume: 47.5 mL
Wavelength: 254 nm

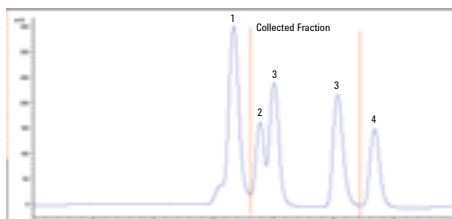
The GPC is calibrated using a Restek EPA mix (p/n 32041) containing compounds with varying molecular weight. To enable us to collect the target fractions whilst removing lipids, other macromolecules, and sulphur, a mix containing the following 5 test probes is used:

EPA Method Test Mixture

1. Corn Oil
2. Bis (2-ethylhexyl) phthalate
3. Methoxychlor
4. Perylene
5. Sulphur

This mixture of test samples is recommended for use by the USEPA method 3640A to enable the correct fractionation window to be established.

The elution order of these compounds is given in the following chromatogram.



Fraction collector is setup to collect the GPC eluent from approximately 18 min to 27 min i.e. after the corn oil fraction and before sulphur elutes from the column.

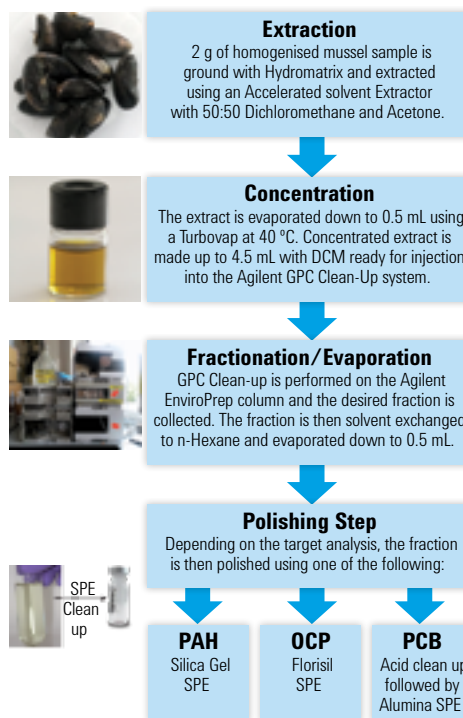
The sample extraction is non-specific and hence many other compounds are extracted along with the target molecules including macromolecules such as lipids in shellfish and elemental sulphur in marine sediments. Gel Permeation Chromatography (GPC) is a separation technique whereby compounds within a sample mixture are separated based on their molecular size, or their hydrodynamic volume. Large molecular weight compounds such as lipids elute early in the separation and are diverted to waste whereas smaller target molecules are retained in the GPC column and are collected. Since these compounds are relatively non-volatile, their

removal from the extract prior to GC/MS analysis of pesticides and other pollutants extends the lifetime of the GC column, and improves chromatographic efficiency. A mixture containing reference compounds with known molecular weights helps to evaluate the separation efficiency of the GPC column and together with a fraction collector facilitates the isolation of cleaned-up target molecules.

The extract is then further treated using Solid Phase Extraction and acid clean-up stages to remove polar and non-polar interferences before the final extracts are analysed by Gas Chromatography Mass Spectrometry.

Sample Preparation and Clean-Up

The process of sample preparation, fractionation, and collection can be shown through a flow chart below:



Final Chromatogram Showing analysis of the fully cleaned mussel extract using GC/MS

The final extract is analysed on an Agilent GCMS System in SIM mode. Using a PCB calibration standard we can overlay with the sample extract to quickly see evidence of target compounds.



TIC of Mussel tissue extract (Black) overlaid with PCB Calibration Standard (Red) (1.5 µg/kg level).

Conclusion

GPC has been used for many years in various forms for post-extraction sample clean-up, prior to analysis by HPLC, LC/MS, GC or GC/MS. The overall methodology is supported by the US EPA mandated procedures 3640 and 3640a, and is widely applicable to environmental samples such as sludge, soil, sediment, wastewater and biota matrices, together with a wide variety of applications in the food industry, such as animal fats, oils, plants, grasses and grains.

High performance gel permeation chromatography (GPC) provides a simple, convenient, yet important, clean-up procedure removing large molecules such as fats, oils, lipids and resins, together with sulphur, that typically interfere with subsequent analytical techniques such as LC/MS and GC/MS. GPC sample clean-up facilitates the improved separation and quantification of trace organic compounds at very low concentrations.

The Agilent GPC Sample Clean-Up system is a pre-configured combining Agilent Infinity 1260 Series HPLC modules and Agilent EnviroPrep Clean-Up columns. The system will deliver a reliable, tested solution for the clean-up of a variety of Environmental and Food samples.

Reference

1. Clean Seas Environmental Monitoring Program – GREEN BOOK, Marine Assessment and Review Group (MARG), UK.
2. EPA Method 3640A – Gel Permeation Cleanup.

www.agilent.com/chem/gpcresources

This information is subject to change without notice.

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