ENVIRONMENTAL ANALYSIS

AN AUTOMATED SYSTEM FOR THE ROUTINE CLEAN-UP OF ENVIRONMENTAL SAMPLES PRIOR TO INSTRUMENT ANALYSIS

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Markets and Applications Programs

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ABSTRACT

An automated system for the routine clean-up of environmental samples prior to instrument analysis has been developed. The overall methodology is supported by the US EPA mandated procedures 3640 and 3640A, and is applicable to a wide range of environmental samples. The Agilent GPC Sample Clean-Up system is pre-configured, combining Agilent Infinity 1260 Series HPLC modules and Agilent EnviroPrep Clean-Up columns. The system delivers a reliable, tested solution for the clean-up of a variety of environmental and food samples.

INTRODUCTION

The Clean Seas Environment Monitoring Programme (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 polyaromatic 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.

This solution note describes an automated system for the routine clean-up of environmental samples prior to instrumental analysis. Samples are extracted using Accelerated Solvent Extraction (ASE), before undergoing Gel Permeation Chromatography (GPC) Clean-Up using an Agilent EnviroPrep column. The collected fraction is then polished using Solid Phase Extraction (SPE) before finally being analysed.

ANALYTICAL TECHNIQUE

Sample Preparation and Clean-Up

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 SPE before final analysis by Gas Chromatography Mass Spectrometry (GC/MS).

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

<table>
<tr><td>  </td><td colspan="3">Extraction
2 g of homogenised mussel sample is ground with Hydromatrix and extracted using an Accelerated Solvent Extractor with 50:50 Dichloromethane and Acetone.</td></tr>
<tr><td></td><td colspan="3">⇣</td></tr>
<tr><td>  </td><td colspan="3">Concentration
The extract is evaporated down to 0.5 mL using a Turbovap at 40°C. Concentrated extract is made up to 4.5 mL with DCM ready for injection onto the Agilent GPC Clean-Up system.</td></tr>
<tr><td></td><td colspan="3">⇣</td></tr>
<tr><td>  </td><td colspan="3">Fractionation / Evaporation
GPC Clean-Up is performed on the Agilent EnviroPrep column and the desired fraction is collected. The fraction is then solvent exchanged to n-Hexane and evaporated down to 0.5 mL.</td></tr>
<tr><td></td><td colspan="3">⇣</td></tr>
<tr><td>  </td><td colspan="3">Polishing Step
Depending on the target analysis, the fraction is then polished using one of the following:</td></tr>
<tr><td></td><td>⇣</td><td>⇣</td><td>⇣</td></tr>
<tr><td>  </td><td>PAH
Silica Gel SPE</td><td>OCP
Florisil SPE</td><td>PCB
Acid Clean Up followed by Alumina SPE</td></tr>
</table>

INSTRUMENTATION

All experiments were carried out on an Agilent 1200 Infinity Series System, comprising:

Agilent 1260 Infinity Quaternary Pump (G1311B)
Agilent 1260 Infinity Standard Autosampler (G1329B) with Extended injection range (option #20) and multi-draw (option #21) *
Agilent 1260 Infinity Variable Wavelength (G1314B) UV Detector
Agilent 1260 Infinity Fraction Collector (G1364C)
Agilent 1290 Infinity Thermostat (G1330B) for cooling the fraction collector

*Agilent 1260 Infinity Prep Autosampler (G2260A) optional alternative

GPC Clean-Up Conditions

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

RESULTS & DISCUSSION

The GPC system was calibrated using the Restek EPA Test Mixture (p/n 32041) containing compounds with varying molecular weight. To ensure the target fractions were collected whilst removing lipids, other macromolecules and sulphur, the mixture containing the following 5 test compounds was used:

EPA Method Test Mixture	
Peak	**Compound**
1	Corn Oil
2	Bis (2-ethylhexyl) phthalate
3	Methoxychlor
4	Perylene
5	Sulphur

This mixture of test compounds is recommended for use by the USEPA method 3640A [2] to enable the correct fractionation windows to be established. The elution order of these compounds is shown in Figure 1.

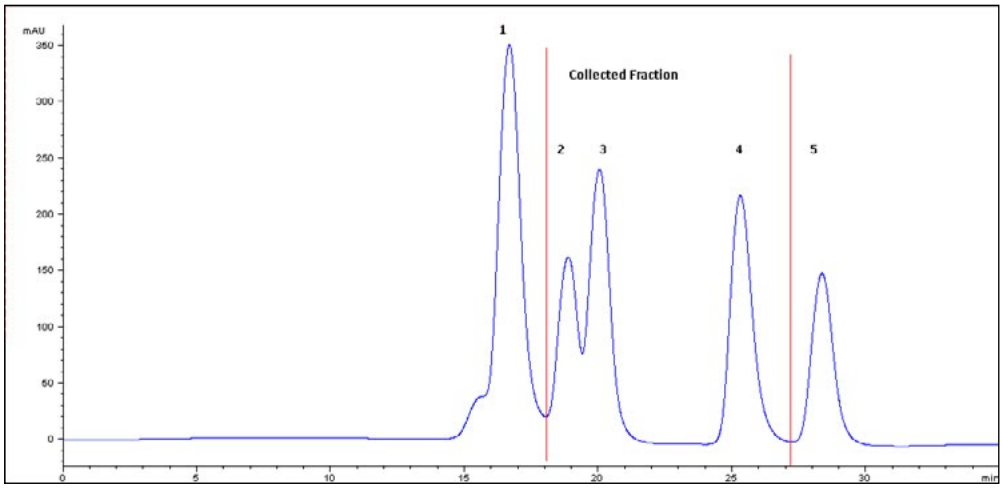

Figure 1. System calibration using the EPA Test Mixture.

The Fraction collector was 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. 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 target compounds all eluted in the fractionation window shown in Figure 2. High molecular weight material is removed by the clean-up process. Also, any residual sulphur present is retained by the GPC column so it elutes after the fraction 'window'. Removal of sulphur is important since it is a common interference in GC analysis.

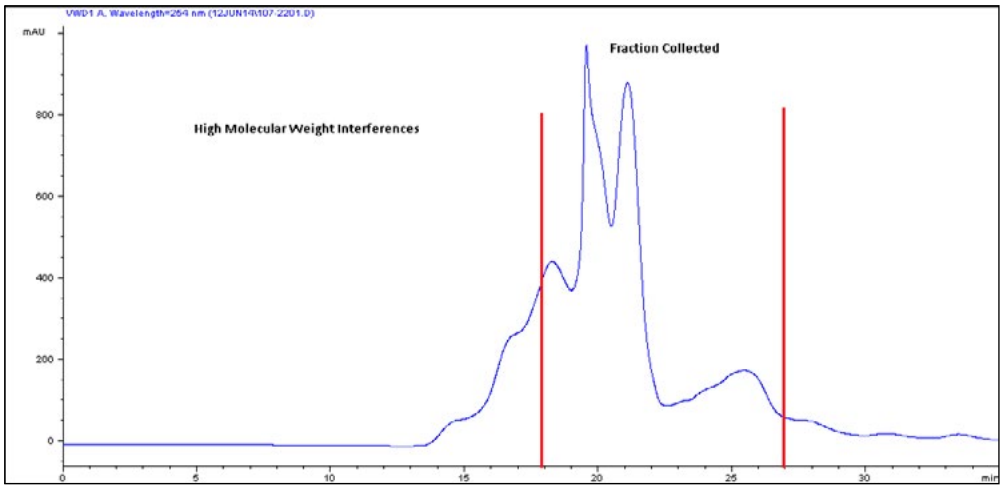

Figure 2. Chromatogram depicting the mussel extract from GPC.

The extract was then further treated using SPE and acid clean-up stages to remove polar and non-polar interferences before the final extract was analysed by GC/MS.

The final fully cleaned mussel extract was analysed on an Agilent GC/MS System in SIM mode. The chromatogram of the mussel extract is shown in Figure 3. A PCB calibration standard chromatogram is overlaid, showing evidence of the presence of target compounds in the mussel extract.

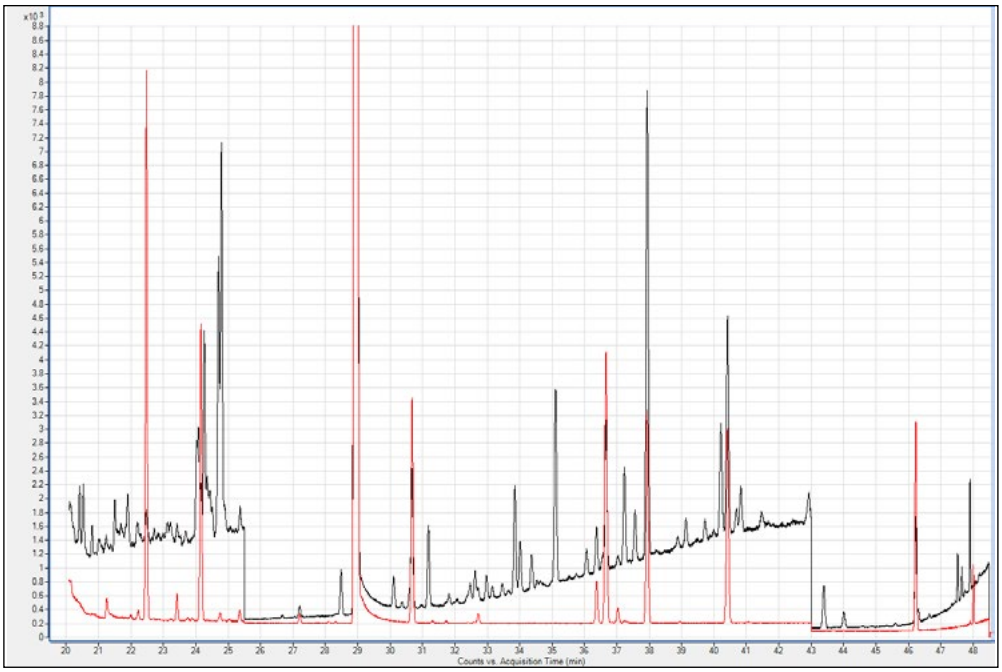

Figure 3. TIC of Mussel Tissue Extract (Black) overlaid with 1.5 µg/kg PCB Calibration Standard (Red).

CONCLUSIONS

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 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 quantitation of trace organic compounds at very low concentrations.

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

REFERENCES

1. Clean Seas Environment Monitoring Programme - GREEN BOOK, Marine Assessment and Review Group (MARG), UK.

2. EPA Method 3640A – Gel Permeation Clean-up.

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