

ARTICLES

Simultaneous Determination of Polychlorinated Biphenyls and Polyaromatic Hydrocarbons in Natural Waters by Dispersive Liquid–Liquid Microextraction and Gas Chromatography–Mass Spectrometry

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Abstract—The test samples are polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), highly toxic and widely prevalent organic pollutants in natural waters. The feasibility of using dispersive liquid–liquid microextraction (DLLME) for extracting PCBs and PAHs, followed by their determination by GC–MS, was assessed in the presence of both contaminants. A DLLME method employing a binary dispersing agent was proposed, ensuring the simultaneous extraction of analytes with efficiency ranging from 80 to 97%. The proposed procedure enabled the GC–MS determination of 16 PAHs and 7 PCBs in natural waters in a wide concentration range of 2.0×10^{-5} – $0.04 \mu\text{g/mL}$ with an average error of 7–18% for PAHs and 11–18% for PCBs. The relative standard deviations for repeatability and reproducibility were found to be 3.1–6.5 and 4.3–7.7%, respectively, for PAHs, and 2.8–5.3 and 3.4–6.0%, respectively, for PCBs.

Keywords: polycyclic aromatic hydrocarbons, polychlorinated biphenyls, simultaneous extraction, dispersive liquid–liquid microextraction, natural water

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Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are super-ecotoxins and widely distributed organic pollutants of natural waters. Environmental monitoring data indicate a possibility of their simultaneous presence in natural waters, especially in industrially developed regions [1, 2]. The danger of PAHs and PCBs lies in their acute toxicity, cumulative effects, and long-term consequences on living organisms. These super-ecotoxins are unevenly distributed in natural waters, ranging from 1.0×10^{-8} to $1.0 \times 10^{-3} \mu\text{g/mL}$ and higher [3–5]. In Russia, maximum permissible concentrations were established only for benzo[*a*]pyrene, tri-, and pentachlorobiphenyls, with no established limits for most individual PAHs and PCBs [6].

The level of pollution of a studied aquatic ecosystem is assessed by the concentrations of indicator compounds. Among the PAHs, there are 16 such pollutants: naphthalene, acenaphthene, fluorene, acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*g,h,i*]perylene, and indeno[1,2,3-*c,d*]pyrene [7]. Among the PCBs, the indicator compounds are PCB-28, PCB-52, PCB-101, PCB-138, PCB-153,

and PCB-180, as they are the most frequently found in environmental samples, along with the dioxin-like PCB-118 [8].

Gas chromatography–mass spectrometry (GC–MS) is used for the determination of super-ecotoxins in natural waters [9–11], ensuring the effective separation of a large number of isomers and the high reliability of their identification. The known certified methods for determining super-ecotoxins mainly involve the extraction and preconcentration of analytes, the purification of the extract, and chromatography [12, 13]. These methods are labor- and time-intensive in practice and are accompanied by significant analyte losses, which affects the performance characteristics of PAH or PCB determination in natural waters. Currently, approaches are being developed that involve the simultaneous determination of PAHs and PCBs, enhancing the informativeness and rapidity of water analysis [14, 15]. In [16], a possibility of the solid-phase extraction of both classes of super-ecotoxins was demonstrated, with the recoveries ranging from 80 to 100% for PCB and up to 90% for PAHs.

The sensitivity, reproducibility, and speed of analyte determination are enhanced through various microextraction techniques [17]. A possibility of the simultaneous solid-phase microextraction of PAHs

and PCBs from water has been demonstrated using fibers with polydimethylsiloxane coatings [18, 19], as well as coatings applied to magnetic stirrers followed by the thermal desorption of analytes [20]. In the latter case, the recovery rates for 14 PAHs with three or more aromatic rings varied from 73 to 91%, and for six PCBs, from 48 to 83%.

Dispersive liquid–liquid microextraction (DLLME) is a promising approach to the extraction and preconcentration of organic pollutants taking into account the principles of green chemistry. Using DLLME minimizes the volumes of organic reagents used [21, 22]. Various modifications of this extraction method are employed to extract chlorinated pesticides, PAHs, and PCBs. These modifications differ in the dispersing method and the nature of the extractant, and they can be combined with other sample preparation techniques and subsequent analyte detection methods [23–28]. In the conventional version of DLLME, an organic solvent (extractant) and a dispersing agent, which is miscible with both water and the extractant, are rapidly introduced into the sample. Under these conditions, an equilibrium is reached within 3 min due to the large surface area of contact between the extracting solvent and the water sample [22]. For PAHs and PCBs, chlorinated solvents, such as tetrachloroethylene, chloroform, dichloromethane, and chlorobenzene are primarily used as extractants. Acetone, methanol, and acetonitrile are used as dispersing agents [22, 23, 29]. The high extraction efficiency of PAHs with varying molecular weights from water was achieved using a binary dispersing agent (acetone + acetonitrile) [30].

The approaches used for the extraction and detection of PAHs and PCBs are similar, suggesting a potential for developing a universal sample preparation method that allows the simultaneous extraction and GC–MS determination of both classes of analytes.

This study aims to evaluate the feasibility of the DLLME extraction of polychlorinated biphenyls and polycyclic aromatic hydrocarbons and their GC–MS determination in natural waters in the presence of both contaminants.

EXPERIMENTAL

Material and reagents. The solvents used included chloroform (ACS reagent grade, $\geq 99.8\%$ purity, Acros Organic, Belgium), acetonitrile (gradient grade, $\geq 99.9\%$), acetone (HPLC grade, $\geq 99.8\%$), hexane (ACS reagent grade, $\geq 99.5\%$; Sigma-Aldrich (United States) and Merck (Germany)), and ultrapure water prepared using a Milli-Q system (Millipore, United States).

We used standard samples of polychlorinated biphenyls (PCBs), including a mixture of PCB (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153,

and PCB-180) and PCB-118 in *iso*-octane, each in a concentration of 10 $\mu\text{g/mL}$ (Dr. Ehrenstorfer, Germany). PCB-166 in *iso*-octane in a concentration of 10 $\mu\text{g/mL}$ (Dr. Ehrenstorfer, Germany) was used as an internal standard.

The determination of PAHs was conducted using standard solutions of individual compounds in acetonitrile: naphthalene, fluorene, acenaphthene, acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, and benzo[*g,h,i*]perylene (Ekros, Russia). Analytical standards of PAHs in cyclohexane included indeno[1,2,3-*c,d*]pyrene and benzo[*e*]pyrene (Sigma-Aldrich, United States). Benzo[*e*]pyrene was used as an internal standard. The concentration of each PAH in the solutions was 200 $\mu\text{g/mL}$, except for standards of dibenzo[*a,h*]anthracene, benzo[*g,h,i*]perylene, benzo[*e*]pyrene, and indeno[1,2,3-*c,d*]pyrene, which were in a concentration of 100 $\mu\text{g/mL}$.

Equipment. For chromatographic analysis, we used a Shimadzu GCMS-QP2020 system, consisting of a chromatograph and a single quadrupole mass spectrometric detector (Japan). Sample ultrasonication was performed using a Grand 57-35 unit (Russia). Chloroform extracts were separated using a Liston C 2201 centrifuge (Russia). Integrated mass spectral libraries, including Wiley8 and NIST-17.1, were employed for analyte identification, alongside retention times of individual standard solutions of PAHs and PCBs.

The GC–MS analysis of PAHs and PCBs in water was conducted in a temperature-programmed mode using a thermostat as follows: initial temperature 60°C for 1 min, followed by heating at a rate of 15 K/min to 170°C for 3 min, further heating at 10 K/min to 280°C for 8 min, and final heating at 10 K/min to 290°C for 25 min. Sensitivity enhancement and matrix interference minimization were achieved using the selected ion monitoring (SIM) mode for specific ions: PCBs at m/z 252, 296, 326, 360, and 392; PAHs at m/z 128, 152, 154, 166, 178, 202, 228, 252, 276, and 278. Analyte separation was achieved in a Zebron-5ms capillary column (60 m \times 0.25 mm) with a 0.25- μm thick coating of 5% polysilarylene and 95% dimethylpolysiloxane. Peak integration in the chromatograms was performed using the GCMSsolution software, version 4.45.

Extraction of analytes. To prepare the samples, 10 mL of water free from the analytes of interest were spiked with standard solutions of PCBs and PAHs in the ratios 1 : 1, 1 : 5, and 1 : 10 respectively. Then, 0.1 μg of internal standards (PCB-166 and benzo[*e*]pyrene) were added using a 2-mL syringe. Next, a mixture for dispersive liquid–liquid microextraction (DLLME) was swiftly introduced into the solution. This mixture consisted of 0.15 mL of chloroform (an extractant), 0.5 mL of acetone, and 0.5 mL

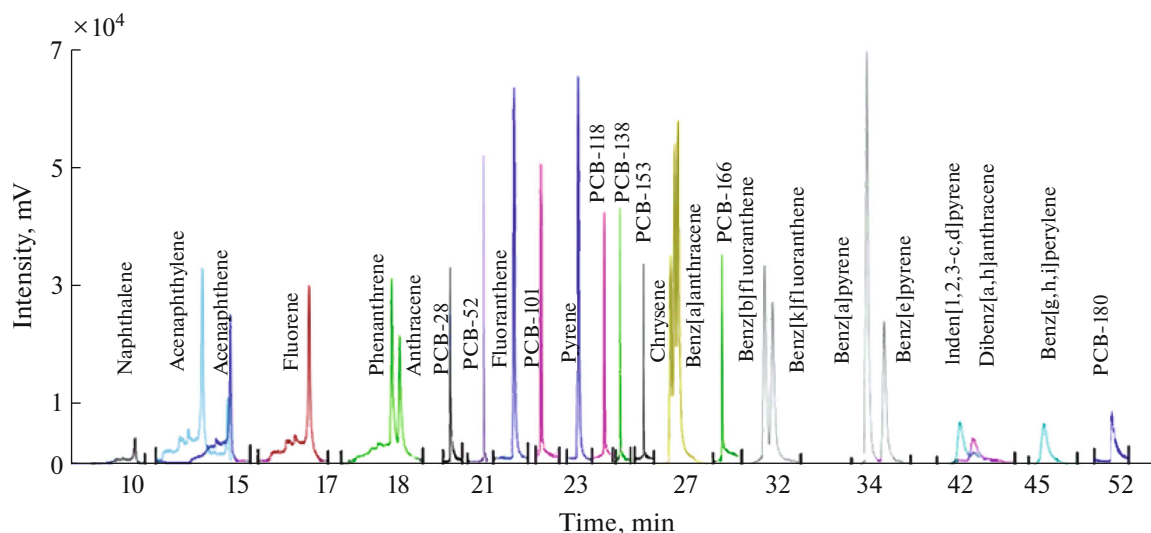


Fig. 1. A GC–MS chromatogram of a hexane solution containing a mixture of PAHs and PCBs in a concentration of 0.05 $\mu\text{g/mL}$ each, with PCB-166 and benzo[e]pyrene as internal standards.

of acetonitrile a dispersing mixture). The extraction system was vigorously shaken and subjected to ultrasonic treatment at 35 kHz for 6 min to enhance the extraction efficiency. Following ultrasonication, the resulting mixture was centrifuged for 2 min at 2600 rpm to separate the organic phase, which was subsequently used for chromatographic analysis.

RESULTS AND DISCUSSION

GC-MS determination of structurally diverse PAHs and PCBs in natural waters on their simultaneous presence. The chloroform extract obtained after the DLLME of the analytes was directly used for a GC–MS analysis without redissolution, simplifying the analytical procedure. The effective chromatographic separation of 17 PAHs and 8 PCBs was achieved using a specific 60 m capillary column coated with 5% polysilarylene and 95% dimethylpolysiloxane, with a gradual temperature ramp from 60 to 290°C in three stages (Fig. 1). The use of the selected ion monitoring (SIM) mode enhanced the reliability of identifying the target components in complex matrices of natural waters.

After preparing water samples for analysis, a GC–MS determination of PAHs and PCBs was conducted using a calibration curve plotted based on standard samples. Correlation coefficients (R^2) close to 0.99 were established, along with limits of (LOD, 3σ) and quantification (LOQ, 10σ). Under the specified conditions, naphthalene, acenaphthene, acenaphthylene, and fluorene were detected at the levels 10^{-5} $\mu\text{g/mL}$, while phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene, chrysene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene were detected at the levels 7.5×10^{-6} $\mu\text{g/mL}$. The lim-

its of detection for PCBs in water were determined at as low as 2.0×10^{-5} $\mu\text{g/mL}$.

The DLLME of PAHs and PCBs on their simultaneous presence involved optimizing conditions for extracting analytes into a chlorinated organic solvent. Using a mixture of chloroform, dichloromethane, carbon tetrachloride, isopropyl and ethyl alcohols, acetone, and ethyl acetate, analytes can be extracted with recovery rates of 90–105%. Subsequently, a GC–MS analysis can detect these compounds at the levels 2.5×10^{-5} $\mu\text{g/mL}$ [31]. To decrease the amount of organic solvents used, a binary mixture of acetone and acetonitrile has been proposed as a dispersing agent for DLLME [30], aligning more closely with the principles of green chemistry. This extraction procedure has proven to be versatile, achieving high recovery rates (91–99%) for PAHs of various molecular weights from natural waters.

Based on the results obtained, we assessed the feasibility of the simultaneous DLLME of PAHs and PCBs from natural water samples using the dispersing agents acetone + acetonitrile. A series of experiments was conducted under the optimized conditions, involving the addition of 0.15 mL chloroform, 0.5 mL acetone, and 0.5 mL acetonitrile to 10 mL of a water sample for microextraction, followed by ultrasonic treatment at 35 kHz for 6 min and subsequent centrifugation at 2600 rpm for 2 min. The capability of co-extracting PAHs and PCBs from water was assessed using model samples of natural water containing 2.0×10^{-5} , 4.0×10^{-4} , and 4.0×10^{-3} $\mu\text{g/mL}$ of each PAH, into which a mixture of the studied PCBs was introduced in equivalent ratios. The selected range of analyte concentrations was based on the most frequently

Table 1. Recovery (%) of PCBs from natural water samples

$c_{\text{PAH}}, \mu\text{g/mL}$	$c_{\text{PCB}}, \mu\text{g/mL}$	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
2.0×10^{-5}	2.0×10^{-5}	85 ± 3	83 ± 1	82 ± 2	81 ± 6	83 ± 1	80 ± 3	81 ± 2
	1.0×10^{-4}	83 ± 3	86 ± 1	86 ± 2	85 ± 6	81 ± 1	85 ± 3	82 ± 2
	2.0×10^{-4}	88 ± 3	89 ± 1	84 ± 2	80 ± 6	84 ± 1	82 ± 4	83 ± 2
4.0×10^{-4}	4.0×10^{-4}	84 ± 3	88 ± 1	86 ± 2	83 ± 6	85 ± 1	84 ± 3	82 ± 2
	2.0×10^{-3}	88 ± 3	91 ± 1	84 ± 2	83 ± 6	84 ± 1	83 ± 4	82 ± 2
	4.0×10^{-3}	88 ± 3	87 ± 1	87 ± 2	85 ± 3	82 ± 1	82 ± 4	83 ± 2
4.0×10^{-3}	4.0×10^{-3}	87 ± 3	88 ± 1	85 ± 2	83 ± 3	83 ± 1	81 ± 4	85 ± 2
	2.0×10^{-2}	84 ± 3	85 ± 1	84 ± 2	81 ± 4	80 ± 1	83 ± 3	81 ± 1
	4.0×10^{-2}	81 ± 2	79 ± 1	73 ± 2	74 ± 5	71 ± 1	70 ± 4	73 ± 2

encountered data in the literature regarding the environmental monitoring of natural waters [3, 4], as well as the maximum permissible concentrations (MPCs) for the studied components. The results demonstrated no interfering effects for the analytes: the recovery rates for PAHs (91–99%) were consistent with the previously obtained data [30]. For PCBs, the recovery rates were as follows (%): PCB-28 (81–88), PCB-52 (80–91), PCB-101 (82–87), PCB-118, PCB-138, and PCB-153 (80–85), and PCB-180 (81–85).

The proposed procedure for PCB extraction with recovery rates ranging from 81 to 91% falls short compared to the characteristics of a method for the simultaneous determination of 22 analytes (90–105%), which considers individual extraction [31]. However, under these conditions, PCBs and PAHs can be extracted by DLLME from water simultaneously. We hypothesized that, on the simultaneous presence of both classes of ecotoxins, their mutual interaction may occur due to PCBs' higher affinity to the chlorinated organic solvent during co-extraction with PAHs. To assess the degree of this interaction, a series of experiments was conducted using five- and tenfold excesses of PCB relative to low ($c_{\text{PAH}} = 2.0 \times 10^{-5} \mu\text{g/mL}$), medium ($c_{\text{PAH}} = 4.0 \times 10^{-4} \mu\text{g/mL}$), and high ($c_{\text{PAH}} = 4.0 \times 10^{-3} \mu\text{g/mL}$) levels of PAH concentrations (Table 1). At PAH concentrations of 2.0×10^{-5} , 4.0×10^{-4} , and $4.0 \times 10^{-3} \mu\text{g/mL}$ in water and high concentrations of PCBs (up to $0.02 \mu\text{g/mL}$), no decrease in the recovery rates for all investigated components was observed. At a tenfold excess of PCBs ($0.04 \mu\text{g/mL}$), extraction efficiencies for all PAHs

decreased by 10–15% (Fig. 2a), while those for PCBs decreased by 6–11% (Fig. 2b).

Under the conditions of natural waters highly contaminated with super-ecotoxins, the volume of the extractant droplet becomes insufficient for the complete extraction of PAHs and PCBs (Fig. 3). In the cases where the analyte concentration exceeds $0.02 \mu\text{g/mL}$, extraction can be conducted in two stages, and, based on the results of analysis of the first and second extracts, the total concentration of analytes can be determined up to $0.04 \mu\text{g/mL}$.

The proposed procedure enables the determination of analytes in the presence of both PAHs and PCBs in natural waters within a wide concentration range (2.0×10^{-5} – $0.04 \mu\text{g/mL}$), encompassing concentrations both below and significantly above the MPC levels.

The repeatability of the results of analysis using the proposed procedure was evaluated based on 16 replicate determinations in model water samples at two concentration levels, 5.0×10^{-5} and $0.02 \mu\text{g/mL}$ for each analyte. The reproducibility parameters were assessed over a 6-day experiment at these concentration levels for PAHs and PCBs (Table 2). The relative standard deviations of repeatability ranged from 3.1 to 6.5% for PAHs and from 2.8 to 5.3% for PCBs. Reproducibility was within the range 4.3 to 7.7% for PAHs and 3.4 to 6.0% for PCBs. The accuracy of analyte determination ranged from 7 to 18% for PAHs and 11 to 18% for PCBs.

Thus, the simultaneous determination of PAHs and PCBs in natural waters can be achieved using dis-

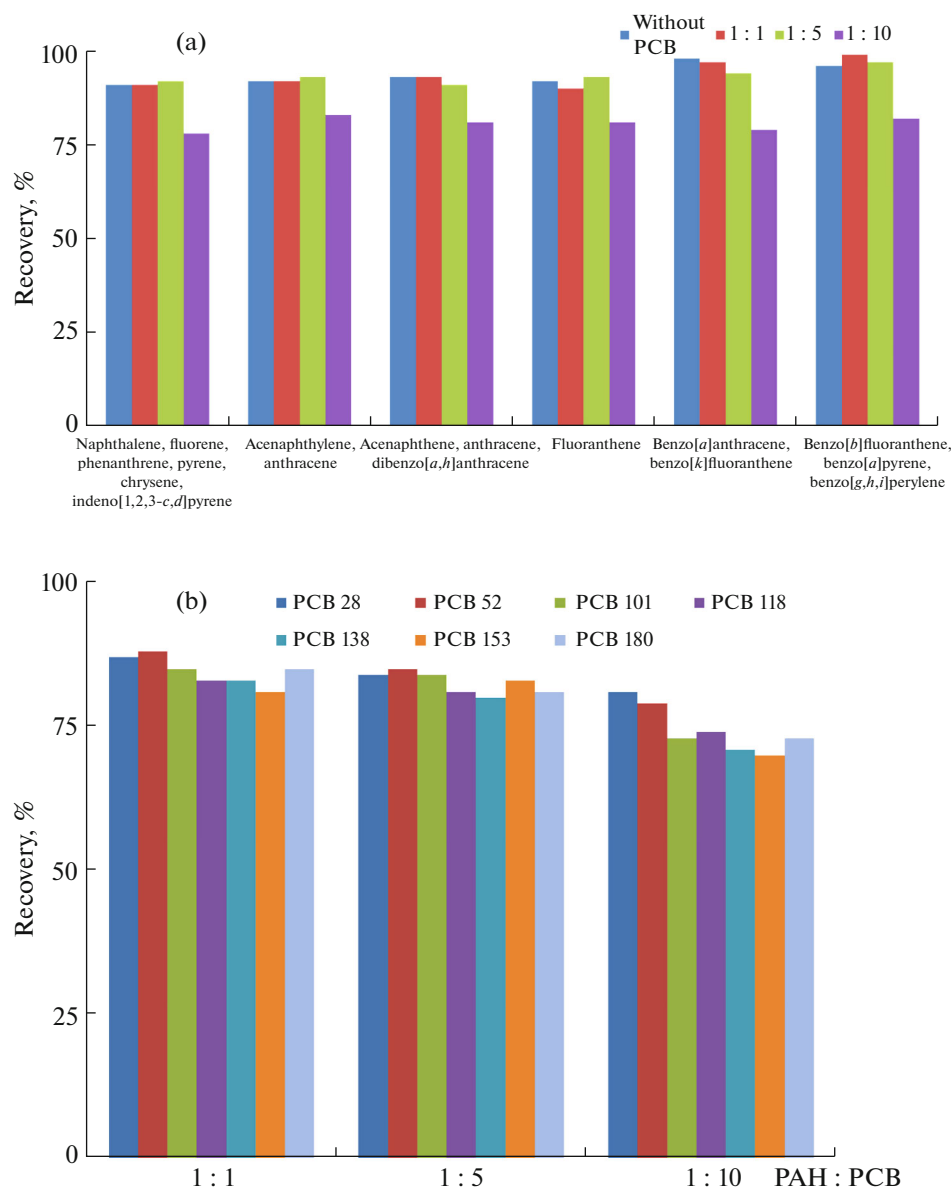


Fig. 2. DLLME recoveries of ecotoxics under co-extraction conditions at different ratios: (a) PAHs and (b) PCBs.

persive liquid–liquid microextraction with a binary dispersing agent followed by the GC–MS determination of the analytes.

CONCLUSIONS

Dispersive liquid–liquid microextraction of analytes with a binary dispersing agent (acetone + acetonitrile) enables the simultaneous recovery of PAHs and PCBs at levels ranging from 80 to 97%. The developed analytical procedure allows the determination of 16 PAHs and 7 PCBs in natural waters by GC–MS within the concentration range 2.0×10^{-5} to 0.04 $\mu\text{g/mL}$, with precision of 7–18% for PAHs and 11–18% for PCBs.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

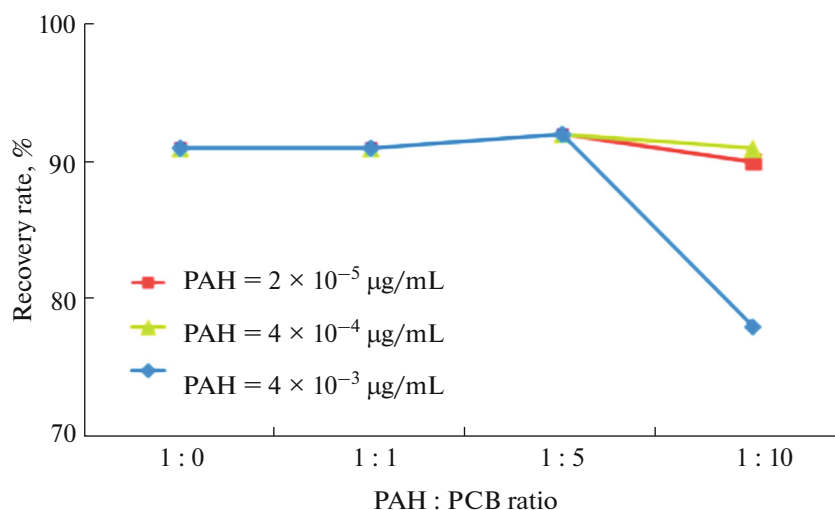


Fig. 3. Dependence of the DLLME recovery of the total amount of PAHs (from 2.0×10^{-5} to 4.0×10^{-3} µg/mL) on the total concentration of PCBs in water.

Table 2. Performance of the procedure for the simultaneous DLLME and GC–MS determination of PCBs and PAHs in waters

Analyte	Repeatability ($n = 16$), %		Reproducibility ($n = 6$), %		Precision, %
	5.0×10^{-5} µg/mL	2.0×10^{-2} µg/mL	5.0×10^{-5} µg/mL	2.0×10^{-2} µg/mL	
PCB-28	5.7	5.1	6.5	6.1	18
PCB-52	6.1	5.6	6.9	6.3	15
PCB-101	4.8	4.5	6.1	6.0	15
PCB-118	4.7	4.5	5.7	5.5	14
PCB-138	5.0	4.4	5.8	5.3	15
PCB-153	4.3	4.0	5.3	5.0	12
PCB-180	4.8	4.2	5.6	5.2	11
Naphthalene	6.9	6.5	7.7	7.0	17
Acenaphthene	6.3	6.2	7.3	6.8	18
Fluorene	5.5	5.3	6.8	6.3	16
Acenaphthylene	5.7	5.2	6.2	6.1	15
Phenanthrene	5.1	4.8	6.3	6.2	16
Anthracene	5.2	4.9	6.4	6.0	14
Fluoranthene	4.8	5.0	5.7	5.2	14
Piren	5.0	4.8	6.0	5.8	13
Benzo[a]anthracene	4.4	4.4	6.3	6.0	12
Chrysene	4.3	4.3	5.5	5.2	10
Benzo[b]fluoranthene	3.9	4.5	4.9	4.3	9
Benzo[k]fluoranthene	3.8	4.0	4.7	4.5	11
Benzo[a]pyrene	4.0	3.8	4.9	4.4	7
Dibenzo[a,h]anthracene	3.1	3.7	4.3	4.0	8
Benzo[g,h,i]perylene	3.5	3.7	4.3	4.1	9
Indeno[1,2,3-c,d]pyrene	3.7	3.5	4.2	3.9	8

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