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SELECTIVE ACCELERATED SOLVENT EXTRACTION FOR THE ANALYSIS OF SOIL POLYCYCLIC AROMATIC HYDROCARBONS AND STEROLS

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Abstract

Accelerated solvent extraction (ASE) has been successfully used in the analysis of a wide range of chemicals from many sample matrices. However, the main problem with accelerated solvent extraction is low selectivity towards the analyte because during the extraction process, many interfering components are co-extracted together with target analytes and thus requires post-extraction clean-up processes. In this study, a selective accelerated solvent extraction with clean-up step incorporated inside the extraction cell was developed for the analysis of polycyclic aromatic hydrocarbons (PAHs) and sterols in soil. PAHs (naphthalene, acenaphtene, anthracene, pyrene) and sterols (coprostanol, cholesterol, stigmasterol, stigmastanol) were extracted separately using two elution steps. The selectivity and efficiency of this approach were evaluated using several sorbents and proper choice of solvents. Using polar sorbents, PAHs were recovered in the first extraction using n-hexane while sterols were recovered in the second extraction using a more polar solvent such as methanol, isopropanol, acetone and mixture of DCM:MeOH (40:60, v/v). Recoveries for PAHs ranged from 76.5-99.2 % and sterols from 83.7.7-91.4% using silica as the sorbent, n-hexane as the first eluent, and methanol as the second eluent.

Keywords: Accelerated solvent extraction, polycyclic aromatic hydrocarbons, sterols.

Introduction

Sample preparation prior to the determination of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) or herbicides in soil and sediments involved multi-step procedures typically based on exhaustive extraction and subsequent removal of co-extracted material by successive clean-up steps. Accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE) has gained wide acceptance for the extraction of persistent organic pollutants (POPs) from various environmental and biological matrices [1,2,3]. However, the presence of co-eluting interferences such as lipids, humic and fulvic substances in the soil requires an effective clean-up step prior to chromatographic analysis. Moreover, the presence of interferences could impair the limit of detection or even damage the chromatographic system. Most of the reported applications of ASE, an exhaustive clean-up of the extracts prior to injection to the chromatographic system was necessary [1,4]. The use of sorbent in ASE is of special interest where integrated clean-up strategies are utilized to combine extraction and clean-up to simplify the sample-preparation chain [2,5,6]. The possibility of performing selective ASE by packing adsorbents in the extraction cells has been illustrated by several researchers for the extraction of PCBs from fatty samples [2,7]. The main aim of the clean-up stage is to remove substances that could interfere with the final determination and quantitation of target analytes. Many clean-up procedure employed column chromatography using florisil, silica and alumina either separately or in a combination [8]. The objective of this study is to develop method for analysis of PAHs and sterols from soil using selective ASE with minimum specific procedures. The suitability of silica and florisil in extracting PAHs and sterols in soil sample was evaluated. Due to the fact that the PAHs and sterols are of different polarity, their join extraction requires the use of two solvents. Using polar sorbents, PAHs were recovered in the first extraction using n-hexane while sterols were recovered in the second extraction using a more polar solvent such as methanol, isopropanol, acetone and mixture of DCM:MeOH (40:60, v/v).

Experimental

Sample preparation

Soil samples were dried at room temperature, ground in a mortar with a pestle, sieved through a 600μm pore sieve and stored in an air tight container at 4°C before extraction.

Standards and reagents

GC grade dichloromethane (DCM) and n-hexane were purchased from Merck (Darmstadt, Germany). Acetone, methanol, isopropanol (analytical grade) were obtained from Merck (Darmstadt, Germany). Florisil (60-100 mesh) (Fisher Scientific Loughborough, United Kingdom) and ultra pure silica gel (70-230 mesh) (Silicycle, Quebec, Canada) were activated for 24h at 130°C before use. Diatomaceous earth non-washed was purchased from Sigma-Aldrich (Steinheim, Germany). Individual standards of PAHs: naphthalene, acenaphtene, antracene and pyrene were obtained from Dr. Ehrenstorfer, GmbH (Augsburg, Germany). Individual standards of sterols: 5-α-cholestane (internal standard), 5β-cholestan-3β-ol (coprostanol), 5-cholesten-3β-ol (cholesterol), 5β-cholestan-3α-ol (stigmasterol) and stigmastanol were purchased from Sigma Aldrich (Steinheim, Germany). A 50 μg/mL mixture of PAHs and sterols were prepared in acetone and dichloromethane respectively from the individual stock solutions. A five-point calibration standard of PAHs was prepared in the concentration interval 5-100 μg/mL. A four-point calibration standard of sterols was prepared in the concentration interval 10-100 μg/mL. Two internal standards; tetradecane for PAHs and 5α-cholestane for sterols were added into all calibration standards at concentration of 25 μg/mL.

Selective accelerated solvent extraction (SASE)

Extractions were done using ASE 200 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA) equipped with 33-mL stainless-steel cells. Cell loading was done in the following sequence: Cellulose filter was placed at the bottom of cell, then 10 g of activated silica (or Florisil), topped by cellulose filter followed by a spiked soil (5g) blended with diatomaceous earth. The packing of the extraction cell is illustrated in Figure 1. The cell was inserted into the cell tray for the extraction. Two solvents: n-hexane and DCM/MeOH (40:60, v/v) were utilized as extraction solvents. *n*-hexane was pumped into the cell and the cell was then preheated for 2 min to reach the set temperature of 100°C, pressure of 1500psi followed by a static extraction step of 10 min (USEPA Method 3545). After its conclusion, the pressure was released and the extract was collected in a 60 mL glass vials. The cell was rinsed with fresh solvent (about 80% of extraction cell volume) and was purge using pure nitrogen for 1 min. For the second extraction, the same sample cell was extracted again using a more polar solvent (DCM/MeOH (40:60,v/v)). Extract was collected into a second collection vial. Internal standards (tetradecane and 5α-cholestane, 1 mL each) were added to the extracts and rotary evaporated to 1 mL prior to gas chromatograph analysis.

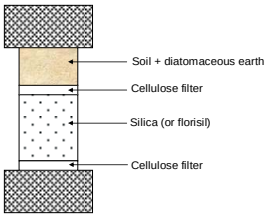

Figure 1: Packing of extraction cell

GC-FID Analysis

Gas chromatographic separation and identification of PAHs and sterols were performed using HP6890 series II (Agilent Technologies Inc., Palo Alto, CA, USA) with splitless injection and flame ionization detection. A 30 m x 0.25 mm id x 0.25μm film thickness HP5-MS capillary column (Agilent technologies) was used to achieve separation of PAHs and sterols with the following temperature program: initial temperature, 50°C; hold for 3 min; increase at 18°C min^{-1} to 250°C, increase at 10°C min^{-1} to 310°C; hold for 7 min. The detector temperature was set at 310°C. PAH quantification was carried out using five-point calibration plot containing 5, 10, 25, 50 and 100 mg/L PAHs standard mixtures and 25 mg/L internal standard (tetradecane). Sterol quantification was carried out using four-point calibration plot containing 10, 25, 50 and 100 mg/L sterols standard mixtures and 25 mg/L internal standard (5α-cholestane).

Results and discussion

Selective accelerated solvent extraction (SASE)

In this work, a selective ASE technique that can simultaneously extract and separate PAHs and sterols into two fractions was developed. Sorbent was added into the extraction cell and the suitability and selectivity of sorbent were studied using several sorbents such as silica, florisil, alumina and activated carbon. Quantitative extraction and successful separation of the two classes of compounds were obtained using extraction cells packed with 10g activated silica and florisil as shown in Table 1. PAHs were extracted in the first extraction using n-hexane followed by sterols in the second extraction using solvent of higher polarity (DCM:MeOH, 40:60,v/v). In the first extraction using *n*-hexane, sterols being relatively polar compounds will retained on silica or florisil (polar sorbents) while PAHs being relatively non polar compounds can be quantitatively extracted. The recoveries of PAHs were in the range of 76.5–99.1% and 70.4-93.3% when silica and florisil was used as sorbent respectively. The recoveries of sterols in the second extraction were slightly higher (above 80%) from both sorbents. Lower recoveries for naphthalene and acenaphthene observed using both sorbents may be due to loss during the concentration step as reported in previous study [9]. The recoveries of sterols are slightly higher (80.1 to 105.4%) as compared to PAHs for both sorbents. Based on the results, both sorbents gave comparable recoveries of PAHs and sterols. However, silica was chosen as a suitable sorbent for in-cell clean-up in selective ASE approach because it is much cheaper compared to florisil.

Table 1. Recoveries of PAHs and sterols using silica and florisil sorbent

Compound	% Recovery (n=3)			
	Silica		Florisil	
	n-hexane (1^{st} extraction)	DCM: MeOH (2^{nd} extraction)	n-hexane (1^{st} extraction)	DCM: MeOH (2^{nd} extraction)
Naphtalene	76.5 ± 4.1		70.4 ± 8.0	
Acenaphtene	85.2 ± 4.6		86.1 ± 1.7	
Antracene	85.0 ± 6.9		93.3 ± 7.6	
Pyrene	99.2 ± 7.4		81.2 ± 6.4	
Coprostanol		99.2 ± 5.4		80.1 ± 9.9
Cholesterol		83.7 ± 7.3		90.0 ± 3.0
Stigmasterol		91.4 ± 8.0		105.4 ± 11.8
Stigmastanol		87.0 ± 10.7		85.6 ± 5.9

Solvent selection

A mixture of hexane-acetone (1:1, v/v) has been used in extracting non-polar compounds such as OCPs. PCBs and PCDDs from soil samples by several researchers [10,11,12]. In this work, n-hexane was used in the first extraction to elute the non-polar compounds (PAHs). Acetone was not used due to fact that it is highly capable of building hydrogen bonding and can disrupt the strong analyte-matrix interaction, increasing the extracting power and thus will extract sterols together with PAHs. In the second extraction cycle, the suitability of various polar solvents; acetone, isopropanol, methanol, DCM:n-hexane (40:60,v/v) and DCM:MeOH (40:60,v/v) was studied for the extraction of sterols from soil and the recoveries (%) of sterols are shown in Table 2. All solvents except for DCM:hexane (40:60, v/v) gave comparable amount of sterols with the recoveries above 80%. Methanol, the most polar solvent gave the highest recoveries of sterols (91.7-97.6%) compared to acetone (75.5-96.6%), isopropanol (86.4-98.6%) and DCM:MeOH (40:60, v/v) (83.7-93.3%). Therefore, methanol was chosen as the extraction solvent for sterols.

Table 2. Recoveries (%) of sterols using different solvent with silica clean-up

Compound	% Recovery (n=3): 2nd extraction				
	DCM:hexane (40:60,v/v)	Acetone	Isopropanol	DCM:MeOH (40:60,v/v)	MeOH
Coprostanol	72.7 ± 5.9	86.6 ± 2.7	86.4 ± 2.8	93.3 ± 5.4	91.7 ± 7.5
Cholesterol	73.1 ± 6.6	92.4 ± 1.2	98.6 ± 5.9	83.7 ± 7.3	97.6 ± 4.3
Stigmasterol	77.7 ± 7.8	75.2 ± 5.9	86.9 ± 10.2	91.4 ± 8.0	93.2 ± 6.2
Stigmastanol	73.7 ± 4.6	96.6 ± 6.4	80.1 ± 7.8	87.0 ± 10.7	94.7 ± 6.7

Effect of activated silica sorbent on the extraction of PAHs and sterols in soil

In this study, selective extraction of PAHs and sterols was achieved using activated silica sorbent added to the extraction cell. As shown in Table 3, using the proposed selective ASE using silica, PAHs were extracted in the first extraction cycle and sterols in the second extraction. Without the addition of activated silica, most of PAHs and sterols were extracted together in the first extraction, and a small fraction of these compounds were detected in the second extraction cycle. In addition, activated silica also functions as a clean-up step and cleaner extracts were obtained. Clear extract containing PAHs was obtained in the first extraction cycle and slightly yellow extract containing sterols was obtained in the second extraction cycle. As reported by several researchers, silica gel is the most polar sorbent available and it is very useful for extract clean-up in the determination of non-polar compounds such as PAHs [10,13,14]. The main advantage of the clean-up step is an increase in lifetime of the analytical GC column.

Table 3. Recoveries PAHs and sterols with and without activated silica sorbent in the extraction cell.

Compound	% Recovery (n=3)			
	With sorbent		Without sorbent	
	1st extraction	2nd extraction	1st extraction	2nd extraction
Naphtalene	72.5 ± 5.3	nd	70.3 ± 7.0	0.8 ± 1.1
Acenaphtene	94.2 ± 3.8	nd	85.2 ± 3.1	2.2 ± 0.8
Antracene	88.1 ± 5.5	nd	81.3 ± 5.6	2.1 ± 1.3
Pyrene	87.6 ± 6.4	nd	90.1 ± 3.4	3.3 ± 2.1
Coprostanol	nd	93.3 ± 5.4	74.5 ± 6.8	2.6 ± 0.9
Cholesterol	nd	83.7 ± 7.3	83.4 ± 5.3	4.6 ± 2.5
Stigmasterol	nd	91.4 ± 8.0	95.3 ± 4.5	1.6 ± 2.2
Stigmastanol	nd	87.0 ± 10.7	87.0 ± 4.7	4.4 ± 3.1

nd – not detected

Conclusion

In this study, a selective ASE method with clean-up step was developed and this approach significantly reduced the time of analysis and yield extracts ready for gas chromatographic analysis. Using activated silica gel placed in the extraction cell, the extraction of PAHs was achieved in the first extraction cycle using n-hexane while sterols in the second cycle using methanol.

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