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Trace-Level Determination of Polycyclic Aromatic Hydrocarbons in Packaged Water Using DLLME Prior to HPLC-FLD Analysis

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) represent the largest group of compounds recognized as carcinogenic agents. The International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (EPA) have classified these compounds based on evidence of carcinogenicity and mutagenicity. Accordingly, a list of 16 compounds, known as EPA-priority PAHs, has been identified as high-risk environmental pollutants.

In this study, dispersive liquid-liquid microextraction (DLLME) combined with high-performance liquid chromatography equipped with fluorescence detection (HPLC-FLD) was employed for the extraction, preconcentration, and determination of PAHs in drinking water. The extraction conditions were optimized, and under optimal conditions, 14 PAH compounds were successfully quantified. Recoveries ranged from 65.5% to 94%, with limits of quantification (LOQ) between 0.003 and 0.12 $\mu\text{g L}^{-1}$. The linear dynamic range for most compounds spanned 0.05–1.5 $\mu\text{g L}^{-1}$. Relative standard deviations (RSD%) at a concentration of 0.7 $\mu\text{g L}^{-1}$ were in the range of 4.5–12.7%. Method validation was performed by simultaneous analysis of a spiked sample based on the standard ISIRI 9387-2 method, with comparison of mean results via the statistical t-test. Finally, the optimized method was applied to determine PAH levels in 40 packaged drinking water samples, revealing no significant contamination in any of the samples. The developed method is simple, cost-effective, and suitable for rapid determination of PAHs in aqueous samples, with potential for adaptation to other matrices with minor modifications.

Keywords Polycyclic aromatic hydrocarbons, Dispersive liquid-liquid microextraction, High-performance liquid chromatography

Introduction

Ensuring access to water of acceptable quality is fundamental to human health, ecosystem integrity, and sustainable development. However, increasing industrial activities have led to the release of a wide range of chemical contaminants that compromise water quality. These contaminants are commonly classified into three major cate-

gories: persistent trace elements (such as heavy metals), organic pollutants, and radionuclides. Among them, organic pollutants include both persistent organic pollutants (POPs) and emerging contaminants, such as pharmaceutical components [1].

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic organic compounds characterized by 2–7 fused ar-



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omatic rings. Ranging from naphthalene ($C_{10}H_8$, MW 128 Da) to coronene ($C_{24}H_{12}$, MW 278 Da), PAHs are semi-volatile with low vapor pressure and ubiquitous in atmospheric, terrestrial, and aquatic environments [2]. Classified by molecular weight, low-molecular-weight PAHs (LPAHs; e.g., naphthalene, phenanthrene) contain 2–3 rings, while high-molecular-weight PAHs (HPAHs; e.g., benzo[α]pyrene, fluoranthene) have 4 or more fused rings [3, 4]. With increasing molecular weight, PAHs exhibit higher hydrophobicity, stronger resistance to degradation, greater environmental persistence, and enhanced potential for bioaccumulation [1]. In recent years, growing concern has been directed toward alkylated PAHs owing to their substantial contribution to total PAH concentrations in crude oil, refined petroleum products, and diesel fuels. Compared to their non-alkylated counterparts, these compounds exhibit slower degradation rates and a greater tendency to accumulate in aquatic environments, particularly following oil spills and industrial discharges, thereby posing serious ecological risks [1, 5, 6]. From a toxicological perspective, sixteen PAHs have been identified by the United States Environmental Protection Agency (USEPA) as priority pollutants due to their widespread occurrence and potential carcinogenicity [7]. Several of these compounds, particularly high molecular weight PAHs such as benzo[α]pyrene, dibenzo[α,h]anthracene, and benzo[α]anthracene, have been classified by the International Agency for Research on Cancer (IARC) as carcinogenic or probably carcinogenic to humans [4]. To evaluate the combined toxicity of PAH mixtures, toxicity equivalency factors (TEFs) have been established relative to benzo[α]pyrene, enabling the calculation of toxicity equivalent concentrations (TECs) and total toxicity equivalent concentrations (TTECs) in environmental samples [1].

Because PAHs exhibit low aqueous solubility and are typically present at trace levels in water, sensitive extraction and preconcentration procedures are required prior to instrumental determination. Various techniques have been developed for this purpose, including liquid–liquid extraction (LLE)[8], solid-phase extraction (SPE) [9], solid-phase microextraction (SPME)[10], hollow-fiber liquid-phase microextraction (HF-LPME)[11], and dispersive liquid–liquid microextraction (DLLME)[12]. Among these methods, DLLME has gained considerable attention due to its simplicity, rapidity, low solvent consumption, high enrichment factor, and environmental compatibility. The technique is based on the rapid dispersion of a small volume of extraction solvent into an aqueous sample using a disperser solvent, producing a cloudy solution with a large interfacial area that enables fast mass transfer and efficient analyte extraction[12].

Following extraction, PAHs are commonly quantified using validated analytical techniques recommended by international organizations such as USEPA and ISO. These methods primarily rely on chromatographic techniques, particularly high-performance liquid chromatography (HPLC) and gas chromatography (GC), coupled with sensitive detectors including fluorescence, ultraviolet, and mass spectrometry, owing to their superior selectivity, sensitivity, and reliability for PAH determination in complex environmental matrices [1, 13].

In this study, a dispersive liquid–liquid microextraction method combined with high-performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD) is proposed for the determination of priority PAHs in packaged drinking water. The extraction parameters were optimized, and the analytical performance of the method was validated through comparison with the Iranian National Standard method ISIRI. 9387-2 [14]. The developed method was subsequently applied to the determination of USEPA priority PAHs in packaged drinking water samples.

2. Material and methods

2.1. Chemicals and Reagents

A certified standard mixture of 16 PAHs, including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[α]anthracene, chrysene, benzo[β]fluoranthene, benzo[k]fluoranthene, benzo[α]pyrene, dibenzo[α,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3- cd] pyrene, at a concentration of $10\text{ }\mu\text{g mL}^{-1}$ in acetonitrile was obtained from Supelco (USA). A stock solution ($1\text{ }\mu\text{g/mL}$) was prepared by diluting 1.0 mL of the certified standard mixture to 10 mL with acetonitrile. Working standard solutions were freshly prepared by serial dilution of the stock solutions using acetonitrile.

All organic solvents, including methanol, acetone, acetonitrile, carbon tetrachloride, bromobenzene, chloroform, tetrachloroethylene, cyclohexane, and *n*-hexane, as well as sodium chloride (NaCl), were of analytical grade and purchased from Merck (Germany). A 10% (w/v) sodium chloride solution was prepared by dissolving 10 g of NaCl in deionized water and diluting to 100 mL.

A total of forty packaged drinking water samples representing 29 commercial brands were collected from retail markets in Tehran and Karaj. In addition, municipal tap water samples from Tehran and Karaj and a groundwater sample were analyzed for comparative purposes.

2.3. Instrumentation

Chromatographic analysis was performed using an HPLC system (Young Lin, Model 9100, Korea) equipped with a

column oven and a programmable fluorescence detector (FLD) capable of time-dependent excitation and emission wavelength switching. Separation was achieved using a PAH-specific reversed-phase column (250 mm × 4.6 mm i.d., 5 µm particle size; CNW Technologies).

The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), applied under gradient elution conditions as summarized in Table 1. The injection volume was 60 µL, introduced via a 20 µL sample loop. The column temperature was maintained at 30 °C.

Time-programmed excitation and emission wavelengths for the fluorescence detector were optimized for the determination of the 15 target PAHs and are presented in Table 2. Due to minor instrumental fluctuations affecting retention times, a mixed PAH standard solution was injected daily alongside samples to ensure accurate compound identification and appropriate adjustment of detector settings.

Table 1. HPLC gradient elution program

	Time (min)	Solvent A(%)	Solvent B(%)	Flow[mL/min]
1	0	50	50	1.0
2	8	30	70	1.0
3	16	60	40	1.0
4	18	10	90	1.2
5	20	10	90	1.2
6	30	0	100	1.2
7	45	0	100	1.2

2.3. Extraction procedure

2.3.1 Dispersive Liquid–Liquid Microextraction Procedure

For optimization of the DLLME parameters, 30.0 mL of distilled water was transferred into a 50 mL conical centrifuge tube and spiked with a PAH standard mixture at a concentration of 0.5 µg L⁻¹. Different volumes (0.5–4.0 mL) of a 10% (w/v) NaCl solution were added to evaluate the salting-out effect. Subsequently, a mixture consisting of 2–7 mL of disperser solvent containing 10–40 µL of extraction solvent per milliliter was rapidly injected into the aqueous sample using a syringe, resulting in the formation of a stable cloudy solution. The mixture was gently shaken to ensure efficient dispersion of the extraction solvent throughout the sample.

The resulting suspension was centrifuged at 4000 rpm for 5 min, allowing the extraction solvent to sediment at the bottom of the conical tube. The sedimented phase was completely collected and transferred into a small vial, evaporated to dryness under a gentle nitrogen stream at 35 °C, and reconstituted in 200 µL of acetonitrile to improve repeatability and ensure compatibility with the HPLC system. The reconstituted extracts were stored at –18 °C until analysis, and a volume of 60 µL was injected into the HPLC–FLD system.

Under the optimized conditions, the developed DLLME procedure was applied to real water samples. Briefly,

Table 2. FLD time-program for the determination of 15 PAHs

Group	Compound	Wavelength switching time (min) (a)	Excitation wavelength (nm)	Emission wavelength (nm)
1	Naphthalene	0	270	324
	Acenaphthene			
	Fluorene			
2	Phenanthrene	13.3	248	375
	Anthracene			
3	Fluoranthene	16.6	280	462
4	Pyrene	18.3	270	385
	Benzo[a]anthracene			
	Chrysene			
5	Benzo[b]fluoranthene	24.5	256	446
	Benzo[k]fluoranthene			
	Benzo[a]pyrene			
6	Dibenzo(h,a)anthracene	27.1	292	410
	Benzo(i,h,g)perylene			
7	Indeno(1,2,3-c,d)pyrene	34.1	274	507

(a) Due to instrumental limitations, retention times were not constant throughout the study. Therefore, standard solutions were injected daily along with samples to ensure proper identification of the target PAHs and to adjust the instrumental conditions accordingly.

30.0 mL of each water sample was mixed with 1.0 mL of a 10% (w/v) NaCl solution, followed by rapid injection of 6.0 mL of acetone containing tetrachloroethylene as the extraction solvent (disperser-to-extraction solvent ratio of 50:1, corresponding to 20 μL of extraction solvent per milliliter of disperser solvent). The subsequent steps, including centrifugation, collection of the sedimented phase, solvent evaporation, reconstitution, storage, and injection into the HPLC–FLD system, were performed as described above. Standard solutions were processed following the same extraction procedure to ensure consistency.

2.3.2. Standard Extraction Method Based on a National Standard Procedure

To validate the developed DLLME method, a spiked water sample was analyzed in parallel using both the optimized DLLME procedure and the Iranian National Standard method No. 9387-2 (ISIRI) [14]. The analytical results obtained by the two methods were statistically compared using a t-test.

To extract PAHs through the standard method, 100 mL of the water sample was transferred into a glass container and extracted with 2.5 mL of cyclohexane under mechanical stirring for 1 h. The mixture was transferred to a separatory funnel, and the organic phase was collected. The remaining aqueous phase was re-extracted twice with 1.0 mL portions of cyclohexane. The combined organic extracts were evaporated to dryness under a nitrogen atmosphere at 35 °C, and the residue was reconstituted in 600 μL of acetonitrile. Finally, 60 μL of the resulting solution was injected into the HPLC-FLD system for analysis.

3. Results and Discussion

3.1. Direct Calibration and Compound Identification

To evaluate method performance and calculate analyte recoveries during both the optimization and final analytical stages, direct calibration was initially performed for the sixteen target PAHs. For this purpose, a series of PAH standard solutions with concentrations of 5, 10, 20, 40, 60, 80, and 100 $\mu\text{g L}^{-1}$ were prepared in acetonitrile and directly injected into the HPLC-FLD system. Among the sixteen PAHs included in the standard mixture, fifteen compounds were successfully identified and calibrated. Acenaphthylene could not be quantified due to insufficient detector sensitivity at the investigated concentration levels. The chromatogram obtained from direct injection for calibration at 60 $\mu\text{g L}^{-1}$ is shown in Figure S1, while the identified compounds, their corresponding abbreviations used throughout the study, and their retention times are summarized in Table S2. For confirmation of compound identity, daily injections of standard mixtures

were performed, and retention times were monitored to account for minor instrumental variations.

3.2. Optimization of DLLME Conditions

To achieve maximum extraction efficiency and analytical sensitivity, the DLLME procedure was optimized by systematically evaluating parameters expected to significantly influence PAH recovery. Based on literature review and preliminary experiments, the type of extraction solvent and disperser solvent, the volume ratio of extraction solvent to disperser solvent, the total volume of the injected solvent mixture, and salt concentration were selected as critical variables for optimization. Parameters such as extraction time and centrifugation time were not optimized, as previous studies have demonstrated their negligible influence on DLLME recoveries. The effect of salt addition was also investigated, since variations in salinity among water matrices may influence extraction performance through salting-out or, in some cases, salting-in effects, thereby affecting both analytes partitioning and the volume of the sedimented phase.

3.2.1. Selection of the Extraction Solvent

Extraction solvents with densities higher than water and low water solubility were evaluated, including carbon tetrachloride, tetrachloroethylene, trichloroethylene, and a hexane/tetrachloroethylene (1:1, v/v) mixture. Although carbon tetrachloride provided slightly higher recoveries, it showed poor repeatability due to its volatility. The hexane/tetrachloroethylene mixture improved recovery for high-molecular-weight PAHs but performed inadequately for lighter compounds. Considering overall recovery, repeatability, and chromatographic compatibility, tetrachloroethylene was selected as the extraction solvent. The average recovery values of each compound using different extraction solvents are presented in Figure 1(a).

3.2.2. Selection of the Disperser Solvent

The disperser solvent must be miscible with both aqueous and organic phases and capable of forming a stable cloudy solution. Acetone, acetonitrile, and methanol were evaluated. Comparable recoveries were obtained using acetone and methanol, whereas acetonitrile showed no significant improvement (Figure 1(b)). Due to its lower toxicity, cost-effectiveness, and satisfactory extraction performance, acetone was chosen as the disperser solvent.

3.2.3. Optimization of Extraction Solvent Volume

The volume of extraction solvent directly affects enrichment factor and phase stability. Volumes ranging from 10 to 40 μL of tetrachloroethylene per 1 mL of disperser solvent were tested. Although PAH recoveries varied slight-

ly depending on molecular weight, 20–30 μL provided consistently high recoveries for all analytes (Figure 1(c)). To ensure adequate sedimented phase volume and better repeatability, 20 μL mL^{-1} was selected for subsequent experiments.

3.2.4. Effect of Salt Addition

Although PAHs are neutral compounds, salt addition can influence extraction efficiency through salting-out effects and by increasing the sedimented phase volume. Different volumes of a 10% (w/v) NaCl solution were

examined. Low salt content resulted in reduced recoveries, while higher amounts showed no significant improvement (Figure 1(d)). Therefore, 1.0 mL of 10% (w/v) NaCl (final concentration 0.33% w/v) was selected as the optimal salt level.

3.2.5. Optimization of the Extraction Mixture Volume

The volume of the injected extraction mixture (acetone/tetrachloroethylene, 50:1, v/v) was varied between 1 and 6 mL. Recoveries increased with increasing volume and

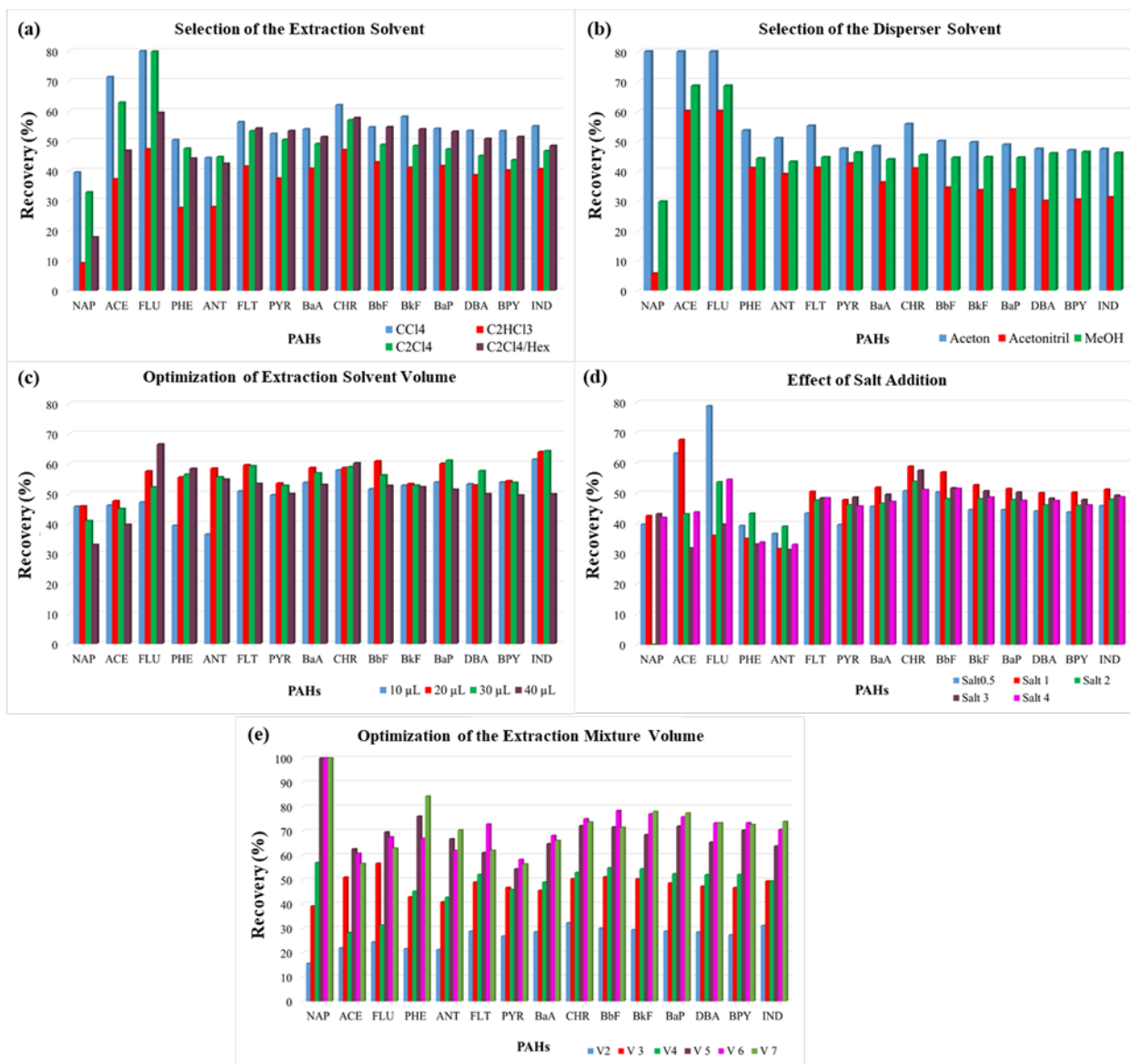


Fig 1. (a) Optimization of DLLME parameters for the extraction and determination of polycyclic aromatic hydrocarbons (PAHs) in water samples. (a) Selection of extraction solvent. (b) Selection of disperser solvent. (c) Optimization of extraction solvent volume (d) Effect of salt addition. (e) Optimization of extraction mixture volume. Recovery percentages are shown for individual PAHs

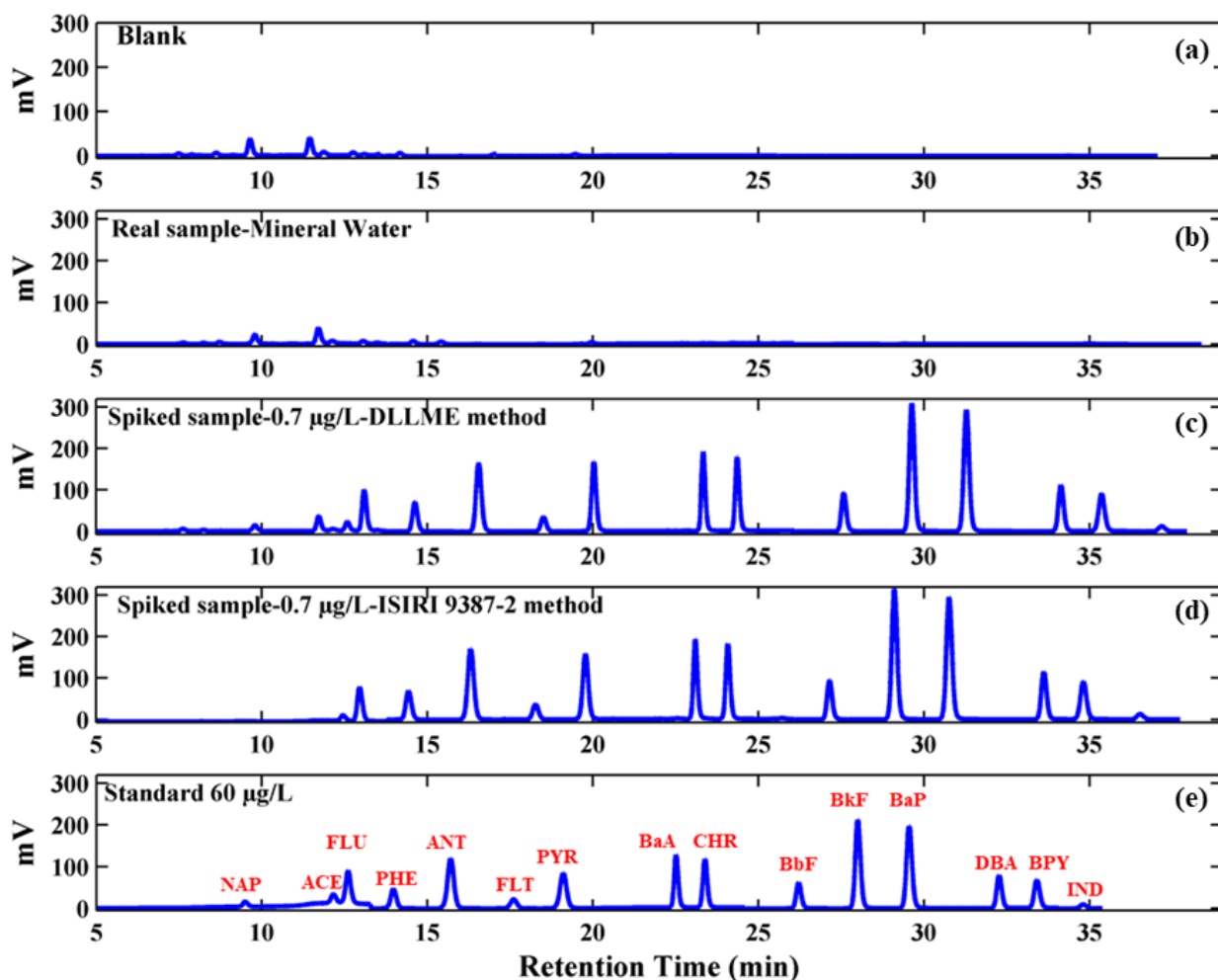


Fig 2. Chromatograms of (a) blank sample, (b) real sample (drinking water), (c) real sample spiked with 0.7 µg/L PAHs mixture and extracted by the optimized DLLME method, (d) real sample spiked with 0.7 µg/L PAHs mixture and extracted by the standard method (ISIRI 9387-2), and (e) direct injection of the standard mixture at 60 µg/L concentration.

reached a plateau at 6 mL, likely due to enhanced extraction capacity (Figure 1(e)). Consequently, 6.0 mL of the extraction mixture was selected as the optimal volume.

3.3. Optimized DLLME Conditions

Based on the optimization results, the optimal DLLME conditions for PAH extraction from drinking water samples were as follows: 30 mL of sample volume, addition of 1.0 mL of 10% (w/v) NaCl, rapid injection of 6.0 mL of acetone containing tetrachloroethylene at a disperser-to-extraction solvent ratio of 50:1, followed by centrifugation at 4000 rpm for 5 min. The sedimented phase was evaporated under nitrogen at 35 °C, reconstituted in 200 µL of acetonitrile, and analyzed by HPLC-FLD.

3.4. Figures of Merit and Method Validation

Calibration curves for each analyte were constructed by spiking ultrapure water samples with different concentrations of the standards, followed by extraction and analysis under the optimized conditions. The corresponding calibration parameters, along with the limits of detection (LOD), limits of quantification (LOQ), recovery, and repeatability data, are summarized in Table 3. LOD and LOQ for the DLLME method were determined using three blank samples alongside standard solutions. Noise levels were calculated as the standard deviation of the baseline signal (SD_b) at the analytes' retention times. LOD and LOQ values were computed as $\frac{3 \times SD_b}{\text{slop}(m_{cal})}$ and $\frac{10 \times SD_b}{\text{slop}(m_{cal})}$, respectively.

Table 3. Calibration equations and figures of merit for DLLME extraction of PAHs, compared to standard method.

No.	Compound	Abbr.	Calibration Equation	R ²	LOD (µg/L)	LOQ (µg/L)	Sample (µg/L)	Spiked Sample (µg/L)	Recovery %	RSD % (n=3)	Spiked Sample (Standard Method) (µg/L)	Recovery % (Standard Method)	RSD % (n=3)
1	Naphthalene	NAP	-	-	-	-	-	-	-	-	0.019	2.76	73.19
2	Acenaphthene	ACE	$y = 392.89x + 1.068$	0.997	0.005	0.017	<LOD	0.658	93.99	12.87	0.203	28.94	22.03
3	Fluorene	FLU	$y = 1723.5x + 43.392$	0.997	0.029	0.097	<LOD	0.535	76.46	10.99	0.303	43.31	12.73
4	Phenanthrene	PHE	$y = 1466.8x + 57.11$	0.984	0.035	0.117	<LOD	0.472	67.38	4.97	0.261	37.31	2.15
5	Anthracene	ANT	$y = 4059.4x + 4.832$	0.982	0.002	0.007	<LOD	0.536	76.54	4.54	0.441	63.00	4.34
6	Fluoranthene	FLT	$y = 883.88x + 4.693$	0.982	0.007	0.022	<LOD	0.458	65.50	4.24	0.415	59.24	2.14
7	Pyrene	PYR	$y = 3380.2x + 44.302$	0.985	0.001	0.003	<LOQ	0.485	69.37	6.16	0.385	54.97	2.25
8	Benzo[α]anthracene	BaA	$y = 3208.3x + 1.703$	0.986	0.002	0.007	<LOD	0.504	72.00	7.44	0.424	60.61	2.47
9	Chrysene	CHR	$y = 3205.8x + 7.43$	0.989	0.010	0.034	<LOD	0.513	73.27	7.18	0.472	67.38	1.63
10	Benzo[β]fluoranthene	BbF	$y = 1921.1x + 6.23$	0.989	0.008	0.028	<LOD	0.515	73.58	7.21	0.483	68.98	1.75
11	Benzo[k]fluoranthene	BkF	$y = 7012.2x + 8.74$	0.980	0.005	0.016	<LOD	0.498	71.08	7.13	0.492	70.28	1.93
12	Benzo[α]pyrene	BaP	$y = 6964.9x + 4.24$	0.982	0.001	0.004	<LOD	0.498	71.08	7.50	0.462	65.99	2.00
13	Dibenz[a,h]anthracene	DBA	$y = 2541.9x + 1.411$	0.980	0.002	0.005	<LOD	0.494	70.64	7.26	0.468	66.82	1.92
14	Benzo[g,h,i]perylene	BPY	$y = 2368x + 12.794$	0.975	0.012	0.040	<LOD	0.511	73.04	4.04	0.429	61.29	2.41
15	Indeno[1,2,3-cd]pyrene	IND	$y = 370.79x + 1.228$	0.983	0.001	0.004	<LOQ	0.495	70.69	8.65	0.476	68.00	1.50

Method recovery was evaluated by spiking a real water sample with 0.7 µg/L of the PAHs standard mixture. Recoveries ranged from 65.5% to 94%, with an average recovery of approximately 70%. Repeatability was assessed through triplicate extraction of the spiked sample; relative standard deviations (RSDs) were below 10% for all analytes except for phenanthrene.

Figure 2 shows representative chromatograms of (a) a blank sample, (b) a real sample, (c) the spiked sample, and (d) a direct injection of the 60 µg/L standard solution, demonstrating the method's sensitivity and selectivity.

3.5. Method Validation of DLLME

To validate the developed DLLME extraction method, a spiked real sample was simultaneously extracted using both the DLLME method and the standard method (ISIRI 9387-2). The resulting concentrations obtained from both methods were statistically compared using a t-test. The concentration results for all tested PAHs are presented in Table 3.

Since only six PAH compounds (fluoranthene, benzo[β]fluoranthene, benzo[k]fluoranthene, benzo[α]pyrene,

benzo[ghi]perylene, and indeno(1,2,3-cd)pyrene) are included in the scope of the standard method, the t-test comparison was performed only for these compounds. According to Table 3 and the recovery data, the standard method showed poor recovery for lighter PAHs, unlike the DLLME method, while both methods showed acceptable agreement for heavier PAHs. The chromatogram obtained by the standard method is shown in Figure 2(e). The results of the t-test comparing the mean concentrations of the six PAHs measured by the standard and DLLME methods are summarized in Tables S3-S8. Both one-tailed and two-tailed tests were conducted. In all cases, the calculated t-values (t stat) were less than the critical t-values (t critical), indicating no significant difference between the two methods. It is noteworthy that concentration measurements in the standard method were based on direct injection calibration, whereas in the DLLME method they were determined using the calibration curve derived from the extraction procedure.

3.6. Measurement of 15 PAHs in Real Samples

Forty bottled water samples from 29 different brands,

Table 4. Determined levels of polycyclic aromatic hydrocarbons (PAHs) in real water samples

[illegible]

types, and production dates were collected (Table 4) and analyzed for concentrations of polycyclic aromatic hydrocarbons (PAHs). Additionally, well water samples from Karaj and tap water samples from Tehran and Karaj were tested. The results are summarized in Table 4.

According to the Iranian national drinking water standard (INSO 1053) [15], the maximum allowable concentration of benzo[α]pyrene in drinking water is 0.0007 mg/L (equivalent to 0.7 μ g/L). As the results in Table 4 suggest, all samples contain PAHs at concentrations well below this limit, indicating compliance with safety guidelines. It should be noted that the identified compounds in these chromatograms represent preliminary findings, and many of the detected levels are effectively below the limit of detection (LOD) when compared to blank sample values. In 20 out of 43 samples (46.5%), the concentrations of all 14 PAHs were below the quantifiable level, and in 17 samples (39.5%), only one PAH was detected at a quantifiable level, which was just slightly above the method's LOQ, indicating very minor contamination. Sample B25 showed relatively higher PAH levels compared to other samples, although still well below the maximum permissible limit.

All samples were free of fluorene (the observed peak corresponds to blank contamination). Pyrene was the most frequently detected contaminant, though its concentration was generally very low, mostly below 0.02 μ g/L. The highest contamination was benzo[ghi]perylene in the B35 sample at 0.047 μ g/L. Naphthalene peaks were present in all samples; however, due to blank sample signals, accurate assessment of naphthalene contamination was not possible.

4. Conclusion

In the present study, a dispersive liquid–liquid microextraction (DLLME) procedure coupled with high-performance liquid chromatography–fluorescence detection (HPLC–FLD) was successfully optimized for the determination of polycyclic aromatic hydrocarbons (PAHs) in drinking water samples. It should be noted that this extraction approach had previously been optimized in combination with gas chromatography–flame ionization detection (GC–FID) for PAH analysis in aqueous matrices. The proposed DLLME–HPLC–FLD method is simple, rapid, and cost-effective, while providing satisfactory sensitivity and repeatability for the target analytes.

Method validation was performed for six PAHs, including fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[α]pyrene, benzo[ghi]perylene, and indeno(1,2,3-c,d)pyrene, through simultaneous analysis of a sample using the Iranian National Standard method

(ISIRI 9387-2). Based on the validation results, the proposed method demonstrated acceptable recoveries and analytical figures of merit for six out of the sixteen EPA priority PAHs, while yielding reasonably satisfactory performance for an additional eight compounds.

The optimized method was subsequently applied to the analysis of 40 bottled drinking water samples and three municipal water samples. The results obtained from real sample analysis indicated that the investigated waters were essentially free of significant levels of PAHs. These findings further confirmed the capability and reliability of the developed method for trace-level determination of PAHs in aqueous matrices.

It is noteworthy that the determination of acenaphthylene at very low concentrations remains challenging when using HPLC–FLD. Additionally, naphthalene exhibited a high and poorly reproducible signal in blank samples under the optimized conditions, suggesting the presence of an interfering peak. This limitation could potentially be addressed through modification of the extraction procedure or by changing the solvents used, which may allow reliable calibration and quantification of naphthalene. Alternatively, coupling the extraction method with gas chromatography–mass spectrometry (GC–MS) could enable the determination of a broader range of PAHs especially for LPAHs.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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