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# Air- agitated Liquid–Liquid Microextraction and gas chromatographic determination of some polycyclic aromatic hydrocarbons in hookah water and hookah smoke

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**Abstract**

In this study, Air-agitated liquid–liquid microextraction (AALLME) with low solvent consumption was demonstrated for gas chromatographic determination of some polycyclic aromatic hydrocarbons (PAHs) as carcinogenic and mutagenic compounds in aqueous samples. Factors affecting the extraction process, such as extracting solvent type, extracting solvent volume, number of agitation, ionic strength, and centrifugation time, were investigated thoroughly and optimized. Under the optimized conditions, the method provided a good linearity in the range of 0.05 to 120 ng $mL^{-1}$ ($R2 \geq 0.995$), low limits of detection (0.015 to 0.05 ng $mL^{-1}$), good repeatability of the extractions (RSDs below 9.8%, n = 5), and the enrichment factors (EFs) were found to be in the range of 327 to 773. This is the first report on the utilization of AALLME for extraction of PAHs and results showed that AALLME can be a good choice for efficient micro-extraction of these compounds. In order to investigate this common view among some of people that hookah smoke can be healthy due to passing through water, both the hookah water and the hookah smoke were analyzed.

**Keywords**: Air-agitated liquid-liquid micro-extraction, Gas chromatography, Hookah water, Hookah smoke

## 1. Introduction

Sample extraction plays a key role in modern analytical methodology, which affects the accuracy and precision of the final results. However, traditional sample extraction procedures, based on conventional liquid–liquid extraction (LLE) is often time-consuming, labor-intensive, and environmentally unfriendly due to the utilization of large amounts of potentially toxic organic solvents. Solid phase extraction (SPE) utilizes much less solvent and is less time-consuming than LLE, but requires column conditioning and is relatively expensive [1,2]. In the past few years, the promising objectives of green chemistry caused many research efforts focusing on the development of efficient, miniaturized and environmentally friendly extraction methods such as liquid-phase micro-extraction (LPME) [3-5], and solid-phase micro-extraction (SPME) [6-8]. SPME is a solvent-free extraction technique which unifies extraction and pre-concentration in a single step. However, SPME fiber is expensive, fragile and has a limited lifetime, also sample carry-over can be a great problem in this method [9]. LPME approaches are much more cost-effective and can be divided into two broad categories: membrane-protected solvent and exposed solvent. The protected LPME modes include hollow-fiber-protected 2-phase micro-extraction, hollow-fiber-protected 3-phase micro-extraction, and electro-

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membrane micro-extraction (EME) [9]. Single-drop micro-extraction (SDME), head-space single-drop micro-extraction (HS-SDME), liquid-liquid-liquid micro-extraction (LLLME) can be some of main subdivide of exposed-solvent techniques [10]. Also in 2006, a rapid, simple, high enrichment factor and low cost method namely dispersive liquid–liquid micro-extraction (DLLME), as another method of LPME modes was proposed [11]. It is based on a ternary component solvent system in which the utilization of the co-solvents (disperser solvents) has been led to some disadvantages such as decrease in partition coefficients of analytes into the extracting solvent and increase in the cost and environmental pollution.

To overcome this problem some efficient and disperser-free methods such as vortex-assisted liquid–liquid micro-extraction (VALLME) [12,13], ultrasound-assisted emulsification micro-extraction (USAEME) [14-16], and air-agitation liquid-liquid micro-extraction (AALLME) were introduced [17,18]. AALLME needs very simple equipment that can be easily found in laboratories [19]. The utilization of micro liter of extracting solvent, rapidity, as well as achieving to the high enrichment factor caused AALLME to be a proper choice in trace analysis. The determination of PAHs in environmental samples, especially in water samples is not an easy task, as their concentrations in such samples are very low, because of their low solubility. Therefore, pre-concentration and pre-separation are needed to achieve the sufficient sensitivity and selectivity [20]. These compounds are carcinogenic, mutagenic, and ubiquitous environmental pollutants, resulting from the incomplete combustion or pyrolysis of organic materials during industrial processing and various human activities. It is predictable that by burning tobacco PAHs can be produced, and by passing of the hookah smoke in water, these compounds can be trapped in water. In contradiction with some common views, it is possible that these compounds as carcinogenic agents can pass through the water and be inhaled by the smoker. To investigate these objectives, the aim of this study was based on the utilization of very simple and efficient method for micro-extraction of PAHs in the hookah water. For this purpose the effects of various experimental parameters on the extraction efficiency of the method was investigated. In order to study of the existence of PAHs in hookah smoke, by using an efficient design of two consecutive two-piece vacuum trap, these analytes were extracted from the hookah smoke stream and they were analyzed by GC-FID.

## 2. Experimental

### *2.1. Reagents and solutions*

Standards of PAHs (naphthalene, fluorene, anthracene, chrysene and benzo[a]pyrene), and biphenyl were purchased from Aldrich (Milwaukee, WI, USA). Chloroform, 1,2-dichloroethane, and 1,2-dichlorobenzene were purchased from Sigma-Aldrich. Analytical-reagent grade acetonitrile, methanol, carbon tetrachloride and Sodium chloride were obtained from Merck (Darmstadt, Germany). Stock standard solution of 1000 mg $L^{-1}$ of each PAH was prepared in acetonitrile. Working standard solutions were daily prepared at the appropriate concentration by dilution of the stock standard solutions in methanol. All solutions were stored at 4°C while protected from light. All of the other used chemicals were of reagent grade or of the highest purity available.

### *2.2. Samples*

Fifteen grams of tobacco (Borazjan tobacco) was loaded in the water pipe head during each smoking session, then two charcoal pieces were lit and placed a top of the tobacco at the beginning of the smoking session. The duration of each smoking session was 10 min and two consecutive two-piece vacuum traps were used for sampling of the hookah smoke. In one of them, 300 mL of water was poured that acted like a hookah (the operation mechanism of this device exactly is similar to hookah device). The produced smoke from the burning of tobacco was passed through the water (this passed smoke is normally inhaled by smoker), then it was directly ferried to the organic solvent (10 mL carbon tetrachloride) which poured in the second two-piece vacuum trap. All solutions were centrifuged to sediment the interfering compounds.

*2.3. Apparatus*

Separation and detection of PAHs were performed by a gas chromatograph (GC-17A, Shimadzu, Japan) equipped with a splitless/split injector and a flame ionization detector. Helium (purity 99.999%) was used as the carrier gas at the constant flow rate of 4 mL min-1. The temperatures of injector and detector were set at 280 °C and 290 °C, respectively. The injection port was operated at splitless mode. For FID, hydrogen gas was generated with a hydrogen generator (OPGU-2200S, Shimadzu, Japan). A 30 m BP-1 SGE fused-silica capillary column (0.32 mm i.d. and 0.25 μm film thickness) was applied for separation of PAHs. Oven temperature program was: 70 °C for 2 min, increased to 115 °C at 8 °C $min^{-1}$, held for 2.5 min, increased to 200 °C at 8 °C $min^{-1}$, held for 0 min, increased to 290 °C at 10 °C $min^{-1}$ and then held at 290 °C for 3 min. The Hettich centrifuge, model EBA20 (Tuttlingen, Germany) was used for accelerating phase separation and a 10.0 μL ITO (Fuji, Japan) micro syringe was applied for the collection of sedimented organic solvent and injection into the GC, also two-piece vacuum trap was homemade.

*2.4. Extraction procedure*

Five milliliter of aqueous solution containing PAHs and internal standard (IS) was poured into a 10-mL glass centrifuge tube with conical bottom. Extracting solvent, carbon tetrachloride (15 μL), was added, and then the mixture was repeatedly sucked from the tube and dispensed into it with a 10 mL glass syringe. In both sucking and dispensing steps, the solution became more and more turbid. After performing predetermined number of suction – dispersion cycles (seven times), the mixture was centrifuged for 4 min at 4500 rpm. This made the finely dispersed droplets of the extractant was settled down at the bottom of the centrifuge tube (5 ± 0.5 μL). Two microliter of the sedimentary phase was withdrawn and injected into the GC system for analysis.

**3. Results and discussion**

*3.1. Optimization of AALLME*

*3.1.1. Extracting solvent*

The selection of an extracting solvent is of great importance in solvent microextraction methods in order to obtain efficient extraction. In the selection of extracting solvent, some factors should be considered. The selection of a suitable extraction solvent is limited by several characteristics, it must have a low water solubility, to have the ability to extract the analytes of interest and still be compatible with the analytical instrumentation to be used. Accordingly, four organic solvents were evaluated as extracting solvents including carbon tetrachloride, 1,2-dichloroethane, chloroform and 1,2 dichlorobenzene. To achieve similar volumes of the sedimentary phase (10 ± 0.5 μL), 27 μL carbon tetrachloride, 57 μL 1,2-dichloroethane, 72μL chloroform and 25 μL 1,2 dichlorobenzene were used for extraction of target analytes from 5 mL aqueous sample solution spiked with 25 ng mL−1 of each PAHs. The results revealed that 1,2 dichlorobenzene peak, interferes with the analytes. However, carbon tetrachloride was the most effective extracting solvent and gave the highest extraction efficiency for the target analytes among the three solvents investigated (Fig. 1). Hence, carbon tetrachloride was chosen as the extracting solvent.

*3.1.1. The volume of the extracting solvent*

The volume of extracting solvent is a very important parameter. This parameter can affect the achievable enrichment factor (EF) of target analytes in the method, according to the equation (1):

$$EF=C_{sed}/C_0=((n_{sed})(V_w))/((n_0)(V_{sed})) \quad (1)$$

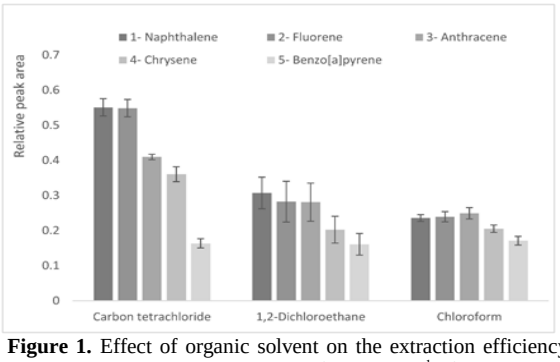


**Figure 1.** Effect of organic solvent on the extraction efficiency. Conditions: sample solution: 5 mL of 25 ng $mL^{-1}$ of each PAH in doubly distilled water; volume of sedimented phase for each extracting solvent: 10 ± 0.5 μL; extraction number: 7 times; centrifugation rate, 4500 rpm; and centrifugation time, 4 min. (1-5: left to right).

Where $C_{sed}$ is the analyte concentration at the sedimented phase, $C_0$ is the initial concentration of analyte, $n_0$ is the total analyte amount, $n_{sed}$ is the amount of analyte which was extracted to the sedimented phase, and $V_{sed}$ and $V_w$ are the volumes of sedimented phase and sample solution, respectively. As can be expected from Eq. (1), the enrichment factor can increase by decreasing the volume of sedimented phase. Also, the extraction recovery (ER) was defined by equation (2):

$$ER=n_{sed}/n_0\times100=((Cs_{ed})(Vs_{ed}))/((C_0)(V_w))\times100=EF\times V_{sed}/V_w\times100 \quad (2)$$

Increasing the volume of the extracting solvent increases the amount of extracted analyte for all chemicals, but by increasing the amount of extracted analyte the volume of sedimented phase is increased too, and enrichment factor is decreased. To study the effect of volume of extracting solvent, different volumes of carbon tetrachloride (15, 20, 25 and 30 µL) were used with the same extraction procedure. The results showed that by increasing the volume of carbon tetrachloride from 15 to 30 µL, the analytical signals were rapidly decreased. It was also found that by increasing the volume of carbon tetrachloride, the volume of the sedimented phase at the bottom of the test tube was increased from 5 to 14 µL. As a result (Fig. 2) at lower volumes of the extracting solvent, high analytical signals could be obtained. Therefore, 15 µL was selected as the optimum volume of the extracting solvent.

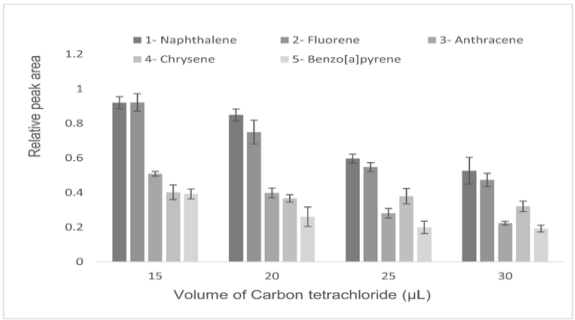


**Figure 2.** Effect of extracting solvent volume on the pre-concentration of PAHs. Conditions: sample solution: 5 mL of 25 ng $mL^{-1}$ of each PAH in doubly distilled water; extraction number: 7 times; centrifugation rate, 4500 rpm; and centrifugation time, 4 min. (1-5: left to right).

### *3.1.2. Effect of salt addition*

The salting out effect has been universally used in SPME and LLE methods. The addition of salt to an analytical sample can potentially increase the analyte extraction recovery in the microextraction procedures. The influence of the ionic strength on the performance of the method was evaluated by adding different amounts of NaCl (0–30%, m/v) into the sample solution. The other experimental conditions (except volume of the extracting solvent) were kept constant. In order to obtain a constant volume of sedimented phase, the experiments were performed using different volumes of the extracting solvent to achieve 5 ±0.5 µL of sedimented phase after applying AALLME (15, 14, 14, and 13 µL for 0, 5, 10, and 30% (m/v) NaCl, respectively). On the other hand, by addition of salt, the viscosity of sample can be increased and this phenomenon can decrease the dispersion of extracting solvent into the aqueous sample. To deal with this problem, before each salt addition, one cycle of extraction (aspiration – dispersion cycle) was performed. The results of GC-FID analysis (Fig. 3) show that in the AALLME method the extraction efficiency of PAHs are not change by increasing the concentration of NaCl. Thus all the experiments were performed without any salt addition.

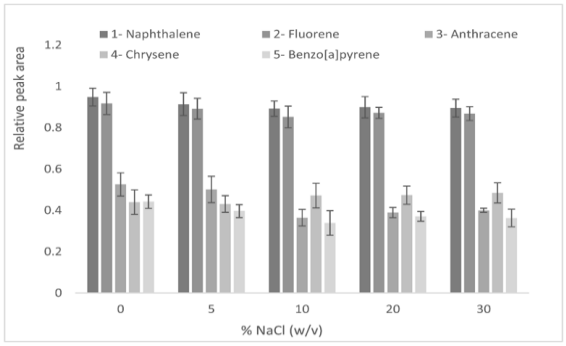


Figure 3. Effect of ionic strength on the extraction efficiency. Conditions: sample solution: 5 mL of 25 ng $mL^{-1}$ of each PAH in doubly distilled water; volume of sedimented phase: 5 ± 0.5 µL; extraction number: 7 times; centrifugation rate, 4500 rpm; and centrifugation time, 4 min. (1-5: left to right)

### *3.1.4 Number of extraction (number of aspiration–dispersion cycles)*

The number of air-agitation cycles in the AALLME method was defined as the number of times which repeatedly aspirated and dispensed the mixture of extracting solvent and the sample solution using a glass syringe. It is expected that by increasing the number of

cycles, there will be an improvement in the extraction efficiency. However, it should be noted that concurrent with rising the cycles, vaporization of the extracting solvent and the other volatile compounds can be increased. When air-agitation is performed more than 10 times, vaporization of the extracting solvent is significant. Therefore, to achieve the best performance, the number of air-agitation cycles was investigated in the range of 1–10. By increasing the number of air-agitation cycles, analytical signals also increased up to the 7th air-agitation cycle and then remained constant (Fig. 4). Hence, 7 times of air-agitations were selected for the following studies.

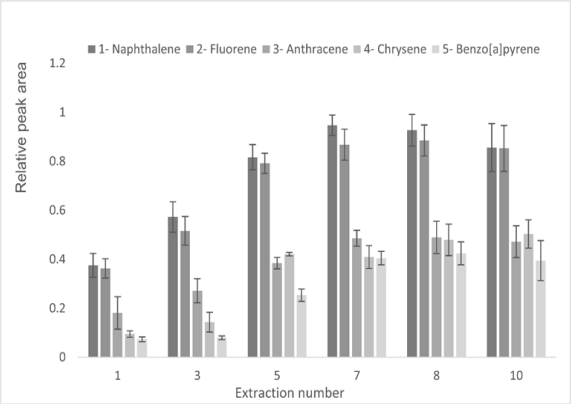


Figure 4. Study of number of air-agitation cycles. Extraction conditions: sample solution: 5 mL of 25 ng $mL^{-1}$ of each PAH in doubly distilled water; volume of sedimented phase: 5 ± 0.5 µL; centrifugation rate, 4500 rpm; and centrifugation time, 4 min. (1-5: left to right)

*3.1.5 Effect of centrifugation time*

Centrifugation is required to break down the emulsion and accelerate the phase-separation process. Centrifugation times at 4500 rpm were examined in the range of 0–5 min. The obtained results show that this parameter is less effective and the volume of the sedimentary phase are not change in higher than 4 min. Thus, 4 min was selected as centrifugation time.

*3.2. Method evaluation*

*3.2.1. Analytical performance of AALLME*

Calibration curves were obtained by extraction and determination of the PAHs by using AALLME method and GC determination of analytes. They were plotted by using a series spiking levels of PAHs in the concentration range of 0.015-120 ng $mL^{-1}$. The method demonstrated good linearities in the concentration range of 0.050-120 ng $mL^{-1}$ for PAHs with the correlation of determinations ($r^2$) ≥ 0.995. The LODs (based on S/N = 3) for PAHs were obtained as 0.015-0.05 ng $mL^{-1}$ also the LOQs (based on S/N = 10) were obtained as 0.05-0.2 ng $mL^{-1}$. The EFs and the ERs% of the 5 mL aqueous sample (10 ng $mL^{-1}$) calculated by the mentioned equations in section 3.3. They were in the ranges of 327-773 and 33-77% for AALLME. Precision was expressed as RSD of five replicate analyses of samples spiked with 25 ng $mL^{-1}$ for method. Results showed that the RSDs were in the range of 4.8-7.7% for AALLME. The results are summarized in Table 1.

**Table1: Analytical performance data for selected PAHs by AALLME.**

| Analyte | LOD[a] | LOQ[b] | LDR[c] | $R^{2[d]}$ | RSD% (n=5)[e] Intra-day (Inter-day) | EF[f] | E[g] % |
|---|---|---|---|---|---|---|---|
| Naphthalene | 0.015 | 0.05 | 0.05-100 | 0.998 | 5.1 (6.8) | 668 | 67 |
| Fluorene | 0.015 | 0.05 | 0.05-100 | 0.999 | 4.8 (5.2) | 691 | 69 |
| Anthracene | 0.05 | 0.2 | 0.2-120 | 0.996 | 5.7 (4.8) | 327 | 33 |
| Chrysene | 0.015 | 0.05 | 0.05-90 | 0.999 | 7.7 (9.8) | 773 | 77 |
| Benzo[a]pyrene | 0.05 | 0.2 | 0.2-120 | 0.995 | 7.5 (9.3) | 397 | 40 |

[a] Limit of detection (S/N = 3) (ng $mL^{-1}$).
[b] Limit of quantification (ng $mL^{-1}$).
[c] Linear dynamic range (ng $mL^{-1}$).
[d] Correlation of determinations.
[e] Relative standard deviation (n = 5, C = 25 ng $mL^{-1}$).
[f] Enrichment factor.
[g] Extraction recovery.

*3.2.2. Real sample analysis*

In order to demonstrate the applicability and reliability of the proposed methods for real samples, the method

was successfully applied to some real samples including tap water from a gas station (Semnan, Iran) and hookah water. Sample preparation for the real samples was performed according to section 2.2. The results showed that tap water sample was all free of PAHs contaminations. To ensure absence of the matrix effects, this sample was spiked with 10 ng $mL^{-1}$ of standard solutions of the analytes, and was extracted under the optimized conditions. Each treatment was in triplicates, and the results are provided in Table 2. These results demonstrated that the different matrix of tap water used in this experiment had little effect on the extraction efficiencies. The existence of PAHs in the hookah smoke was investigated by analysis of these analytes in carbon tetrachloride. Concentration of the trapped PAHs in 10 mL of carbon tetrachloride was determined by using standard addition method (without micro-extraction process). Finally, the recoveries for each PAHs from hookah water were determined by standard addition method and the results are provided in Table 2. The method showed high relative recoveries for hookah water from 90 to 102% which ensured the accuracy of the amount of PAHs detected in non-spiked hookah water. Fig. 5 depicts the AALLME/GC-FID chromatograms of the PAHs in the tap water from a gas station, before and after spiking the sample with PAHs.

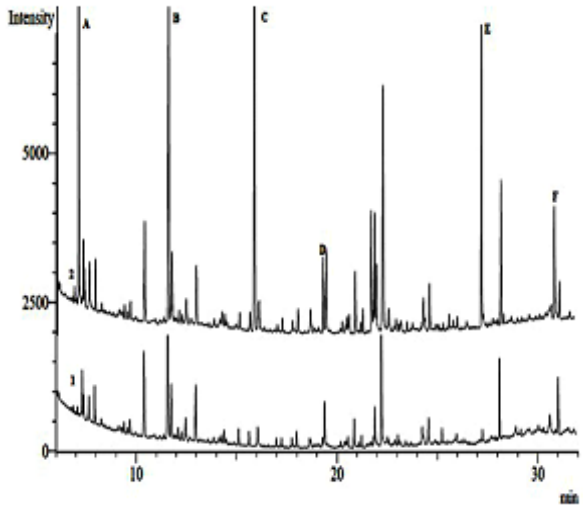


Figure 5. GC-FID chromatograms of the (1) non-spiked and (2) 25 ng mL−1 of each PAH spiked tap water sample. (A) naphthalene, (B) biphenyl (internal standard), (C) fluorene, (D) anthracene, (E) chrysene and (F) benzo[a]pyrene.

**Table 1: Results obtained from analysis of some natural water samples by AALLME.**

| Compound | Hookah water | | | | Tap water from a gas station | | | | Hookah smoke |
|---|---|---|---|---|---|---|---|---|---|
| | Initial[a] | Found[b] | RSD%[c] | RR%[d] | Initial | Found | RSD% | RR% | Concentration (ng $mL^{-1}$)[e] |
| Naphthalene | 0.86 | 9.51 | 2.1 | 95 | <LOD | 10.07 | 3.2 | 101 | 407 |
| Fluorene | 0.42 | 9.02 | 5.7 | 90 | <LOD | 9.30 | 7.8 | 93 | 112 |
| Anthracene | 1.53 | 10.21 | 9.6 | 102 | <LOD | 9.63 | 10.4 | 96 | 104 |
| Chrysene | 2.91 | 10.16 | 16.8 | 102 | <LOD | 10.40 | 10.3 | 104 | <LOD |
| Benzo[*a*] pyrene | 2.88 | 9.64 | 14.3 | 96 | <LOD | 9.71 | 9.1 | 97 | <LOD |

[a]Initial concentration (ng $mL^{-1}$) of PAHs in non-spiked samples that found by AALLME.
[b] The real samples were spiked with 10 ng $mL^{-1}$ of each analyte.
[c] Relative standard deviation based on three replicates of each real sample.
[d] RR, relative recovery.
[e] Concentration of the trapped PAHs in 10 mL of carbon tetrachloride (the Hookah smoke was passed from carbon tetrachloride).

**4. Conclusion**

A free-disperser liquid-phase micro-extraction method abbreviated to AALLME has been successfully utilized for the determination of PAHs in aqueous samples. The method is comfortable, simple, fast, and convenient with organic solvent consumption at μL levels. In this method, the extracting solvent is dispersed into the sample solution in the absence of disperser solvent. In this way, elimination of disperser solvent can reduce the toxicity, solubility of the analytes in the sample solution, and overlapping of analyte peaks with interfering peaks originated from the disperser solvent. Results showed that AALLME with very simple tools can be an efficient

method for extraction of PAHs. Also, analysis of the hookah water and hookah smoke showed this common view among some people that hookah smoke can be healthy because it passes through water, is not true. Based on the obtained results in this study, PAHs (as carcinogenic compounds) can exist in the hookah smoke as well.

**Acknowledgements**

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