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Determination of low levels of polycyclic aromatic hydrocarbons in soil by high performance liquid chromatography with tandem fluorescence and diode-array detectors

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HIGHLIGHTS

- We set up a method for determination of low levels of 16 PAHs in soil.
- Analyses were performed by HPLC with fluorescence and diode-array detectors.
- The analytical performance of the proposed method were demonstrated.

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ABSTRACT

Risk assessment of polycyclic aromatic hydrocarbons (PAHs) contaminated soil and source apportionment require accurate analysis of the concentration of each PAH congener in the soil. However, determination of low level PAH congeners in soil is difficult because of similarity in the chemical properties of 16 PAHs and severe matrix interferences due to complex composition of soils. It is therefore imperative to develop a sensitive and accurate method for determination of low level PAHs in soil. In this work, high performance liquid chromatography equipped with fluorescence and diode-array detectors (HPLC–FLD–DAD) was used to determine the concentration of 16 PAHs in soil. The separation of the 16 PAHs was achieved by optimization of the mobile phase gradient elution program and FLD wavelength switching program. Qualitative analysis of the 16 PAHs was based on the retention time (RT) and each PAH specific spectrum obtained from DAD. In contrast, the quantitative analysis of individual PAH congeners was based on the peak areas at the specific wavelength with DAD and FLD. Under optimal conditions the detection limit was in the range 1.0–9.5 μg L^{-1} for 16 PAHs with DAD and 0.01–0.1 μg L^{-1} for 15 PAHs with FLD, and the RSD of PAHs was less than 5% with DAD and 3% with FLD. The spiked recoveries were in the range 61–96%, with the exception of NaP (<40%). The results show that HPLC–FLD–DAD can provide more accurate and reliable analysis of low level PAH congeners in soil samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of diverse organic compounds that typically contain two or more fused aromatic rings. They are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials, in particular fossil fuels such as coal, oil and natural gas, and other hydrocarbons (Lu et al., 2008; Zhang et al., 2011). Due to their carcinogenicity, teratogenicity and mutagenicity (White, 1986), 16 PAHs have been listed by the US Environmental Protection Agency (EPA) as priority pollutants. These are naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracne, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene.

PAHs may enter the soil via wastewater discharge, dry and wet deposition and oil leaks. It has been shown that soil is one of the main sinks for PAHs in the environment (Wilcke, 2000). PAHs that have accumulated in soils may directly or indirectly pose a risk to human and ecosystem health (Jones, 1991). Risk assessment and

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source apportionment of PAH-contaminated soils require accurate analysis of the concentration of each PAH component in the soil. Because of similarity in the chemical properties of 16 PAHs and severe matrix interferences due to complexity of soils, it is imperative to develop a sensitive and accurate method for determination of low level PAHs in soils.

At present the analytical equipment used for the measurement of PAHs in soils mainly comprises gas chromatography (GC) (Kuosmanen et al., 2003; Sikalos and Paleologos, 2005; Zuazagoitia et al., 2009), gas chromatography–mass spectrometry (GC–MS) (Ma et al., 2005; Ozcan et al., 2009; Ene et al., 2012) and high performance liquid chromatography (HPLC) (Chen et al., 2002; Lim et al., 2007; Yang et al., 2011). When GC is used for analysis of PAH components with high boiling points it needs higher temperatures to vaporize, and this may result in a discrimination effect. Moreover, some PAH isomers such as Phenanthrene and Anthracene cannot be easily quantified by GC (Wang et al., 2009). GC–MS equipped with selected ion monitoring (SIM) outperforms GC in PAH isomer separation. However, SIM mode is not useful in further identification of the compound structure, especially when non-target PAH components such as benzo[e]pyrene are present in the sample. In addition, as with GC, GC–MS also needs higher temperatures to vaporize the PAH components with high boiling points (Buco et al., 2004). HPLC is suitable for analysis of compounds with higher molecular weights and boiling points, and has therefore been widely used for PAH analysis. HPLC may be equipped with one of three detectors, namely an ultraviolet (UV), fluorescence (FLD) or diode array detector (DAD). FLD has the characteristics of high sensitivity, high resolution and low detection limits, therefore HPLC–FLD has higher sensitivity for the determination of PAHs exhibiting fluorescent effects. For example, Criado et al. reported that the sensitivity is 4–20 times higher using FLD compared with UV (Criado et al., 2004). However, one main drawback of HPLC–FLD is that the analytes are identified only by their RT. Identification has to be confirmed when samples are complex and many peaks are detected. This can be achieved by using a DAD, which provides the match with specific UV spectra for PAH components (Bouzige et al., 1999). Kicinski et al. used HPLC connected with UV/VIS DAD and FLD to analyze PAHs in drinking water and soil. They concluded that DAD is useful for qualitative and quantitative analysis of PAHs in soil samples and FLD is recommended for the analysis of PAHs in water samples (Kicinski et al., 1989). HPLC–DAD/FLD has been successfully applied in the analysis of PAHs in sewage sludges (Miègea et al., 2003) and food supplements (Danyi et al., 2009). However, the PAHs were detected at a fixed wavelength of 254 nm with DAD. To our knowledge, there is no report on the use of DAD scanograms to obtain the specific UV spectra of the 16 PAHs for peak identification and peak purity checks, as well as for quantitative analysis of PAHs at each specific UV wavelength.

This present work was aimed at developing a sensitive and reliable method for detection of low level PAHs in soils. Separation of 16 PAHs was achieved by optimizing the mobile phase gradient elution program and the FLD wavelength switching program. The specific UV spectra of the 16 PAHs obtained from DAD were used to confirm the identification of PAH components and to quantitatively analyze PAHs at each specific UV wavelength.

2. Experimental methods

2.1. Apparatus

Analyses were performed using an HPLC system (Shimadzu, Kyoto, Japan) consisting of a LC-20AT binary pump, a DGU-20A on-line degasser, a SIL-20A autosampler, a CTO-20A column oven, a RF-20A fluorescence detector, a SPD-M20A diode array detector and a CBM-20A lite system controller. The data were collected and analyzed using an LC Solution Chromatogram Workstation (Shimadzu, Kyoto, Japan).

Table 1
Mobile phase gradient elution program for separation of 16 PAHs.

Time (min)	Total flow rate (mL min^{-1})	Acetonitrile (%)	Water (%)
0	0.5	65	35
25	0.5	65	35
40	↓	90	10
60	1.0	↓	↓
70	1.0	100	0

2.2. Reagents

HPLC grade acetonitrile was obtained from Tedia Company Inc. (Fairfield, OH); analytical grade Dichloromethane, n-Hexane, Methanol and Acetone were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China); ultra-pure water was prepared daily with a Milli-Q water purification system (Millipore, Billerica, MA). All other reagents used were of analytical grade quality.

2.3. Standard solution

A standard mixture of the 16 PAHs (100.0 mg L^{-1}) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), comprising Naphthalene (NaP), Acenaphthylene (AcPy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phe), Anthracene (AnT), Fluoranthene (F1uA), Pyrene (Pyr), Benzo(a)anthracene (BaA), Chrysene (Chry), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Dibenzo(a,h)acenaphthene (DBA), Benzo(a)[ghi]perylene (BghiP), and Indeo[1,2,3-cd] pyrene acenaphthene (In-[1,2,3-cd]P) congener.

A 1.0 mg L^{-1} mixture of the 16-PAH stock solution was obtained by diluting the standard solution with acetonitrile, charging into ampoules and then sealing. All stock and standard solutions were stored at 4 °C. All working solutions were prepared immediately before the experiment by diluting the stock solution.

2.4. Soil sample preparation

2.4.1. Sample extraction

2.00-g aliquots of dried and homogenized soils sieved through 0.15 mm mesh were extracted in a Soxhlet extraction system with 65 mL of mixed n-hexane/acetone solvent (1:1, v/v) for 24 h. The resulting crude extracts were evaporated to dryness using a Model 850 rotary evaporator (Büchi, Flawil, Switzerland) with the water bath at 40 °C, a pressure of 500 mbar and a rotation rate of

Table 2
Wavelength switching program for FLD.

Time (min)	λ_{ex} (nm)	λ_{em} (nm)	PAHs determined
0.01	270	323	1. NaP; 2. AcPy (no fluorescence); 3. Ace; 4. Flu
24.1	252	370	5. PhA
27.0	252	402	6. AnT
30.0	280	460	7. FluA
33.0	270	390	8. Pyr; 9. BaA; 10. Chry
42.5	290	410	11. BbF; 12. BkF; 13. BaP; 14. DbA; 15. BghiP
60.0	290	500	16. In-[1, 2, 3-cd]P