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# Removal of carotene-like colored compounds by liquid–liquid extraction during polycyclic aromatic hydrocarbons analysis of plant tissue

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**Abstract**

Plants contain a wide variety of chemicals, some of which may have similar chromatographic behavior to polycyclic aromatic hydrocarbons (PAHs). During solid phase extraction (SPE) with Si-gel for instance, the co-elution of carotene-like colored compounds with PAHs has been observed. In this paper, liquid–liquid extraction was applied for the separation and subsequent analysis of PAHs from plant extracts. PAHs containing 2–6 rings, which include naphthalene, phenanthrene, pyrene, benzo[*a*]pyrene and benzo[*ghi*]perylene, were used as representative target chemicals. Carotene-like compounds extracted from Komatsuna (*Brassica campestris*) shoot by acetone followed by Si-gel treatment were incorporated as undesired components in the model matrix. Results showed the feasibility of employing either acetonitrile or 2% (w/v) KOH–methanol as solvents for high PAHs recovery and low extraction of colored fraction. For acetonitrile, 86.9–93.5% of each PAH could be recovered after three extraction cycles (relative standard deviation, RSD < 1.6%) with only about 10% co-extraction of colored fraction. For 2% KOH–methanol, PAHs recoveries ranging from 79.3% to 83.1% after five cycles (RSD < 1.5%) were achieved while the percent extraction of colored fraction was also low at 10%. The relatively higher selectivity of the solvents for PAHs over the colored fraction as well as the solubility of the matrix solution in the solvent may have contributed to these results. On this basis, liquid–liquid extraction is very useful for the pre-treatment of plant extracts for PAHs analysis.
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*Keywords:* Clean-up; Phytoremediation; Sample pre-treatment; Solid phase extraction (SPE)

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of the most important classes of toxic pollutants that are ubiquitously found in the environment (Juhasz and Naidu, 2000; Mastral et al., 2003; Chen and White, 2004). Their occurrence is mainly attributed to a variety of anthropogenic activities such as incomplete combustion of fossil-fuels (Junk and Ford, 1980; Wilcke, 2007). Several PAHs, such as fluoranthene and benzo[*a*]pyrene, which are likely carcinogens, are listed as priority pollutants by United States of Environmental Protection Agency (USEPA, 1985). In recent years, much attention has been paid to the exposure of plants to PAHs via atmospheric deposition and adsorption from the soil (Simonich and Hites, 1994; Kipopoulou et al., 1999; Howsam et al., 2001; Kazerouni et al., 2001; Berber et al., 2004). On the other hand, there are also numerous studies regarding the application of phytoremediation to treat PAHs contaminated soil using a variety of plant species (Liste and Alexander, 2000; Fismes et al., 2002; Maila and Cloete, 2002; Ke et al., 2003; Gao and Zhu, 2004; Parrish et al., 2006). In all of these studies, accurate and efficient analysis of PAHs content of plant tissues is very important.

Plants contain a wide variety of chemicals (e.g. pigments, fatty acids and alcohols) and some of these may have very similar chromatographic behavior to PAHs.

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The direct injection of untreated plant extracts into the GC/MS may not be appropriate for instrument safety and accuracy of PAHs analysis. Clean-up of plant extracts is therefore very important. Solid phase extraction (SPE) method with silica (Tao et al., 2004; Lin et al., 2007; Yang and Zhu, 2007), florisil (Kazerouni et al., 2001), alumina (Ratola et al., 2006) and $C_{18}$ (Reilley et al., 1996) has been used for sample purification. However, during SPE with silica gel as stationary phase and dichloromethane as eluent, the co-elution of a yellow-colored fraction, which has a similar visible spectrum pattern with β-carotene, has been quite persistent. In addition, carotene, sterol and alcohol are also co-eluted with PAHs during SPE using florisil (Meudec et al., 2006) to indicate the general difficulty of separating certain compounds. In order to improve sample pre-treatment, a combination of the above-mentioned stationary phases (Tremolada et al., 1996; Wenzel et al., 1998) has been employed during SPE. Size exclusion chromatography (Hubert et al., 2003) has also been considered. However, these methods prolong and thus generally complicate the overall analysis.

In the present paper, liquid–liquid extraction was evaluated as a simple alternative to remove colored components from PAHs-containing samples. Specifically, the optimization of extraction conditions (i.e. extraction solvent and number of extraction cycle) for separating 2–6 rings PAHs from contaminating compounds was considered. An extract from the shoot of Komatsuna (*Brassica campestris*), which is known to contain high amounts of carotene-like chemicals (Hels et al., 2004), was used as model matrix to simulate the yellow-colored fraction commonly encountered during purification of plant extract.

## 2. Materials and methods

### 2.1. Reagents and plant material

Five types of powdered PAHs (purity >98%) were used in the present study: naphthalene and phenanthrene from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); pyrene from Nacalai Tesque, Inc. (Kyoto, Japan); benzo[*a*]-pyrene and benzo[*ghi*]perylene from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were of special grade obtained from Nacalai Tesque or Kanto Chemical Co., Inc. (Tokyo, Japan).

The shoot of Komatsuna (*B. campestris*) was used for the preparation of matrix solution. The vegetable was purchased at a local marketplace, washed with tap water and then stored in the dark at −20 °C until use. The 5 PAHs contents of the plant sample were below the detection limit.

### 2.2. Preparation of PAHs-spiked solution

Komatsuna shoots were cut into small pieces and homogenized for 3 min with acetone in an Erlenmeyer flask. The mixture was ultrasonicated for 30 min and filtered through Whatman GF/F glass filter (Whatman Japan KK., Tokyo). The filtrate was transferred in a separatory funnel and then hexane and distilled water were added. The funnel was shaken and then allowed to stand for a few minutes after which the hexane phase including the remaining stable emulsion was obtained. The hexane extract was washed twice with distilled water, and the excess water was removed over anhydrous $Na_2SO_4$. The extract was concentrated in a rotary evaporator at 30 °C. To remove chlorophyll and other interfering components, the concentrated solution was deposited onto a silica gel column (Wakogel C300, Wako pure chemical Industries; 12 cm length by 3.2 cm diameter). The column was eluted with hexane (60 ml), and then with dichloromethane. A yellow-colored fraction was eluted from top of the column as soon as the solvent was changed to dichloromethane. The solvent was loaded until the eluent became almost colorless. The hexane and dichloromethane eluents were combined and the resulting solution was evaporated in a rotary evaporator at 30 °C to remove the dichloromethane. The concentrated yellow-colored solution was used as matrix solution. It was spiked with the stock solutions of the 3 PAHs (3-ring phenanthrene, 4-ring pyrene and 5-ring benzo[*a*]pyrene; 500 mg $l^{-1}$ for each PAH in acetone) to come up with concentrations of 0.1 and 1.0 mg $l^{-1}$ for each PAH. The PAHs-spiked solutions were stored in the dark at −20 °C until use.

### 2.3. Optimization for PAHs determination by liquid–liquid extraction

Methanol, HCl–methanol, KOH–methanol and acetonitrile were used as solvents for liquid–liquid extraction and the number of extractions was varied from one to five cycles. The concentrations of HCl (v/v) and KOH (w/v) were 1–5%. The volume of extraction solvent was 1.5 ml per one cycle except for acetonitrile, where 3 ml per one cycle was employed. The detailed procedure was as follows: 5 ml of PAHs-spiked solution and the specified volume of extraction solvent were combined in a glass test tube and then subjected to vortex mixing for 30 s. The lower phase was collected and the upper hexane phase was re-extracted with an equal volume of extraction solvent. The operation was repeated up to the specified cycles. In the case of methanol as solvent, 3 ml of hexane was added into the tube before the first extraction. The initial addition of hexane in this case was made to compensate for its high affinity for methanol as compared with the other solvents. This would assure that even after five cycles of extraction, an upper hexane phase would remain. The remaining hexane phase was stored for estimation of percent extraction of colored fraction as described in Section 2.5. All the extracts collected in each cycle were combined in a 100 ml screw glass vial and then 70 ml of 1% (w/v) aqueous NaCl and 10 ml of hexane were added. The vial was shaken and the upper hexane layer was obtained. The hexane extract was washed with distilled water and passed through anhydrous $Na_2SO_4$ column. The solution was concentrated to a final

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