



Optimization of HPLC method for polycyclic aromatic hydrocarbons quantification in aqueous samples

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Abstract: The current study successfully optimized the liquid-liquid extraction technique/reverse-phase high performance liquid chromatography and ultraviolet detection (LLE-HPLC-UV) approach for the measurement of 16 polycyclic aromatic hydrocarbons (PAHs) in aqueous samples. LLE is revealed to be effective in the extraction of 16 PAHs. The methodology's performance was assessed in terms of linearity, detection and quantification limits, and recovery. Good linearity for all PAHs were obtained. Low LOD values (0.003-0.131 µl/mL) were obtained making the HPLC-UV method particularly sensitive for PAHs determination. Reasonable recovery was obtained for all PAHs. Under the analytical conditions utilized, no PAH was identified in the genuine water samples examined.

Abbreviations— ACEE: acenaphthene; ACEY: acenaphthylene; ANTA: anthracene; BAAA: benzo(a)anthracene; BAPY: benzo(a)pyrene; BBFA: benzo(b)fluoranthene; BGPY: benzo(g, h, i)perylene; BKFA: benzo(k)fluoranthene; CHYS: chrysene; DBAT: dibenzo(a, h)anthracene; FLRE: fluorene; FLAT: fluoranthene; HPLC: high performance liquid chromatography; IDPY: indeno(1,2,3-cd)pyrene; LOD: limit of detection; LOQ: limit of quantification; NAPT: naphthalene; PAHs: polycyclic aromatic hydrocarbons; PHNT: phenanthrene; POP: persistent organic pollutant; PYRY: pyrene; r: correlation coefficient; RSD: relative standard deviation; SD: standard deviation.

Keywords:

high-performance liquid chromatography and ultraviolet detection (HPLC-UV); liquid-liquid extraction; polycyclic aromatic hydrocarbons; water analysis

1. INTRODUCTION

Environmental pollution is one of the most serious global challenges, as pollution provides a direct threat to human health and other living organisms. Polycyclic aromatic hydrocarbons (PAHs) are common permanent organic contaminants that are mostly discharged into the environment as a result of insufficient burning of organic matter during natural or anthropogenic processes [1–3]. Sixteen unsubstituted PAHs have been approved as preference pollutants by the US Environmental Protection Agency (EPA) [4–5]. The US Department of Health and Human Services recommends a maximum permissible concentration of PAHs in water of 0.005–3.0 ppm [6]. The largest anthropogenic sources of PAHs include power plants, waste incineration, industrial activities, household heating, and, most critically, motor vehicle exhaust emissions [1–3]. These compounds are of great importance to many researchers due to their carcinogenic, mutagenic and immunotoxic properties [3]. As a result, there is an increasing interest in determining PAHs in environmental samples such as air, water, and soils in order to

conserve human health and the ecosystem [7–8]. Coal tar, which is used to save iron pipes from rust, is the principal cause of PAHs contamination in drinking water in underdeveloped nations [1, 9]. PAHs can also be introduced into the aqueous media by industrial waste water and particulate matter transported by the wind and rains [8]. As a result, developing methods for identifying and quantifying PAHs in water samples at trace levels to validate drinking water quality criteria became critical [10]. Instrumental analysis usually requires a sample pre-concentration procedure to determine trace PAHs in aquatic environments [7]. However, extracting PAHs at trace levels from aquatic samples is a difficult task because no single approach is capable of successfully analyzing all organic contaminants prevalent in this matrix [11]. Many methods for extracting and pre-concentrating PAHs from aquatic materials have been devised, including liquid-liquid extraction (LLE) [11–18], Solid-phase extraction (SPE) [4, 11–12, 19–21], solid-phase microextraction (SPME) [6], stir bar sorptive extraction (SBSE) [22], liquid-phase microextraction (LPME) [23–25], Salting-out Liquid-Liquid Extraction (SALLE) [9], dispersive liquid-liquid microextraction (DLLME) [26–27], portable micro-solid phase extraction

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(μ -SPE) [28], fabric phase sorptive extraction (FPSE) [29], ultrasonic assisted emulsification micro-extraction (USAEME) [30], directly suspended droplet microextraction (DSDME) and Indirectly suspended droplet microextraction (ISDME) [31]. Although LLE is one of the oldest technique for isolating PAHs from water, it gives excellent results with high recoveries [13]. For the determination of PAHs, high-performance liquid chromatography and gas chromatography with multiple detectors are widely used [32-33].

For the quantification of PAHs in various aqueous samples at low concentrations (0.003-0.131 μ L/mL), this work offers the optimization of a liquid-liquid extraction technique/reverse-phase high performance liquid chromatography and ultraviolet detection methodology. The methodology's performance was assessed in terms of linearity, detection and quantification limits, and recovery.

2. MATERIALS AND METHODS

Extraction Methods

A 1L water sample was placed into a 2-L separator funnel to extract PAHs. 60 mL methylene chloride was added to the separator funnel, and the sample was extracted after two minutes of shaking. After that, the organic layer was separated from the aqueous phase for at least 10 minutes. The extraction method was repeated three times, with the extracts being combined in an Erlenmeyer flask. Thereafter, 10 g of anhydrous sodium sulfate was passed through a solvent-rinsed drying column. The Erlenmeyer flask and column were washed with 30 mL of methylene chloride. The methylene chloride extract was then evaporated to dryness using rotary evaporator. PAHs were extracted and redissolved in 2 mL acetonitrile before being stored in HPLC vials.

Determination

PAHs were separated and quantified using an HPLC equipment that followed the parameters and conditions listed in Table 1. Before being injected into the HPLC equipment, all solutions were degassed for 10 minutes in an ultrasonic bath. PAH mixed standard solutions at five concentration levels were used to calibrate the HPLC apparatus. To ensure the procedure's reproducibility, each solution was injected three times. Under ideal analytical conditions, the PAHs compounds were identified using the UV spectrum of individually injected PAHs standard solutions and the correspondence of their retention times in samples with those in standards. The linear least squares calibration equations were used to calculate PAH concentrations. Equations 1 and 2 were used to obtain the LOD and LOQ, where S is the slope of the calibration curve and SD is the standard deviation of the response.

$$\text{LOD} = 3 \sigma/S \quad (1)$$

$$\text{LOQ} = 10 \sigma/S \quad (2)$$

Table 1: Analytical circumstances for HPLC equipment

Equipment	Shimadzu HPLC System LC-10AT		
Integrator	Shimadzu Class-VP		
Detector	Shimadzu UV-VIS Detector SPD 10A		
Column	Supelcosil LC-PAH; 250 x 4.6 mm, 5 μ m		
Temperature:	30 $^{\circ}$ C		
Injection volume	10 μ l		
Pump: Flow rate	1.5 ml/min		
Run time	55 minutes		
Wavelength	254 nm		
Mobile Phase	Channel A: 100 % Water (purified) Channel B: 100 % Acetonitrile		
Gradient	Time	% Channel A	% Channel B
	[min]		
	0	60	40
	5	60	40
	45	0	100
	55	0	100

Chemicals and Reagents

Acetonitrile, dichloromethane and Sodium sulfate were obtained from Sigma-Aldrich, USA. All the chemicals used were analytic grade chemicals and were employed in this study without further purification. The PAHs single reference materials were obtained from Supelco, USA as specified in Table 2. Mixture of sixteen PAHs reference materials in the concentration rang of 100 – 2000 μ g/mL were also purchased from Supelco, USA.

Table 2: PAHs single reference materials

Analyte	Product Number	Concentration [μ g/ml]	Supelco Lot No.
Acenaphthene	48643	200	LB90931
Acenaphthylene	48630	200	LB90672
Anthracene	48647	200	LB91669
Benzo(a)anthracene	48651	200	LB83394
Benzo(a)pyrene	48665	200	LB88272
Benzo(b)fluoranthene	48637	200	LB80011
Benzo(g,h,i)perylene	48667	200	LB80145
Benzo(k)fluoranthene	48668	200	LB88549
Chrysene	48650	200	LB90308
Dibenz(a,h)anthracene	48652	200	LB80169
Fluoranthene	48662	200	LB83295
Fluorene	48664	200	LB78464
Indeno(1,2,3-cd)pyrene	48669	200	LB87865
Naphthalenr	48641	200	LB80528
Phenanthrene	48661	200	LB80963
Pyrene	48649	200	LB80171

Samples

Table 3: LOD, LOQ and Calibration equations, for various PAHs standards (mg/ml).

PAH	tr (min)	Equation	R	LOD (µg/mL)	LOQ (µg/mL)	Recovery%
NAPT	19.230	$y = 5681x + 294.34$	0.9978	0.131	0.456	39.64
ACEY	22.077	$y = 4489x - 5.5221$	0.9999	0.082	0.272	66.17
ACEE	22.907	$y = 2600x - 122.22$	0.9997	0.065	0.227	84.64
FLRE	24.923	$y = 29264x - 99.054$	0.9999	0.009	0.035	86.19
PHNT	26.732	$y = 79221x - 4.1383$	0.9999	0.005	0.025	76.36
ANTA	28.857	$y = 136631x + 245.10$	0.9997	0.007	0.023	74.57
FLAT	30.289	$y = 19506x - 104.11$	0.9998	0.011	0.024	94.34
PYRY	35.185	$y = 15411x - 96.25$	0.9993	0.011	0.024	77.52
BAAA	36.190	$y = 42247x - 255.02$	0.9999	0.005	0.024	78.39
CHYS	40.192	$y = 64794x - 172.41$	0.9999	0.004	0.010	82.31
BBFA	41.937	$y = 46260x - 220.64$	0.9999	0.007	0.023	68.73
BKFA	43.548	$y = 37106x - 167.37$	0.9999	0.003	0.011	97.48
BAPY	46.010	$y = 24826x - 150.90$	0.9997	0.007	0.024	103.12
DBAT	46.673	$y = 10186x - 222.67$	0.9975	0.041	0.148	109.61
BGPY	47.663	$y = 13735x - 102.61$	0.9895	0.085	0.280	106.62
IDPY	48.590	$y = 43552x - 420.84$	0.9999	0.004	0.017	116.74

Water samples were gathered in glass bottles from three distinct wells located on in Abha, Assir region, Saudi Arabia. Each sampling was carried out in three replicates. All the samples were immediately taken to the laboratory and kept at 4 °C until further use.

3. RESULTS AND DISCUSSIONS

Quality control for PAHs analysis

The HPLC chromatogram of a mixture of sixteen PAHs reference materials at a concentration of 4.0 µg/mL is shown in Figure 1. Table 3 shows the calibration equations, correlation coefficients, LOD, LOQ, and average recovery of each PAH in spiked samples. The slopes of equations for the calibration of ACEE, ACEY, ANTA, BAAA, BAPY, BBFA, BKFA, CHYS, FLRE, FLAT, IDPY, and PHNT were higher than other PAHs, implying that the frontal has better sensitivity than the latter. The correlation coefficients were in the range of 0.9895–0.9999 indicating good linearity for all PAHs. The lowest LODs (0.003–0.009 µg/mL) were acquired for BKFA, CHYS, IDPY, PHNT, BAAA, ANTA, BBFA, BAPY, and FLRE. The highest LODs (0.011–0.131 µg/mL) were acquired for FLAT, PYRY, DBAT, ACEE, ACEY, BGPY and NAPT. Efficiency of extraction and quantification of PAHs in the examined samples were evaluated using the spiking method. The recovery values of each PAH were computed when known amounts of PAHs were injected into fresh parts of previously tested water samples at three levels. Plausible recovery was obtained for all PAHs (66.17–109.61%), except for NAPT (39.64%) and IDPY (116.74%). The limited recovery of NAPT can be ascribed to its high volatility, which prevents the analyte from concentrating in water samples.

Analysis of PAHs

The results of analysis of residue levels of PAHs in the aqueous samples from three different wells, from water tap and drinking water bottles revealed that all the specified

concentrations of PAHs in the studied samples are much less than the LOD and LOQ, and therefore the presence of PAHs in these samples was not detected.

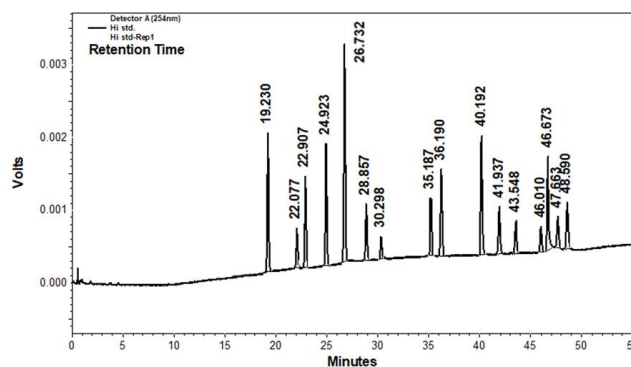


Figure 1: HPLC chromatogram of sixteen PAHs reference materials at the concentration of 4.0 µg/mL.

CONCLUSION

The current investigation found that the LLE-HPLC-UV approach was sufficient for extracting and determining PAHs in various aqueous samples. Good linearity for all PAHs were obtained. Low LOQ values (0.003–0.131 µg/mL) were obtained which make the HPLC-UV technique particularly sensitive for determination of PAHs. Using the spiking approach, the efficiency of extraction and quantification of PAHs in the tested samples were assessed. Reasonable recovery was obtained for all PAHs. Using the optimized method for these compounds, no PAH were found in the water samples when analyzed by HPLC–UV.

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