

# An Accelerated Solvent Extraction-Solid Phase Extraction-High Performance Liquid Chromatographic Method for Determination of Polycyclic Aromatic Hydrocarbons in Soil and Earthworm Samples



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**Abstract:** A high performance liquid chromatographic (HPLC) method was established for the determination of the content of seven polycyclic aromatic hydrocarbons (PAHs) in soil and earthworm samples based on accelerated solvent extraction (ASE) for sample extraction and solid phase extraction (SPE) for sample purification. The samples were firstly extracted by the mixed solution of *n*-hexane and acetone (4:1, *V/V*), and purified by SPE column (silica gel column for soil sample purification and Al<sub>2</sub>O<sub>3</sub>-silica gel column for earthworm sample purification). Then the extract was eluted from the SPE column by 10 mL of *n*-hexane/dichloromethane (9:1, *V/V*), and evaporated to dryness with a rotary evaporator. After that, the extract was re-dissolved in acetonitrile to a constant volume and filtrated by a 0.22- $\mu$ m organic filter membrane for the quantification of the seven PAHs by HPLC. The recoveries of this method for the seven PAHs were 83.5%–110.2% in soil samples and 81.2%–97.1% in earthworm samples. The detection limits of the method for the seven PAHs were 0.15–0.85  $\mu$ g kg<sup>-1</sup>. The method had good reproducibility and could meet the quality control requirements of sample analysis.

**Key Words:** Accelerated solvent extraction; Solid Phase Extraction; High performance liquid chromatography; Soil; Earthworm; Polycyclic aromatic hydrocarbons

## 1 Introduction

Polycyclic aromatic hydrocarbons (PAHs), commonly divided into aromatic fused rings and non-aromatic fused rings, are aromatic compounds whose molecules are composed of two or more benzene rings fused together in a linear, angular, or clustered manner<sup>[1]</sup>. PAHs are typical persistent organic pollutants and widely exist in soil, water and other natural environments, and are also enriched in

earthworms and other organisms as a result of the food chain<sup>[2,3]</sup>. They have good fat solubility and can be enriched in the living body through the food chain. Indeed, PAHs are a great threat to human health due to their carcinogenesis, tetratogenesis and mutagenesis<sup>[4–6]</sup>. Sixteen kinds of PAHs with no branched chains are listed as one of the priority control persistent organic pollutants (POPs) by the United States Environmental Protection Agency (USEPA)<sup>[7]</sup>. The sources of PAHs include natural resources like combustion

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(forests, grassland natural fires and volcanic eruptions) and biosynthesis (sediment into rock, biotransformation processes and tar pit gas). Also, they arise from man-made sources such as the incomplete combustion of coal, oil and wood, as well as organic polymers, waste incineration, landfill, food production, direct transportation emissions and so on<sup>[8–10]</sup>. In recent years, researchers have conducted a large number of field investigations on PAH pollution in soil and the results have shown that for soil in China<sup>[11–13]</sup>, all of the 16 kinds of priority control PAHs have been detected more or less, even with a high detection rate. According to the mass fraction and distribution of PAHs in European agricultural soil, the pollution degree of PAHs in soil is classified into four levels by Maliszewska-Kordybach<sup>[14]</sup>. The soil with the total content of 16 kinds of PAHs less than 200  $\mu\text{g kg}^{-1}$  is considered to be non-contaminated, with the content in the range of 200–600  $\mu\text{g kg}^{-1}$  is considered to be slightly polluted, within the range of 600–1000  $\mu\text{g kg}^{-1}$  to be moderately polluted, with the content higher than 1000  $\mu\text{g kg}^{-1}$  to be heavily polluted. The State Environmental Protection Administration recommends the use of high performance liquid chromatography (HPLC) for the determination of PAHs in water (drinking water, groundwater, rivers, lakes and industrial waste waters)<sup>[15]</sup>. However, there are few methods for the determination of PAHs in soil and living organisms. Soil and organism samples are complex and difficult to be purified as the organic matter and impurity contents are high. Therefore, the samples need to be extracted, concentrated and purified before treatment to improve the detection sensitivity and reduce the detection limit.

At present, the known methods for extraction of total PAHs in soil and organisms (such as earthworms) mainly include Soxhlet extraction and ultrasonic-assisted extraction<sup>[16–18]</sup>. The recovery rate of Soxhlet extraction is higher. However, it is a laborious and time-consuming method with high organic solvent consumption, for instance, sometimes the extraction requires even over 72 hours<sup>[19]</sup>. Ultrasonic extraction method, recommended by the US EPA, is simple and rapid, and only takes a few hours for an extraction<sup>[20]</sup>. Although this method can save resources and time, the extraction rate is low<sup>[21]</sup>. Accelerated solvent extraction (ASE) is a new technology which was developed in recent years. It has many advantages including high speed, high efficiency, low organic solvent consumption and automatic operation, and thus has been widely applied in the extraction of organic pollutants in soil, such as pesticide residues, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyl (PCBs), PAHs, etc<sup>[22–25]</sup>. Earthworms are often chosen as model organisms for soil toxicity tests and are ideally suited for assessing the bioavailability of many chemicals in soil for the following reasons. Firstly, there is a low level of mixed-function oxidase (MFO) activity in earthworms, allowing greater potential for the accumulation of organic compounds that would normally be metabolized in other organisms. Secondly, the exterior

epidermal surface of the earthworm is vascularized with no cuticle, allowing the uptake of contaminants directly from the soil through skin. Thirdly, earthworms are adaptable to different types of soil and can easily survive. Furthermore, earthworms have a large mass, which is advantageous for the determination of contaminant concentrations in soil<sup>[26,27]</sup>. Therefore, it has a very important practical significance for measuring the content of PAHs in earthworms. In this study, the solid phase extraction (SPE) technique was applied to the purification of soil and earthworm samples under the optimized conditions. Based on this, an efficient, fast method with high sensitivity and low detection limits was developed for the determination of PAHs in soil and earthworm samples by HPLC technique.

## 2 Experimental

### 2.1 Instruments and reagents

Chromatographic analysis was performed on a Shimadzu Class-VP HPLC system (LC-20A, Shimadzu, Kyoto, Japan) equipped with a SupelcoSil<sup>TM</sup>LC-PAH chromatogram column (25 cm  $\times$  4.6 mm stainless steel column, 5  $\mu\text{m}$  particle diameter) and a fluorescence detector (RF-10AXL, Shimadzu). Sample extraction was carried out with an accelerated solvent extractor (ASE-200, Dionex, Sunnyvale, CA, USA). Besides, a rotary evaporator (R210/R215, Buchi, Flawil, Swiss) was used for the sample treatment.

PAH standard samples including phenanthrene (Phe, 99%), pyrene (Pyr, 99%), chrysene (Chry, 99%), benzo (a) anthracene (BaA, 99%), benzo (b) fluoranthene (BbF, 99%), benzo (k) fluoranthene (BkF, 99%) and benzo (a) pyrene (BaP, 96%) were obtained from Sigma-Aldrich (Augsburg, Germany). Analytical reagents including chromatography silica gel, diatomite, alumina, anhydrous sodium sulfate and quartz sand, and chromatographic reagents including dichloromethane (99.5%), acetone (99.5%), *n*-hexane (98.0%), and acetonitrile (99.9%) were obtained from Nanjing First Chemical Reagent Co., Ltd., China. Prior to use, diatomaceous earth, alumina ( $\text{Al}_2\text{O}_3$ ), anhydrous sodium sulfate and quartz sand were heated at 200  $^\circ\text{C}$  for 4 h and silica gel (100-mesh) was activated at 130  $^\circ\text{C}$  for 2 h. The earthworms used in experiments were *Eisenia fetida* of *Lumbricidae* (Nanjing, China).

### 2.2 Collection and analysis of soil samples

Clean natural red soil was collected from 10–20 cm surface soil from Hainan, China (19 $^\circ$ 46'16.6"N, 110 $^\circ$ 00'21.5"E). The physical and chemical characteristics were as follows: pH 6.33, 2% organic matter, 41% sand, 27% silt and 32% clay. The texture of the soil was clay loam and it contained no detectable levels of PAHs. The soil was air dried and passed through a 2-mm sieve, then stored under cool condition until use.

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