Simultaneous Determination of 13 Priority Polycyclic Aromatic Hydrocarbons in Tehran’s Tap Water and Water for Injection Samples Using Dispersive Liquid-Liquid Micro Extraction Method and Gas Chromatography-Mass Spectrometry

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are classified as persistent and carcinogenic organic pollutants. PAHs contamination has been reported in water. Many of relevant regulatory bodies such as EU and EPA have regulated the limit levels for PAHs in drinking water. In this study, 13 priority polycyclic aromatic hydrocarbons (PAHs) were determined in tap water samples of Tehran and water for injection. Dispersive liquid-liquid microextraction procedure combined with gas chromatography-mass spectrometry was used for the extraction and determination of PAHs in the samples. Under the optimized conditions, the range of extraction recoveries and relative standard deviations (RSDs) of PAHs in water using internal standard (anthracene-d_{10}) were in the range of 71-90\% and 4-16\%, respectively. Limit of detection for different PAHs were between 0.03 and 0.1 ngmL\textsuperscript{-1}. The concentration of PAHs in all tap water as well as water for injection samples were lower than the limit of quantification of PAHs. This is the first study addressing the occurrence of PAHs in water for injection samples in Iran using dispersive liquid-liquid micro extraction procedure combined with gas chromatography-mass spectrometry.

Keywords: GC-MS; Polycyclic Aromatic Hydrocarbons (PAHs); Water for injection; Tap water; Iran.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous semi-volatile environmental contaminants (1, 2) and have been often monitored in water, air, soil and food matrices (3). They are principally by-product of incomplete combustion of organic substances and also generated by fossil fuels or plants burning. Due to their ubiquitous presence and potential to cause adverse human health effects, PAHs are a concern in all urban water sources (4). Environmental waters can be contaminated with
PAHs from diverse sources such as industrial and municipal wastewaters, runoff, atmospheric deposition via wet and dry particle deposition and gross gas absorption, oil spills, rain water and from asphalt abrasion (2, 4-6). Long-range atmospheric transport of PAHs has been well documented in different countries (5). After the arrival of PAHs to the atmosphere, they are transferred into water by direct surface contact or as a result of precipitation (5). Occurrence of PAHs in water resources, including drinking water have been reported indifferent parts of the world (5). WHO reported that water is a very significant source of PAHs, and in drinking water, fluoranthene, phenanthrene, pyrene and anthracene were usually detected in it (7, 8). Among the routes of exposure of the general public such as inhalation of ambient and indoor air, dermal absorption, and/or dietary intake, drinking water is also important (9, 10). The typical concentration range for sum of selected PAHs in drinking water vary between 1 ngL$^{-1}$ and 11 µgL$^{-1}$ (7, 8).

The incidence of these compounds in the environment is of reasonable concern, as these are known to be mutagenic and carcinogenic (3). PAHs need metabolic activation to exert their carcinogenic effects, and through a three-step sequence resulting in the formation of diol epoxides, which react with DNA to create adducts that can cause mutations and begin the carcinogenic process (11, 12). Various PAHs vary in carcinogenic capability, but even those that are considered as not being carcinogenic, may act in organisms as synergists, increasing the carcinogenicity of other PAHs (13). According to the international agency for research on cancer classification system, benzo [a] pyrene as one of the most known is classified in group 1 and some of them are also categorized as group 2A or 2B carcinogens (14, 15). Regard to the mutagenic and carcinogenic potential of some PAHs, it was not conceivable to set up a threshold level for PAHs below which danger would be unimportant and so a tolerable daily intake could not be set (16). Therefore, it suggested that exposures to PAHs must be as low as reasonably achievable (17).

PAHs are contaminants which undergo the legislative control or are integrated in the monitoring programs of the EU scientific committee on food (SCF), World Health Organization (WHO), Environmental Protection Agency (EPA), and Food and Drug Administration (FDA) (18). For example, WHO has recommended maximum permissible concentrations of 0.7 µgL$^{-1}$ for benzo [a] pyrene (BaP) in drinking water (19, 20). The international organizations, i.e. WHO, EPA and European Community (EC), have recommended the continual detection and quantification of these compounds in drinking waters (21) and established the maximum residue levels for BaP (20). The permissible level for BaP in drinking water that was proposed by WHO is the same as that of Iranian National Drinking Water Standard (22).

Karyab et al. (23) have reported the measurement of 16 priority polycyclic aromatic hydrocarbons (PAHs) in six stations in Karaj River, which is the main resource of drinking water in Tehran. The single PAHs concentrations ranged from not detected to 2,327.8 ng L$^{-1}$, with a mean value of 31.5 ng L$^{-1}$. The total PAHs concentrations ranged from 25.6, in the spring, to 4,040.3 ng L$^{-1}$, in the summer.

The sample preparation step in an analytical process typically consists of an extraction procedure that results in the separation and enrichment of analytes of interest from a sample matrix. Extraction can differ in degree of selectivity, speed and convenience and depends on the approach, conditions and the type of the extraction phase (24, 26, 35-36). Liquid–liquid extraction (LLE) is among the oldest of the preconcentration and matrix separation techniques in analytical chemistry (24, 26). However, LLE is time-consuming and requires large amounts of organic solvent. Solid-phase extraction (SPE) uses much less solvent than LLE, but can be rather expensive (24, 26).

Dispersive liquid-liquid microextraction (DLLME) offers several important advantages over trivial solvent extraction methods: faster operation, no need of large amounts of organic extraction solvent, low cost and time and easy application (20, 24). Thus, since its introduction, DLLME has been frequently used for determination of organic contaminants in liquid samples, including of PAHs in water samples.
The aim of this study was to determine the level of 13 priority PAHs in different brands of water for injection as well as tap water samples collected from Tehran, Iran, in March, 2014 using DLLME procedure and GC/MS.

**Experimental**

**Standards and reagents**

Naphthalene (Naph.), acenaphthene (Ace.), fluorene (Fl.), phenanthrene (Phen.), anthracene (Ant.), anthracene-d10 (Ant.d10), pyrene (Pyr.), benzo [a] anthracene (B[a]A), chrysene (Chy.), benzo [b] fluoranthene (B[b]F), benzo [k] fluoranthene (B[k]F), benzo [a] pyrene (B[a]P), dibenzo [a, h] anthracene (D[ah]A) and benzo [ghi] perylene (B[ghi]P) with purity higher than 98% were purchased from Sigma-Aldrich/Fluka/Supelco (Germany). HPLC grade isoctane, toluene, acetonitrile, dichloromethane, tetrachloroethylene, n-hexane, ethanol, 2-propanol, methanol, acetone and chloroform were obtained from Chem-lab Belgium. Ultrapure water was obtained from a Milli-Q plus ultra-pure water system (Millipore, Molsheim, France).

**Tap water and water for injection sampling**

According to drinking water supply, Tehran was divided into six regions. In 2014, six tap water samples (each 500 mL) were collected from each region during two weeks, totally 36 samples and stored in amber glass bottles. The water samples were stored in a cold box while being carried to the laboratory and stored at 4°C. Water for injection samples commercially available in Tehran were purchased from drugstores (five different brands, six batches each, totally 30 samples).

**Calibration standards**

Individual stock standard solutions (1 mgmL⁻¹) of the PAHs were prepared in toluene. All the solutions were transferred to amber glass vials and stored at 4°C. They were kept for 30 min. at ambient temperature prior to their use. A mixed intermediate standard solution at a concentration of 100 ngmL⁻¹ was prepared via appropriate dilution of the stock solutions in methanol. This solution was used as a spiking solution for validation experiments. Spiked calibration standards at concentration levels of 0.35, 0.7, 1.4, 2.8, and 5.6 ngmL⁻¹ were prepared by addition of 35 μL, 70 μL, 140 μL, 280 μL and 560 μL of mixed standard stock solution to 10 mL of blank water samples in each case.

A stock solution of anthracene-d10 in toluene at concentration of 1 mgmL⁻¹ was used as internal standard. An aliquot of 70 μL of anthracene-d10 in methanol (100 ngmL⁻¹) was added to the spiked water sample as internal standard. The samples so obtained were treated as described in sample preparation section.

**Extraction and clean-up by using DLLME technique**

We have already developed a method using DLLME technique (that is environmentally friendly, a very simple and rapid method for extraction and preconcentration of organic compounds from water samples and reduce the cost of analysis by reduction of organic solvents) and GC/MS analysis for determination of 13 priority PAHs in the mineral water samples (27). This method was applied for analysis of 13 PAHs in tap water as well as water for injection samples as follows: 10 mL ultrapure water was placed in a 10 mL screwed glass tube and 70μL of anthracene-d10 (as internal standard) at concentration 100ngmL⁻¹ was added to the sample. A mixture of 500 μL chloroform and 1000 μL acetone (as extraction and disperser solvent, respectively) was quickly injected into the sample solution with a 500 μL Hamilton syringe. A cloudy solution was formed after adding the mixture extraction and disperser solvent in the glass tube. The glass tube containing ultrapure water, extraction and disperser solvent was placed in a centrifuge (Hettich Zentrifugen Universas 320R) and centrifuged for 3 min at 2500 rpm. The upper layer was discarded and 500 μL of the lower phase (extraction solvent) was removed by a Hamilton syringe and transferred to 1.5 mL amber glass vial. The contents of the vial were dried using a gentle stream of nitrogen at room temperature and re-dissolved with 70 μL chloroform.

**GC–MS instrumentation and conditions**

The analysis was carried out using GC model Agilent 7890A, Washington, USA, equipped with
a split/splitless injection port, an autosampler model Agilent 7693, electronic ionization and a triple quadrupole mass analyzer model Agilent 7000 series. A HP-5MS 5% Phenyl Methyl Silox, Agilent 19091s-433 capillary column was used with 30 m × 0.25 mm I.D. and 0.25 μm film thickness. Helium with a purity of 99.99% and a flow rate of 1 mL min⁻¹ was used as carrier gas. The injector temperature was retained at 280°C and injection performed in the splitless mode. The initial oven temperature was maintained at 60°C for 0.5 min, increased to 230°C at a ramp rate of 3°C min⁻¹ and kept for 0.5 min, then increased to 290°C at 5°C min⁻¹ and hold for 10 min at the final temperature. Data acquisition was delayed for 12 min. The ionization was performed in ion source with electron impact mode (70ev). The ion source and triple quadrupole mass analyser temperature were kept at 230°C and 280°C, respectively. The vial was placed in autosampler and 2 μL of the contents was injected into the gas chromatograph for analysis. All samples were analysed in duplicate.

Quantitation
PAHs concentrations were calculated by intrapolating the peak ratio for PAH to internal standard on the calibration curve. In order to compensate for losses during sample processing and instrumental analysis, anthracene-d10 was used as internal standard.

Recovery studies
For recovery determination, spiked water samples at concentration levels of 0.35, 0.7, 1.4, 2.8, and 5.6 ngmL⁻¹ were prepared in triplicates and then treated according to the procedure described previously. The recoveries were calculated using the spiked calibration curves.

Results and discussion
Method performance characteristics
In the present study, the spike calibration curves (five points) for all the analytes were constructed by plotting the peak area ratio of each compound to internal standard against the concentration of that PAH. The graphs were constructed from triplicate analysis of the ultrapure water spiked at each concentration level (0.35-5.6 ngmL⁻¹). All 12 spiking calibration curves of PAHs were linear and the coefficients of determination (r²) ranged 0.993-0.999. The repeatability and recovery of method for each 12 analytes ranged 4.0-16.0% and 71-90% respectively. Limits of detection (LOD) and limits of quantification (LOQ) were calculated based on the signal-to-noise ratio of equal to 3 and 10, respectively. LOD of different PAHs were between 0.03 and 0.1 ngmL⁻¹. All validation parameters examined were in compliance with EU criteria.

The recoveries and RSDs of 12 PAHs in quality control samples (QC) are summarized in Table1.

The optimized and validated method was utilized for extraction and determination of studied PAHs in 36 tap water samples and 30 water for injection samples. The samples were not treated (such as filtration) prior to extraction process. The results showed that the concentration of 12 PAHs in all samples (tap water and water for injection) were lower than the LOQ (0.1-0.35 ngmL⁻¹) of the method.

Regarding naphthalene, the results showed that it was present in all analyzed samples including tap water and water for injection samples, and no analyte-free sample as blank matrix for determining the blank sample baseline and constructing the calibration line was available. Therefore, it was not possible to construct the calibration curve for naphthalene, and its concentration in samples was not determined. It is recommended that for accurate determination of naphthalene, an isotope-labeled naphthalene such naphthalene-d8 (surrogate analyte) instead of naphthalene is used as calibration standard. By this approach, the true blank matrix, which is not accessible by other methods and is a prerequisite for accurate quantification specially at trace level is provided (28).

Water treatment plants are the hearts and brains of the practice to develop the water quality through chemical mixing, coagulation, flocculation, sedimentation, filtration, post-filtration treatment, including disinfection, pH control, and final storage at the plant (30). Removal effectiveness of PAHs by the mentioned treatment processes are reported to
be in the range of 20-100% (30). In another study, it was shown that the concentration of PAHs in mineral water samples collected from Tehran were also lower than LOQ (27). There are only few published papers for determination of PAH in tap water. In one of these studies, Hou et al. (31) determined six PAHs in the river water and tap water. The results for tap water showed that it was free of contamination. In another study, Maa et al. (32) evaluated sixteen PAHs in three real water samples including tap water, sea water and river water. No PAHs were found in the tap water samples. Also, Zanjani et al. (33) determined the level of twelve PAHs in well water, sea water and tap water, and reported that tap water was free from PAHs. Our results are in agreement with the above mentioned studies.

However, in some studies, PAHs contamination has been found in tap water. For example, in one of these studies, Karyab et al. (30) evaluated sixteen PAHs standard in the 24 drinking water samples and found contamination including chrysene, benzo[k]fluoranthene and Indeno[1, 2, 3-cd] pyrene at concentration levels higher than the European Union’s drinking water maximum limit. In another study, HuiXu et al. (34) found naphthalene and acenaphthene in the tap water samples at 0.059 ngmL\(^{-1}\) and 0.399 ngmL\(^{-1}\), respectively. Pena et al. (20) determined eighteen PAHs in drinking water samples (tap, bottled, fountain, well). Sum of PAHs concentration were between 127.8 ng L\(^{-1}\) and 413.2 ng L\(^{-1}\).

To the best of our knowledge, this is the first study regarding evaluation of the contamination of water for injection samples with PAHs. Our results showed that the concentration of PAHs in all samples were lower than the LOQ (0.1-0.35 ngmL\(^{-1}\)) of the method.

Considering the few studies regarding PAHs contamination in drinking water in Iran, to get a clear picture of contamination of drinking water, comprehensive monitoring of PAHs in tap water in different Provinces and seasons is suggested.

**Conclusion**

In the present study, no contamination of tap water samples and water for injection samples with PAHs were found. However, a comprehensive survey for analysis PAHs in water is recommended.

**Table 1.** The recoveries and RSDs of 12 PAHs in quality control samples (spiking level: 1.0 ngmL\(^{-1}\)) analyzed using DLLME method and GC-MS.

| Compounds               | Recovery | Average recovery | RSD% |
|-------------------------|----------|-----------------|------|
|                         | Day1     | Day2 | Day3 | Day4 | Day5 |          |      |
| Acenaphthene            | 70       | 74  | 68  | 75  | 69  | 71.2     | 8    |
| Fluorene                | 72       | 65  | 77  | 68  | 73  | 71.4     | 7    |
| Phenanthrene            | 58       | 62  | 56  | 63  | 55  | 58.8     | 5    |
| Anthracene              | 77       | 79  | 82  | 74  | 83  | 79       | 6    |
| Pyrene                  | 61       | 68  | 75  | 71  | 76  | 70.2     | 12   |
| Benzo[a]anthracene      | 89       | 83  | 85  | 81  | 79  | 83.4     | 7    |
| Chrysene                | 76       | 82  | 88  | 90  | 86  | 84.4     | 8    |
| Benzo[b]fluoranthene    | 78       | 74  | 81  | 76  | 80  | 77.8     | 7    |
| Benzo[k]fluoranthene    | 89       | 88  | 79  | 87  | 78  | 84.2     | 3    |
| Benzo[a]pyrene          | 91       | 86  | 84  | 88  | 93  | 88.4     | 10   |
| Dibenzo[a,h]anthracene  | 73       | 78  | 82  | 80  | 79  | 78.4     | 18   |
| Benzo[ghi]perylene      | 85       | 79  | 75  | 78  | 84  | 80.2     | 9    |
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