GPC cleanup method for soil samples before PAHs analysis

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SUMMARY

This work is focused on the development of a clean-up method with the AZURA® GPC Cleanup system to purify soil sample extracts before analysis. It was found that GPC cleanup is very useful to remove interferences from extracted soil samples before GC-MS/MS determination of semi-volatile organic compounds, like Polycyclic Aromatic Hydrocarbons. Moreover, the described method enables to perform an automated clean-up procedure and hence to purify many samples efficiently.

INTRODUCTION

GPC (Gel Permeation Chromatography) is a size-exclusion clean-up procedure that readily separates high molecular weight interferents from sample extracts using organic solvents and a porous hydrophobic gel (primarily a crosslinked divinylbenzene-styrene copolymer) [1]. It is possible to distinguish between different types of Bio-Beads resin based on the type of cross-linkage. In this application Bio-Beads S-X3 with 200–400 mesh was used according to EPA method 3640 [1]. GPC clean-up can be used extensively in numerous environmental analysis especially for preparing sample extracts prior to semivolatile compounds determination, such as pesticide, and PAHs analysis by GC/MS or HPLC-UV-DAD. Sample cleanup is particularly important for analytical separations such as GC, HPLC, and electrophoresis because high-boiling materials can cause a variety of problems in analytical systems, like analyte adsorption in the injection port or in front of a GC or LC column [2]. GPC cleanup protects GC and HPLC columns, reduces analytical maintenance costs, improves accuracy, and allows lower detection limits.

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RESULTS

The calibration of the AZURA® GPC Cleanup System was performed with a calibration mixture in dichloromethane containing the following compounds also reported in EPA method 3640: corn oil, methoxychlor; phthalic acid, bis-2-ethylhexyl ester (ester of phthalic acid), perylene, and sulfur [1]. 1 mL calibration standard was diluted with 2 mL dichloromethane and 7 mL cyclohexane to esemble the mixture similar to the mobile phase for GPC. 2 mL solution were injected and calibration test was carried out for 60 min at a flow rate of 1 mL/min.

In **Fig. 1** the chromatogram of diluted calibration mixture solution is reported. According to 3640 EPA method, a reagent blank should be analyzed for the compounds of interest prior to the use of the clean-up method [1]. The level of interferences must be below the estimated quantitation limits of the analytes before the method is performed on samples. Using the information coming from the detector, it could be possible to establish appropriate collect time periods for target analytes. 3640 EPA method suggests to initiate column collection just before elution of bis-(2-ethylhexyl) phthalate, after the elution of the corn oil and to stop eluate collection shortly after the elution of perylene, in order to ensure semi-volatiles collection [1]. In particular, a recovery test was performed using PAH standard solution and it was observed that the proper collection time ranged from 18 to 45 min to ensure a good recovery efficiency for the analytes of interest. GPC cleanup method was successfully applied to different soil samples' extracts derived from the Environmental Chemistry Laboratory of the Department of Biology, University of Bari (separate branch of Taranto, Italy). After performing clean-up method on the selected samples, they were concentrated under nitrogen stream and ready to perform analytical determination.

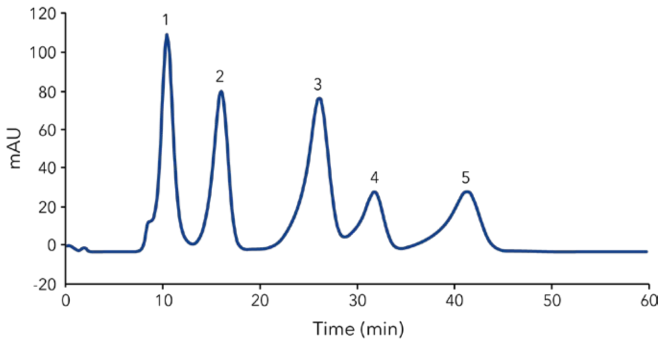


Fig. 1 Chromatogram of a diluted calibration mixture solution 1) Corn oil (5 mg/mL), 2) Phthalic acid, bis-2-ethylhexyl ester (1 g/L), 3) Methoxychlor (0.2 g/L), 4) Perylene (0.02 g/L), 5) Sulfur (0.08 g/L)

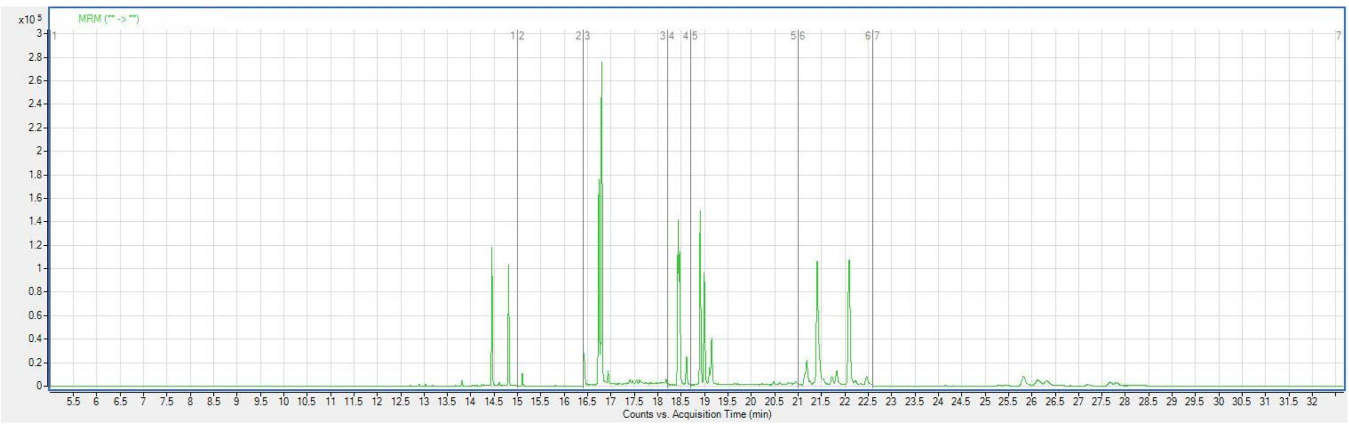


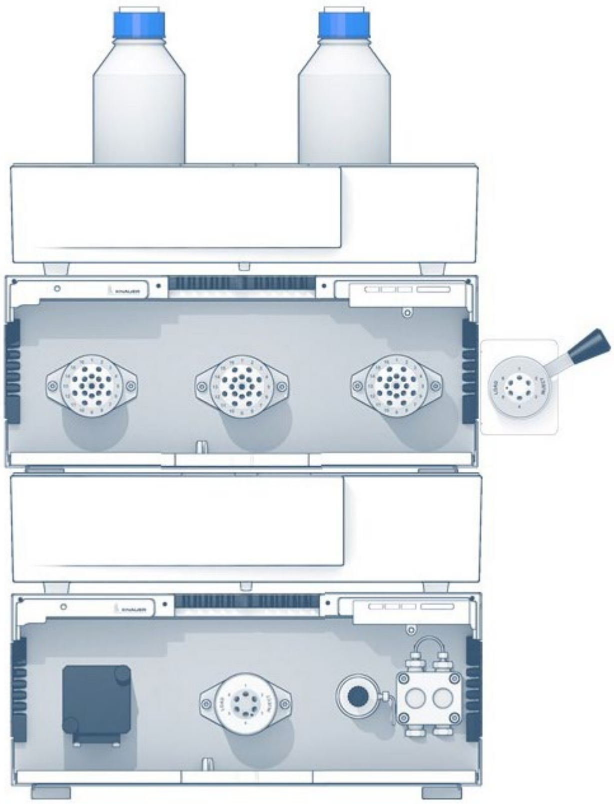
Fig. 2 GC-MS/MS chromatogram of purified soil sample: determination of PAHs

MATRIALS AND METHOD

Extraction of Polycyclic Aromatic Hydrocarbons was carried out using Accelerated Solvent Extraction (ASE), according to the 3545A EPA method [3]. Bio-Beads S-X are supplied dry and must be swollen prior to pack into a chromatographic column. A mixture of cyclohexane and dichloromethane (70:30, v:v) is suitable for clean-up of soil samples. As a general rule, the beads should be swollen in the same solvent chosen as mobile phase, so 10 g of Bio-Beads S-X3 were swelled with 50 mL of cyclohexane and dichloromethane mixture (70:30, v:v) overnight. After the beads were fully swollen, they were packed into a chromatographic column. Before sample cleanup, GPC column was equilibrated with the desired solvent mixture, flushing it almost three times the column volume at 1 mL/min. Cleanup method was performed with AZURA® GPC Cleanup System, which is operated with the Mobile Control Chrom® Software running on a tablet directly mounted on the system. The identification and quantification of PAHs was carried out by GC-MS/MS analysis. Calibration curves were constructed in the concentration range from 10 µg/L to 130 µg/L. To perform GC separation and MRM acquisition, optimization of the best chromatographic and detection conditions was necessary.

CONCLUSION

This application shows how to perform GPC cleanup with KNAUER AZURA® GPC Cleanup System for the analysis of PAHs in soil samples. This report is very detailed to ensure good performance in GPC cleanup for application in environmental area. We can conclude that AZURA GPC Cleanup System is an helpful tool for sample preparation before instrumental analysis because, unlike other techniques, is very useful for the removal of high boiling materials which would contaminate injection ports and column heads, prolonging column life, stabilizing the instrument, and reducing column reactivity. It could be considered an universal cleanup technique for a broad range of semivolatile organics and pesticides. Moreover, AZURA GPC Cleanup System allows the customer to process many extracted samples, with a reduction in time for the cleanup procedures.



REFERENCES

[1] EPA Method 3640A: Gel-Permeation Cleanup

[2] J. D. Winefordner. Chemical analysis: a series of monographs on analytical chemistry and its applications. Vol. 162, p.21 -25

[3] EPA Method 3545A: Pressurized Fluid Extraction (PFE)

ADDITIONAL MATERIALS AND METHODS

Tab. A1 ASE Method parameters

Temperature	100°C
Pressure	1500 psi
Static time	5 min
Cycles	1
Purge	60 s
Solvent	Hexane/DCM (1:1, v:v)
Cell volume	34 mL

Tab. A2 GPC Method parameters

Eluent A	Cyclohexane/Dichloromethane (70:30, v/v)		
Gradient	isocratic 100 % A		
Flow rate	1 mL/min	System pressure	35 psi
Column temperature	RT	Run time	60 min
Injection volume	2 mL	Injection mode	Full loop
Detection wavelength	254 nm	Data rate	10 Hz
Collect time	18-43 min	Time constant	0.1 sec

Tab. A3 System configuration (GPC Cleanup system)

Instrument	Description	Article no.
Injection valve	Manual injection valve 6-port 2-position	AVI26BC
Assistant 1	AZURA ASM 2.1 L left: single variable wavelength UV detector middle: 6 port column bypass valve right: pump with pressure sensor, 10 mL pump head in SST	AYCAEABM
Assistant 2	AZURA ASM 2.1 L left: 16 port multi position valve for fractioning middle: 16 port multi position valve for sample loop right: 16 port multi position valve for sample loop	AYGAGAGA
Flow cell	UV, 3 mm, 2 µL	A4042
GPC tubing guide	16 sample loops with 1 mL	A5329-2
Column	450 mm length, 10 mm ID Resin Bio Beads SX-3	
Software	Mobile control	A9608

Tab. A4 GC-MS/MS method

Injector	Split/Splitless	
Mode	Splitless	
Injector temperature	280°C	
Injection volume	2 µL	
Flow rate	1 mL/min	
Carrier gas	Helium	
Capillary column	HP-5MS 30 m x 250 µm x 0.25 µm	
Oven temperature program	80° for 3 minutes from 80 to 300°C at 15°C/min 15 min at 300°C	
Transfer line temperature	300°C	
Electron impact mode	Positive ion	
Solvent delay	5 minutes	
Source temperature	280°C	
Quadrupole Temperature	150°C	
Scan type	MRM, multiple reaction monitoring	
QQQ Collision cell	Quench gas flow rate	2.25 mL/min
	Collision gas flow rate	1.5 mL7min

RELATED KNAUER APPLICATIONS

VFD0153 – GPC Cleanup of olive oil samples

VEV0081 – GPC vs. SPE and subsequent determination of polycyclic aromatic hydrocarbons using GC/MS

VFD0166 – LC-FLD analysis of 4 PAHs in olive oil samples using AZURA® GPC Clean-up System

