

ANALYSIS OF PAHS IN PLANT TISSUE BY LIQUID-LIQUID EXTRACTION

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Abstract

Plant contains a wide variety of chemicals and part of them may have similar chromatographic behavior to target compounds. During solid phase extraction of plant shoot extract with silica, the co-elution of carotene-like colored fraction with PAHs was observed. In this study, liquid-liquid extraction was considered for the removal of the colored fraction and subsequent analysis of PAHs from plant extracts. Phenanthrene, pyrene and benzo[*a*]pyrene were used as representative PAHs. Hexane solution containing carotene-like fraction from Komatsuna (*Brassica campestris*) shoot extract was used as the matrix solution. Results showed the feasibility of employing acetonitrile and 2% (w/v) KOH-methanol as extraction solvents for high PAHs recovery and low extraction of colored fraction. In the case of acetonitrile, over 90 % of each PAH could be recovered after 3 extraction cycles, whereas only about 10% of colored fraction could be extracted. For 2% KOH-methanol, although PAHs recoveries were little lower than those of acetonitrile (82.8 to 86.4% after 5 cycles), the percent extraction of colored fraction was almost the same value. On the basis of these results, liquid-liquid extraction may be very useful for the pre-treatment of plant extracts for PAHs analysis.

Introduction

There are several studies regarding the application of phytoremediation to treat PAHs contaminated soil using a variety of plant species¹. This would require accurate and efficient analysis of PAHs from plant tissue. Plants contain a wide variety of chemicals and some of these may have very similar chromatographic behavior to PAHs. Solid-phase extraction (SPE) with silica², florisil³, alumina⁴, etc. has been used for clean-up of the plant extract. However, during SPE of plant shoot extracts using silica, a major problem that we encountered was the co-elution of PAHs with a carotene-like colored fraction. In this research, the application of liquid-liquid extraction for the separation and subsequent analysis of PAHs from plant extracts was considered. Specifically, this research describes the optimization of extraction conditions (i.e. extraction solvent and number of extraction cycle) for such technique. Extract from the shoot of Komatsuna (*Brassica campestris*) was used as model matrix to simulate the yellow-colored fraction commonly encountered during SPE purification.

Materials and Methods

Reagents. Three types of PAHs (purity>98%) were used in this study: phenanthrene, pyrene and benzo[*a*]pyrene. All other reagents were of special grade.

Preparation of PAHs-spiked solution. Acetone extract of Komatsuna shoots was transferred with hexane in a separatory funnel and then the hexane solution was concentrated. To remove chlorophyll and other interfering components, the solution was deposited onto a silica gel column. The column was eluted with hexane, 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 both eluents were combined and the solutions were evaporated in a rotary evaporator to remove the dichloromethane. The resulting yellow-colored solution was used as matrix solution. It was spiked with the stock solutions of the 3 PAHs (500 mg L⁻¹ acetone solution) to come up with concentrations of 0.1 and 1.0 mg L⁻¹ for each PAH. The PAHs-spiked solution was stored in the dark at -20°C until use.

Optimization for PAH determination by liquid-liquid extraction. Methanol, HCl-methanol, KOH-methanol and acetonitrile were used as a solvent for liquid-liquid extraction and the number of extraction cycles was varied from 1 to 5. The concentrations of HCl (v/v) and KOH (w/v) were 1%, 2% and 5%. The detailed procedure was as follows: 5 mL of PAHs-spiked solution and the specified volume of extraction solvent (1.5 mL for methanol, HCl-methanol and KOH-methanol, and 3 mL for acetonitrile) were combined and then subjected to vortex mixing. 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 specified cycles. The remaining hexane phase was stored for calculation of percent extraction of colored fraction as described below. All the extracts collected in each cycle were combined in a glass vial and then 1% (w/v) aqueous NaCl (70 mL) and hexane (10 mL) were added. The vial was shaken and the hexane layer was obtained. The hexane extract was washed with distilled water and passed through anhydrous Na₂SO₄ column. The solution was concentrated under a stream of nitrogen. After the addition of internal standard, the solution volume was made up to 10 mL. Aliquot of the solution was used for GC/MS analysis. All experiments were conducted at ambient temperature (20-25°C).

Calculation of percent extraction of colored fraction. The hexane phase, which was stored as described above, was evaporated to dryness and then reconstituted with 5 mL of hexane. Absorption values of the solution at 473 nm and 449 nm were measured using Jasco V-530 spectrophotometer (Jasco Co., Tokyo, Japan). The percent extraction of colored fraction in analytical GC/MS solution was calculated using the following equation:

$$\text{Percent extraction (\%)} = \left(1 - \frac{E_{\lambda_{\text{sample soln.}}}}{E_{\lambda_{\text{spiked soln.}}}} \right) \times 100$$

where E is the absorbance at nm (i.e. 473 nm and 449 nm: the colored fraction displayed absorption maxima at these wavelengths in hexane) of the sample or initially spiked solution before extraction. In this study, though quantitatively similar, the terms “percent recovery” and “percent extraction” were used in the case of PAHs and colored fraction, respectively, to distinguish between the target compounds (PAHs) and the impurities being removed (colored fraction) from the analytical GC/MS solution.

GC/MS analysis. The determination of PAHs concentration was carried out using a Shimadzu GCMS-QP2010 system (Shimadzu Co. Kyoto, Japan) equipped with AOC-20i auto-injector. Percent recoveries of PAHs were calculated from the amounts of PAHs in analytical GC/MS solution and those in the initially spiked solution.

Results

Figure 1 shows the percent extraction of colored fraction from the spiked solution (1.0 mg L⁻¹ PAHs) by HCl-methanol and KOH-methanol at different acid or base concentrations. The percent extraction of colored fraction decreased with increasing HCl or KOH concentration. The percent extraction of colored fraction by KOH-methanol was significantly lower than by HCl-methanol at the same concentration. At 2% KOH concentration in methanol, the percent extraction at 473 nm and 449 nm were only 9.9±1.7% and 10.7±1.5%, respectively, whereas values for 2% HCl were both close to 30% (Fig.1). On the other hand, although the recoveries of PAHs also decreased with increasing HCl or KOH concentration, over 80% of each PAH could still be recovered at 2% acid or base after 5 cycles (e.g. 82.8-86.4% for 2% KOH-methanol).

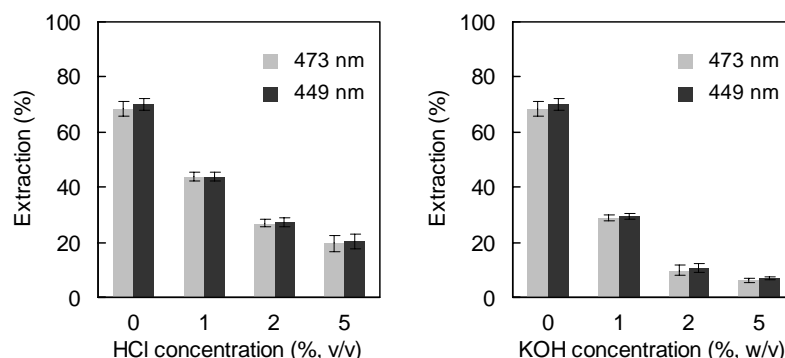


Fig. 1. Percent extraction of colored fraction from 1mg L⁻¹ PAHs-spiked solution by (a) HCl-methanol and (b) KOH-methanol at different acid or base concentration ($n=3$). Number of extraction cycle is 5.

Table 1 shows the effect of number of extraction cycle on the percent recovery of PAHs from the spiked solution (1.0 mg L⁻¹ and 0.1 mg L⁻¹ PAHs) by acetonitrile. The recoveries of each PAH were independent of their initial

concentration of PAHs (Table 1). The recoveries tended to increase with the number of extractions and over 90% of each PAH was recovered after three cycles. Figure 2 shows the percent extraction of the colored fraction at 1.0 mg L⁻¹ PAHs solution. Interestingly, although the extraction percentage also increased with the number of extractions (Fig. 2), the values were quite lower as compared with the methanol data. The percent extraction calculated at 473 nm and 449 nm were only 10.6±1.6% and 8.2±1.5%, respectively, even after 3 cycles, whereas for methanol, the values were much higher (66.2±2.7% and 67.5±2.9%, respectively).

Table 1. Percent recovery of 3 PAHs from spiked solution by acetonitrile at different number of extraction cycle ($n=3$).

PAHs	Extraction cycles				
	1	2	3	4	5
[PAHs]: 1 mg L ⁻¹					
Phenanthrene	58.0 (0.7)	80.6 (0.4)	90.0 (1.2)	91.5 (0.6)	93.3 (1.0)
Pyrene	56.2 (0.1)	80.5 (1.2)	92.6 (0.7)	94.9 (1.7)	99.9 (1.5)
Benzo[<i>a</i>]pyrene	61.8 (1.5)	89.5 (2.0)	99.6 (0.5)	105.3 (1.0)	106.5 (1.5)
[PAHs]: 0.1 mg L ⁻¹					
Phenanthrene	61.6 (0.4)	82.7 (0.2)	93.2 (0.6)	-	-
Pyrene	56.1 (0.2)	80.9 (0.9)	93.5 (0.2)	-	-
Benzo[<i>a</i>]pyrene	58.9 (1.0)	82.2 (2.6)	99.7 (3.0)	-	-

The numbers in bracket indicate standard deviation, -, not determined.

Discussion

In this research, we examined the liquid-liquid extraction technique for the removal of colored components from analysis samples. Specifically, development of a solvent system and conditions that maximize PAHs recovery while minimizing colored component co-extraction was mainly considered.

In the case of methanol solvent systems, we attempted to utilize HCl-methanol or KOH-methanol, taking into account the pH-dependency of colored fraction speciation. As a result, reductions on the extraction percentages of colored fraction were found (Fig. 1). These results suggest that the degree of ionization of the matrix components may affect the extraction percentage of colored fraction. However, we also found that the dissolution volume of hexane into the methanol phase during extraction tended to decrease with increasing HCl or KOH concentrations, suggesting also the significance of this factor on the observed data. Acetonitrile allowed high PAHs recovery as well as low extraction percentage of colored fraction after three cycles (Table 1 and Fig. 2). The low dissolution volume of hexane in the acetonitrile phase as well as acetonitrile's low affinity for colored fraction and good selectivity for PAHs may explain for the observed data.

On the basis of these results, 2% KOH-methanol (5 extraction cycles) and acetonitrile (3 extraction cycles) were found to be appropriate for this purpose. If carbon-labeled PAHs are used as internal standard, it may be possible to further decrease the number of extraction. The range of relative standard deviation values of PAHs recovery for both solvents was satisfactory at 0.2-3.2%, indicating the reproducibility of the procedure. However, it may be necessary to examine whether this method can be applied to other plant species containing different chemical pigments. Nonetheless, this liquid-liquid extraction procedure is very promising for the removal of carotene-like compounds, which is co-eluted with PAHs during the traditional SPE method.

References

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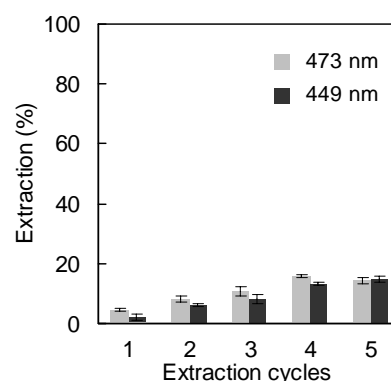


Fig. 2. Percent extraction of colored fraction from 1 mg L⁻¹ PAHs-spiked solution by acetonitrile at different number of extraction cycle ($n=3$).