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Short communication

Biomonitoring method for the determination of polycyclic aromatic hydrocarbons in hair by online in-tube solid-phase microextraction coupled with high performance liquid chromatography and fluorescence detection

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are formed from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities, but human exposure to PAHs has not yet been elucidated in detail. To assess long-term exposure to PAHs, we developed a simple and sensitive method for measuring PAHs in hair by online in-tube solid-phase microextraction using a CP-Sil 19CB capillary column as an extraction device, followed by high-performance liquid chromatography using a Zorbax Eclipse PAH column and fluorescence detection. Seventeen PAHs could be analyzed simultaneously, with good linearity from 20 to 1000 pg/mL each as determined using stable isotope-labeled PAH internal standards. The detection limits of PAHs were 0.5–20.4 pg/mL. PAHs in human hair samples were extracted by ultrasonication in 50 mM NaOH in methanol, and successfully analyzed without any interference peaks, with good recovery rates above 70% in spiked hair samples. Using this method, we evaluated the suitability of using hair PAHs as biomarkers for long-term exposure.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, resulting from the incomplete combustion or pyrolysis of organic matter during industrial processing (e.g., heating, drying, and smoking processes) and various human activities, such as cigarette/tobacco smoking and certain cooking methods (e.g., grilling, roasting, baking, and frying) [1]. PAHs are highly lipophilic, non-polar, and resistant to degradation, and can remain in the environment for long periods with the potential to cause adverse environmental and health effects. Many PAHs are cytotoxic, mutagenic and carcinogenic in laboratory animals and have been implicated in various cancers in humans [1–3]. In particular, benzo[*a*]pyrene (BaP) is classified by the WHO International Agency for Research on Cancer as a human carcinogen (Group 1), whereas other PAHs have been classified as probable/possible human carcinogens. It is difficult, however, to estimate actual levels of human exposure to PAHs from estimated contents in foods and questionnaires about dietary consumption. Therefore, suitable biomarkers of long-term exposure to individual PAHs, and a sensitive, selective, and simple biomonitoring method are required to accurately assess actual exposure and associated cancer risks.

Although PAHs and their metabolites (hydroxyl-PAHs) have been assayed in urine [4,5], these compounds are rapidly eliminated, with the amounts in urine capturing only the previous 24 h of exposure. In contrast, hair has been used to assess and monitor long-term human exposure to exogenous compounds, including environmental pollutants, certain drugs and carcinogens [6,7], because compounds entrapped within hair shafts can remain there for extended periods of time, depending on the length of the hair. Hair has several advantages over the more traditionally used blood and urine specimens, including less invasive sample collection and the inability to easily remove compounds from hair by normal washing. Although long-term exposure to PAHs can be assessed in hair samples [8–10], measuring PAHs and their metabolites in hair is difficult analytically because their concentrations are often below 1 ng/mg. Therefore, highly sensitive assays of PAHs in hair are required for biomonitoring.

PAHs or their metabolites have been measured in hair samples using high performance liquid chromatography with fluorescence detection (HPLC-FLD) [8], gas chromatography-negative chemical ionization-mass spectrometry (GC-NCI-MS) [9], and GC–MS/MS

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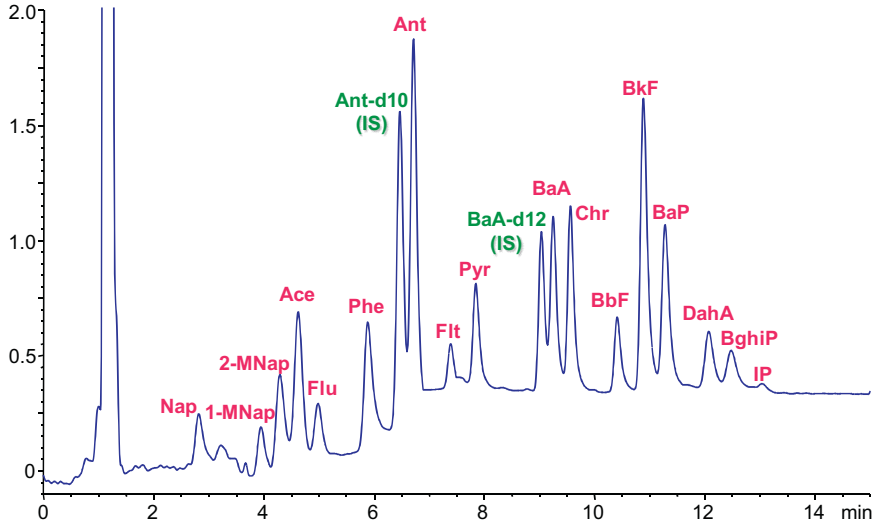

Fig. 1. Typical chromatogram obtained from standard PAHs (1 ng/mL each) by in-tube SPME HPLC-FLD. The in-tube SPME HPLC-FLD conditions are described in the Experimental section.

[10]. Although GC–MS is highly selective and sensitive, PAHs must be converted into volatile derivatives. In contrast, HPLC-FLD is sensitive but does not require derivatization. Nevertheless, these HPLC-FLD and GC–MS methods require time-consuming and laborious sample preparation, including evaporation to dryness, liquid–liquid extraction, or solid-phase extraction, to isolate and preconcentrate PAHs and their metabolites. Moreover, these methods require relatively large amounts of hair samples, of at least 50 mg.

In-tube solid-phase microextraction (SPME), using an open tubular fused-silica capillary with an inner surface coating as an extraction device, is a simple method that can be easily coupled online with HPLC and LC–MS by column switching techniques [11–14]. In-tube SPME enables the convenient automation of the extraction process, not only reducing analysis time, but providing better precision and sensitivity than manual off-line techniques. We have previously described an online analytical method for the determination of PAHs in food samples by in-tube SPME HPLC-FLD [13,14]. However, that method requires two separate analyses, at two different methanol concentrations, because extraction efficiencies differ for low- and high-molecular weight PAHs at methanol concentrations of 5% and 30%, respectively. In this study, compounds were extracted at methanol concentrations of 20%, enabling the simultaneous analysis of low-and high-molecular weight PAHs. In addition, we have improved the performance and precision of this method by using a stable isotope-labeled internal standard (IS). This improved online in-tube SPME HPLC-FLD method was applied to the simultaneous determination of PAHs in hair samples.

2. Experimental

2.1. Materials

A standard mixture of 18 PAHs (2 mg/mL each in 1:1 benzene:methylene chloride) was purchased from Supelco (Bellefonte, PA). This study analyzed 17 PAHs (Fig. S1), all except non-fluorescent acenaphthalene: naphthalene (Nap), 1-methylnaphthalene (1-MNap), 2-methylnaphthalene (2-MNap), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DahA), benzo[*ghi*]perylene (BghiP), and indeno[1,2,3-*cd*]pyrene (IP). The stable isotope-labeled internal standard (IS) compounds, anthracene-d_{10} (Ant-d_{10}, isotopic purity 96%) and benz[*a*]anthracene-d_{12} (BaA-d_{12}, isotopic purity 95%) (2 mg/mL each in methylene chloride) were obtained from Supelco. The standard PAH mixture and IS solution were dissolved in methanol to a concentration of 0.1 mg/mL and stored in amber screw-cap bottles at 4 °C. Stock solutions were diluted with pure water to the required concentrations immediately prior to use. LC–MS grade methanol, acetonitrile, and water used as mobile phases were purchased from Kanto Chemical (Tokyo, Japan).

2.2. Instruments and analytical conditions

The HPLC-FLD system was a Model 1100 series HPLC coupled to a fluorescence detector (Agilent Technologies, Boeblingen, Germany). A Zorbax Eclipse PAH column (100 mm × 2.1 mm i.d., particle size of 3.5 μm; Agilent Technologies) was used for HPLC separation. The gradient elution program and FLD conditions are shown in Table S1. HPLC-FLD data were processed with an HP ChemStation.

2.3. In-tube solid-phase microextraction

The in-tube SPME device consisted of a CP-Sil 19CB (14% cyanopropyl phenyl methylsilicone, Varian Inc., Lake Forest, CA) capillary column (60 cm × 0.32 mm i.d., film thickness 1.0 μm) placed between the injection loop and the injection needle of the autosampler by connection with a 2.5-cm sleeve of 1/16-inch polyetheretherketone (PEEK) tubing, standard 1/16-inch stainless