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Optimization and Validation of the SBSE–HPLC–FLD Method for the Determination of Priority Pollutants PAHs in Several Water Matrices

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are priority pollutants in drinking and environmental waters. Their mutagenic/carcinogenic potential and ng·L^{-1} limits demand methods that are both sensitive and practical. We report a rapid, solvent-sparing workflow coupling stir-bar sorptive extraction (SBSE) to HPLC with fluorescence detection (FLD) for simultaneous determination of six PAHs (fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, indeno[1,2,3-cd]pyrene) in drinking water, groundwater, and surface water. SBSE conditions were optimized, and isocratic RP-HPLC achieved baseline resolution within a 26-min cycle using ~ 39 mL solvent (~20 samples day − 1). Validation under ISO/IEC 17,025 showed linear calibration (r ≥ 0.99941), limits of detection of 0.4–1.8 ng·L^{-1}, and matrix-verified LOQs of 1.5–10.9 ng·L^{-1}. Trueness and precision met predefined criteria across matrices (recoveries 63.3–109.9%; within-laboratory reproducibility ≤ 25% RSD), with expanded uncertainties U(k = 2) ≤ 47.1%. Performance satisfies EU Drinking Water Directive 2020/2184 requirements for benzo[a]pyrene and the regulated PAH sum. By attaining sub-10 ng·L^{-1} LOQs with FLD alone and documenting a complete uncertainty budget, this procedure offers a cost-effective alternative to LC–MS/MS for routine compliance and surveillance. The validated SBSE–HPLC–FLD protocol is fit-for-purpose for regulatory laboratories and environmental services requiring sensitive, robust, and scalable PAH determination across diverse water matrices.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) form a broad family of more than 150 semi-volatile organic compounds released chiefly during the incomplete combustion of fossil fuels, biomass, and other carbon-rich substrates, as well as from petrogenic leaks. Their persistence and lipophilicity facilitate long-range atmospheric transport followed by deposition into soils, sediments, and surface- or groundwaters, giving PAHs a truly global footprint (Wu et al. 2025).

Toxicologically, several high-molecular-weight congeners—including the prototypical benzo[a]pyrene (BaP)—intercalate with DNA and generate bulky adducts that initiate mutagenesis; consequently, BaP is classified by the International Agency for Research on Cancer as carcinogenic to humans (Group 1) (Jameson 2019). Continuous low-level exposure has been epidemiologically linked to oxidative stress, developmental impairment, and elevated cancer risk, underscoring the need for vigilant environmental surveillance.

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In response, the recast European Drinking Water Directive (EU 2020/2184) mandates a parametric value of 0.010 µg L^{-1} for BaP and 0.10 µg L^{-1} for the sum of benzo[b]-, benzo[k]-fluoranthene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene, limits that have been transposed into national legislation across the Union (De Castro 2018). Demonstrating compliance at these ultra-trace levels requires analytical methods capable of sub-nanogram detection with full metrological traceability, as specified in ISO/IEC 17,025.

High-performance liquid chromatography coupled to programmable fluorescence detection (HPLC-FLD) remains the benchmark for the routine determination of PAHs in water, offering signal-to-noise ratios that are orders of magnitude higher than those of UV detection. Yet ng L^{-1} targets cannot be reached without an efficient pre-concentration step; traditional liquid–liquid or solid-phase extraction workflows are labor-intensive, solvent-intensive and prone to matrix-induced bias (Zuin et al. 2005).

Stir-bar sorptive extraction (SBSE) has emerged as a greener, high-enrichment alternative: a polydimethylsiloxane-coated magnetic bar is stirred directly in the sample, achieving enrichment factors > 1000, detection limits below 1 ng L^{-1}, and repeatability typically better than 10%. Recent advances in sorbent chemistries—graphene composites, ionic-liquid or deep-eutectic coatings—and automated thermal or liquid desorption have further expanded SBSE applicability to complex aqueous matrices (Maiga et al. 2023).

Against this background, the present work optimizes and fully validates, to ISO/IEC 17,025 requirements, an SBSE–HPLC–FLD protocol for the simultaneous determination of six priority PAHs (fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, BaP, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene) in drinking, ground- and surface-waters. Extraction variables (temperature, time, stirring rate, organic modifier, and desorption conditions) were systematically tuned to maximize recovery; chromatographic separation was accomplished within 24 min using environmentally benign mobile phases; and the method achieved limits of quantification of 1–10 ng L^{-1} with accuracies and uncertainties fit for regulatory monitoring. The optimized procedure provides a rapid, solvent-sparing, and highly sensitive tool for safeguarding water resources from PAH contamination.

Methods

The reagents, standards, equipment, and materials required for the implementation of this analytical method, as well as the experimental methodology used for this research work, are detailed in this chapter.

Reagents and standards

The PAHs standard mix solution (PAH-Mix 3, product code DRE-L20950003AL in acetonitrile) with the analytes fluoranthene (FLU) (50 µg mL^{-1}), benzo[b]fluoranthene (BBF) (20 µg mL^{-1}), benzo[k]fluoranthene (BKF) (20 µg mL^{-1}), benzo[a]pyrene (BAP) (20 µg mL^{-1}), benzo[ghi]perylene (BPE) (20 µg mL^{-1}), and indeno[1,2,3-cd]pyrene (IND) (40 µg mL^{-1}) was purchased from LGC Standards. The single PAH standard solutions with the concentration of 100 µg mL^{-1} for the analytes FLU (product code 14500-0820-100AN1,5), BBF (product code 14500-0120-100AN1,5), BKF (product code 14500-0140-100AN1,5), BAP (product code 14500-0230-100AN1,5), BPE (product code 14500-0210-100AN1,5) and IND (product code 14500-0880-100AN1,5), were purchased from NEOCHEMA. HPLC grade solvents acetonitrile, methanol, dichloromethane, and acetone were obtained from Fisher Scientific. The sodium thiosulfate pentahydrate, pro analysis (PA), used in water samples as a chlorine inhibitor, was supplied by Chem-Lab.

The stock solutions of PAHs with the concentration of 10 µg L^{-1} for BBF, BKF, BAP, and BPE, 20 µg L^{-1} for IND, and 25 µg L^{-1} for FLU were prepared in acetonitrile by dilution of 50 µL of PAHs standard mix solution (PAH-Mix 3) in a 100 mL volumetric flask with glass stopper and stored in a refrigerator at 5 ± 3 °C. The working standards were prepared by subsequent dilution of stock solutions in ultrapure water (UPW), as shown in Table 1. Stock solutions with a concentration of 10 µg L^{-1} for a single PAH were prepared in acetonitrile by diluting 10 µL of the single PAH standard solution (100 µg mL^{-1}) in a 100 mL volumetric flask with a glass stopper and stored in a refrigerator at 5 ± 3 °C. The single working standards solutions were prepared in ultrapure water (UPW) by dilution of 200 µL of the stock solution (10 µg L^{-1}) in a 200 mL volumetric flask with a glass stopper.

The UPW (with 0.3% acetonitrile) eluent solution was prepared by diluting 3 mL of acetonitrile in a 1000 mL volumetric flask. The concentration levels and number of calibration standards used are detailed in Table 1.

Instrumentation

The chromatographic analyses were performed using an Agilent 1100 Series HPLC system, equipped with an autosampler (G1329A), a degasser (G1322A), a quaternary pump (G1311A), a variable wavelength detector (G1314A), a programmable fluorescence detector (G1321A) and a column compartment (G1316A), fitted with a Inertsil ODS-P column (5 µm particle size; 250 mm × 4.6 mm I.D.) and a Inertsil ODS-P safeguard column (5 µm particle size; 10 mm × 4.0 mm I.D.).

Table 1 PAHs stock solution volume needed to prepare the different levels of the calibration standards, according to the established final volume

PAHs stock solution¥ (µL)	Final volume (mL)	FLU (ng L^{-1})	BBF (ng L^{-1})	BKF (ng L^{-1})	BAP (ng L^{-1})	BPE (ng L^{-1})	IND (ng L^{-1})
75	500	–	–	1.5 (LOQ)	1.5 (LOQ)	–	–
75	250	7.5 (LOQ)	3 (LOQ)	3	3	–	6 (LOQ)
150	250	15	6	6	6	6 (LOQ)	12
200	200	25	10	10	10	10	20
300	200	37.5	15	15	15	15	30
1000	500	50	20	20	20	20	40
3000	1000	75	30	30	30	30	60
2000	500	100	40	40	40	40	80
1000	200	125	50	50	50	50	100
3000	500	150	60	60	60	60	120

¥ PAHs stock solution with a concentration range between 10 and 25 µg L^{-1} for the considered analytes

The chromatographic data were collected and processed using Agilent ChemStation Plus software (Revision A.10.02).

The extraction was carried out on a DLAB ten-position magnetic hot plate stirrer, model MS-H-S10, and for the desorption, a Thermolyne Cimarec magnetic stirrer, model S131120-33, was used.

The eluent solutions were degassed in a J.P. Selecta ultrasonic cleaning bath, model Ultrasons-HD 3000867.

The UPW was acquired using a Millipore ultrapure water treatment system, model Milli-Q Academic. The chemicals were weighed on a Mettler Toledo analytical balance, model AX204.

A digital thermometer (HANNA Instruments, model HI 98501) was used to monitor and control the temperature of the water bath during sample extraction, which was performed in 250 mL Duran beakers.

Materials

Standard solutions were prepared using Class A Duran volumetric flasks (100–1000 mL) and Hirschmann volumetric pipettes (1–100 mL), in combination with Hamilton 1700-series microsyringes (50–500 µL). Water samples, standards and intermediate solutions were handled in amber glass bottles (100–500 mL) with PTFE-lined screw caps. SBSE extraction and desorption were performed using 10 mm PDMS-coated stir bars (1 mm film thickness, Gerstel), 40 mL amber wide-mouth bottles for stir-bar conditioning and 2 mL amber vials fitted with 300 µL conical inserts for extract collection. A 5 mL manual micropipette (Socorex Acura 835) was used to prepare the conditioning solutions for the stir bars.

Procedure

Samples and sampling

Drinking, surface and groundwater samples were collected in 500 mL amber glass bottles containing approximately 40 mg of sodium thiosulfate pentahydrate, filled without headspace and sealed with PTFE-lined caps. Samples were stored in the dark at 5 ± 3 °C, extracted within seven days and analysed within 40 days. Extracts not immediately injected were kept at 5 ± 3 °C in sealed 2 mL vials.

Stir bar sorptive extraction (SBSE)

The selected conditioning, extraction and desorption parameters are summarised in Table 4, and the detailed workflow is described below.

Before use, each stir bar was conditioned into a 40 mL amber wide-mouth glass bottle, containing 4 mL of dichloromethane-methanol mixture (1:1) and magnetically stirred for 5 min at 500 rpm. This procedure was repeated two times with fresh portions of the solvent mixture. Once conditioned, the stir bars were dried with lint-free tissue.

To prevent the adsorption of PAHs onto glassware, 1 mL of methanol was added to 100 mL amber glass bottles with PTFE screw caps, followed by the addition of 100 mL of water sample or standard solution (Table 1) using volumetric pipettes. Afterward, the solution was mixed thoroughly.

The amber glass bottles containing the previous solution were placed in a 250 mL beaker containing approximately 125 mL of ultrapure water (UPW) and positioned on a magnetic hot plate stirrer (brand DLAB, model MS-H-S10) at 65 ± 5 °C. After reaching the desired temperature of 65 ± 5 °C, the conditioned stir bars were added to the amber glass bottle containing the solution and stirred at 500 rpm for 60 min to extract PAHs.

When the extraction time had elapsed, the stir bars were removed from the amber glass bottles using magnetic tweezers, dried on a lint-free tissue, and placed in 2 mL vials with a 300 µL conical glass insert containing 200 µL of acetonitrile. The PAHs desorption in acetonitrile was carried at room temperature, using a magnetic stirrer (brand Thermolyne Cimarec, model S131120-33) for 15 min at 1000 rpm.

Table 2 Chromatographic separation conditions for the HPLC-FLD method

Time (min)	UPW◊ (%, v/v)	Acetonitrile (%, v/v)	Column (ºC)	Flow rate (mL/min)
0–22	10	90	28	1.5
22–24	0	100	28	1.5
24–26	10	90	28	1.5

◊UPW with 0.3% of acetonitrile

Table 3 Time, excitation wavelength, emission wavelength, and PMT gain program used for HPLC-FLD

PAHs	Time (min)	Excitation (nm)	Emission (nm)	PMT gain
Fluoranthene	0.0	237	460	13
Benzo[b]fluoranthene	7.0	255	420	14
Benzo[k]fluoranthene				
Benzo[a]pyrene				
Benzo[ghi]perylene	15.5	255	420	18
Indeno[1,2,3-cd]pyrene	18.0	250	495	18

After the desorption time had elapsed, the stir bars were removed using a magnetic bar retriever and placed in 40 mL amber wide-mouth glass bottles, with PTFE screw caps.

Subsequently, the stirring bars underwent the same conditioning process mentioned above, enabling their reuse in the extraction phase. The 2 mL vials containing the extract were then positioned in the autosampler of the HPLC-FLD.

High-performance liquid chromatography with a fluorescence detector.

PAH analysis was performed on an Agilent 1100 Series HPLC system equipped with an autosampler and a fluorescence detector, using a reversed-phase Inertsil ODS-P column (5 µm particle size; 250 mm × 4.6 mm i.d.) as the stationary phase. The mobile phase consisted of acetonitrile and ultrapure water (UPW) containing 0.3% (v/v) acetonitrile, delivered at a flow rate of 1.5 mL·min^{-1} and a column temperature of 28 °C. An isocratic composition of 90% acetonitrile and 10% UPW (with 0.3% acetonitrile) was applied for 22 min, followed by a 2 min wash at 100% acetonitrile and a 2 min re-equilibration at the initial conditions, giving a total cycle time of 26 min. All injections were made with a fixed-volume loop of 80 µL. Column efficiency (plate number), peak symmetry (tailing factor), and resolution between adjacent PAHs (Rs ≥ 1.5) were verified prior to each analytical batch. The chromatographic separation conditions are summarised in Table 2, and the fluorescence wavelength programme and PMT gain settings are given in Table 3.

The analytes were identified by comparison of the retention time (RT) obtained for the six PAH standard mixture, and the RT achieved by each single PAH standard.

Standard solutions underwent testing using identical conditions to the samples. Analyzing the analyte peak heights at known concentrations facilitated the creation of calibration curves for each analyte, enabling the determination of the PAH concentration in the samples.

Validation procedures

To validate the SBSE–HPLC-FLD method for quantifying six PAHs (fluoranthene, benzo[b]- and benzo[k]-fluoranthene, benzo[a]pyrene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene) in drinking-, ground- and surface-water, the following performance parameters were established: selectivity, linearity, precision, trueness, limits of detection (LOD) and quantification (LOQ), ruggedness and measurement uncertainty.

Selectivity was verified by injecting matrix blanks (ultrapure water samples processed through all extraction and desorption steps without fortification) and PAH standards in the same analytical sequence; retention times had to match within ± 3 σ of the standard value, and no co-eluting peaks could appear in blank chromatograms.

Linearity was evaluated by fortifying ultrapure water with mixed PAH standards with a minimum of five concentration levels (one injection per level) without ever rejecting the upper and lower standards that define the analyte working range (from 7.5 until 150 ng L^{-1} for fluoranthene; from 3.0 until 60 ng L^{-1} for benzo[b]fluoranthene; from 1.5 until 60 ng L^{-1} for benzo[k]fluoranthene and benzo[a]pyrene; from 6.0 until 60 ng L^{-1} for benzo[ghi]perylene; from 6.0 until 120 ng L^{-1} for indeno[1,2,3-cd]pyrene). A plot of peak height versus concentration was constructed, and the equation of the straight line, plus the correlation coefficient (r) was obtain by ordinary-least-squares regression. Acceptance criteria: $r \geq 0.999$, residuals < 15% for every level, and CV of slopes < 25%.

Precision was expressed as relative standard deviation (RSD).

Repeatability was determined from a minimum of ten successive analyses of a single spiked sample at two levels—low (LoQ) and upper end (150 ng L^{-1} for fluoranthene; 60 ng L^{-1} for benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene; 120 ng L^{-1} for indeno[1,2,3-cd]pyrene)—performed under the same conditions (same instrument and analyst).

Intermediate precision was achieved through quality-control samples run on different days (one QC sample every 20 routine samples). The method was accepted when the RSD was ≤25% for all matrices and levels.

Trueness evaluation was checked through recovery tests. Drinking-, ground- and surface-water matrices were fortified at LoQ and mid-level (25 ng L^{-1} for fluoranthene; 10 ng L^{-1} for benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene; 20 ng L^{-1} for indeno[1,2,3-cd]pyrene). Recovery at each level was calculated with Eq. (1):

$$R\,(\%) = \frac{\bar{x}' - \bar{x}}{x_{spike}} \times 100 \tag{1}$$

Where R is the relative spike recovery in percent, $\bar{x}'$ is the mean value of the spiked sample, x¯represents the mean of the experimental sample results, and xspike is the added concentration.

The root mean square of the recovery experiment deviations (brms), was calculated using Eq. (2):

$$b_{rms} = \sqrt{\frac{\sum b_i^2}{n_\eta}} \tag{2}$$

Where bi is the deviation from the mean recovery (if results are corrected with this mean recovery) or from the complete recovery (100%), and nη is the number of recovery experiments.

Limits of detection and quantification were derived from the calibration data. Ten replicate spikes near the expected LOQ furnished the response standard deviation (σ); the slope of the calibration line is S. σ represents the standard deviation of the response and S the calibration slope. LOD and LOQ were obtained with Eqs. (2) and (3):

$$LOD = 3 \times sd_0 \tag{3}$$

Where sd_0 is the standard deviation of a minimum of ten results at LoQ.

$$c' = c_i \times \frac{1}{Rec} \tag{4}$$

LoQs were also verified for the target PAHs at three-tenths of the legislated parametric value for each PAH (or sum of analytes), according to the Portuguese water legislation based on the European Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020.

Ruggedness was assessed by altering the day of analysis. The two-way ANOVA (p=0.05) had to show no significant main or interaction effects, and RSDs had to comply with the precision criterion.

These experiments and calculations demonstrate the method's compliance with international quality requirements for the detection of trace-level PAHs in water.

Results and discussion

Application, selectivity, analytical sensitivity, linearity, working range, limit of quantification, limit of detection, trueness, precision, measurement uncertainty, and fitness for purpose are typical performance characteristics required for validating an analytical method(Relacre 2000; Thompson et al. 2002; Rao 2018). Therefore, this validation study assessed all these criteria based on the experimental results from the SBSE–HPLC–FLD method. A more detailed discussion of these requirements will follow in subsequent chapters.

Sampling and storage

According to some authors, the water samples for PAHs analysis must be collected in amber glass containers (Gachanja 2019; ISO 2018), filled (without overflowing) at the sampling site, and stoppers inserted to leave no air space (Gachanja 2019). Chlorinated samples require the addition of 80 mg of sodium thiosulfate per litre of the sample, followed by thorough mixing before filling the sample vials (EPA 1984; ISO 2018; Relacre 2017). Accordingly, this study utilized 500 mL amber glass bottles (with 40 mg of sodium thiosulfate when needed) and followed the specified container-filling procedure.

From the moment of collection until extraction, all samples should be kept frozen or refrigerated at 4 °C (Barco-Bonilla et al. 2011; Foan et al. 2015). Samples, extracts, and standards must be stored in amber vials to prevent photolytic breakdown due to the light sensitivity of PAHs (EPA 1984), and the screw caps or bottle tops should be PTFE lined or wrapped in aluminium foil (Gachanja 2019), to avoid sample contaminations. Within seven days of sample collection, all samples must be extracted (Aygun and Bagcevan 2019), and they must all be fully analyzed within 40 days of sample extraction (EPA 1984).

Therefore, the PTFE screw caps, and the glass bottle covered in aluminum foil were chosen for PAHs water sampling. Samples were stored at 5±3 °C, with a seven-day extraction period, and sample analysis was completed within 40 days, as determined by the chosen storage and handling conditions.

SBSE optimization

Extraction conditions

The analyte response may be suppressed by insufficient stir bar conditioning (Melo et al. 2009). Therefore, the stir bars were preconditioned in a vial containing 4 mL of a 1:1 mixture of dichloromethane/methanol, and stirred at 500 rpm for 5 min (Foan et al. 2015), to purge the coating of organic impurities (Hu et al. 2014). This step was repeated two times to optimize the conditioning procedure.

In terms of the extraction step, the factors that have received the most research include extraction time, pH adjustment, the addition of an inert salt, the addition of an organic modifier, and stirring rate, followed by extraction temperature and sample volume (Hu et al. 2014). Certain factors, like sample pH adjustments or the introduction of inert salts, alter analytes or sample conditions, thus influencing the chemical equilibrium (Prieto et al. 2010). Since the target PAHs are nonpolar(Zuin et al. 2005; Zuloaga et al. 2020) and non-ionizable substances (Bourdat-Deschamps et al. 2007), pH has no impact on their extraction. As a result, in this validation work, the impact of pH on PAHs extraction was not examined (Hu et al. 2014; Bourdat-Deschamps et al. 2007).

Regarding adding an inert salt, it's noteworthy that nonpolar PAHs in the solution are driven to the water's surface due to the salt's presence (termed the oil effect). This diminishes their interaction with the stir bar's PDMS coating and lowers analyte recovery (Zuin et al. 2005; Bourdat-Deschamps et al. 2007). Overall, the addition of an inert salt to hydrophobic analytes has been noted to decrease extraction efficiency rather than increase it (Prieto et al. 2010); therefore, no salt was used. The sample volume is another crucial factor to consider in the extraction step. Although higher sample volumes reduce extraction effectiveness, the increase in analyte mass may enhance the chromatographic response for SBSE. For the determination of PAHs in ambient waters, minor variations were observed in the 10–60 mL range (Prieto et al. 2010). The selected sample volume can range from 10 mL to 250 mL when PDMS is used as the extraction agent (Dudziak and Bodzek 2009). Therefore, a sample volume of 100 mL was selected to maximize the fluorescence lifetime decay (FLD) response to the analytes.

To reduce analyte adsorption to the glass walls, organic modifiers such as methanol or acetonitrile are tested as additives during solid-phase microextraction (SPME). However, the inclusion of such modifiers can also increase the solubility of the solutes in the aqueous phase and, as a result, reduce extraction efficiency. Usually, methanol is the selected organic modifier when using SBSE (Prieto et al. 2010), and it has been demonstrated that the addition of 10% methanol (concerning the sample volume) enhances the recoveries of PAHs (Bourdat-Deschamps et al. 2007). Hence, in this experimental study, methanol was chosen. However, only 1 mL is added to a 100 mL sample volume to avoid disrupting the extraction efficiency.

Factors such as stirring rate accelerate the process and influence its kinetics. Consequently, the stirring rate is often investigated due to its potential to hasten extraction and enhance responses within a designated extraction time. This phenomenon is attributed to the reduced boundary layer between the stir-bar and the solution bulk. However, increasing the stirring speed may lead to physical damage during the extraction phase, as the stir bar directly contacts the bottom of the sample vial. According to some authors, the stirring rate boosts reactions up to values in the 500–750 rpm range, but greater values have little to no impact (Prieto et al. 2010). Hence, in this experiment, a speed rate of 500 rpm was chosen to optimize the extraction time without compromising the extraction phase.

The extraction temperature is considered a significant factor influencing the effectiveness of extracting PAHs using coated stir bars (Jaworek 2018). According to some authors, the extraction efficiency increases between 40 °C and 60 °C for these analytes (Jaworek 2018; Prieto et al. 2010), and in some studies, an increase in extraction efficiency was observed at temperatures up to 70 °C (Prieto et al. 2010; Mao et al. 2012). Hence, 65 ± 5 °C was selected as the extraction temperature for the target PAHs.

According to some authors, the extraction equilibrium between the target PAHs and the stir bars is reached after 50–60 min (Mao et al. 2012; Hauser et al. 2002). When using SBSE, an extraction time of 60 min allows for the polycyclic aromatic hydrocarbons to equilibrate (at 60 °C), and times longer than 60 min may lead to reduced signal areas (Jaworek 2018). For this reason, an extraction time of 60 min was selected in the following experiments.

The selected extraction parameters were based on both literature reports and preliminary optimization trials performed in our laboratory to confirm recovery and reproducibility.

After the conditioning and extraction steps, to remove any undesired solvent mixture or water droplets, respectively, the stir bars were dried with a lint-free tissue (Melo et al. 2009; Krüger et al. 2011) to prevent the PDMS coating damage. Future studies should include experimental re-testing of individual extraction variables to further confirm their optimal combination.

Desorption conditions

Regarding the desorption step, the most studied variables include desorption solvent, acceptor phase volume, stirring rate, desorption time, and temperature (Foan et al. 2015; Prieto et al. 2010; Zhang et al. 2010; Zuin et al. 2005; Bourdat-Deschamps et al. 2007; Krüger et al. 2011). In this experimental work, liquid desorption was selected because it is usually followed by HPLC-FLD(Foan et al. 2015).

The volume of the acceptor phase must ensure that the coated stir bars are completely submerged in the stripping solvent, promoting the chemical desorption of the extracted solutes. The solvents or mixtures used in this process must be compatible with the PDMS polymer (Bourdat-Deschamps et al. 2007; Prieto et al. 2010). The most popular desorption solvents are acetonitrile, methanol, or combinations of these solvents, along with mixes with water or aqueous buffer (Prieto et al. 2010). However, studies have shown that acetonitrile has the best desorption efficiency for the target PAHs (Jaworek 2018; García-Falcón et al. 2004) and enhances the chromatography peaks (sharp and symmetrical) when HPLC-FLD techniques are employed (Hu et al. 2014). Therefore, acetonitrile was selected and used as the desorption solvent.

Several authors used the liquid desorption into a vial incorporating a 250 µL glass insert (to reduce the solvent quantities) (Prieto et al. 2010; Krüger et al. 2011). Using glass inserts, the common stripping solvent volume ranges from 100 to 200 µL (Prieto et al. 2010; Bourdat-Deschamps et al. 2007). Hence, a 200 µL volume of acetonitrile (Foan et al. 2015; Hauser et al. 2002; Krüger et al. 2011) was selected, and a 300 µL conical glass insert, suitable for the complete immersion of the stir bar, was used.

Experiments conducted at room temperature (20–25 °C) using acetonitrile as the solvent demonstrated that extending the desorption time from 5 to 10 min resulted in increased peak areas. However, peak areas remained mostly constant between desorption times of 15 and 20 min (Popp et al. 2001; Zuin et al. 2005). Occasionally, mechanical agitation or shaking can be employed to reduce this duration, allowing for faster analysis (Melo et al. 2009). According to Hauser et al.(Hauser et al. 2002), at a temperature of 35 °C, the desorbed PAHs increased from 0 to 750 rpm as a result of the stir bars' orbital shaking accelerating. As a result, method validation was performed with a desorption time of 15 min at a stirring speed of 1000 rpm, to enhance the desorption efficiency at room temperature.

All the selected experimental conditions for the SBSE conditioning, extraction, and desorption steps are summed up in Table 4.

HPLC-FLD method

In developing the HPLC-FLD method, a reversed-phase column was chosen, along with acetonitrile and water as mobile phases, as they are commonly used for separating nonpolar polycyclic aromatic hydrocarbons(Kumar et al. 2014; Girelli et al. 2014). A good resolution and selectivity for the six PAHs (Fig. 1) were achieved with an acetonitrile-water mobile-phase gradient (as summarized in Table 2), and the complete chromatographic separation was achieved within 22 min. All sample injections were held constant at 80 µL using a fixed volume injection loop. Injection volume was optimized during preliminary trials; 80 µL ensured sufficient sensitivity without compromising peak symmetry or causing column overloading. The flow rate was set at 1.5 mL min^{-1} (Yang et al. 2019).

The PAHs' intrinsic fluorescence properties make fluorescence detectors one of the most widely employed detection systems for PAHs analysis in water matrices (Bing-Huei et al. 2022). The FLD offers the option of utilizing different excitation and emission wavelengths to enhance selectivity and sensitivity (Yang et al. 2019). This is especially valuable for detecting and quantifying emerging contaminants in aqueous samples with limited analyte volumes. For that reason, an FLD was chosen in this validation work. The FLD and photomultiplier tube (PMT) configuration was selected based on previously published studies in PAHs water samples analysis, using HPLC-FLD (Windal et al. 2008; Hauser et al. 2002; Hu et al. 2014), and the excitation and emission wavelengths, as well as the PMT gain programmers, are described in Table 3.

PAHs were identified by comparing their retention times with those obtained from the reference chromatogram of each analyte. The retention time window criterion for identification was set at three times the standard deviation

Table 4 Summary of the selected experimental conditions in SBSE optimization

SBSE	Solvents and volume	Stirring speed (rpm)	Temperature (ºC)	Time (min)
Stir bars conditioning$^{\varnothing}$	Methanol: Dichloromethane 4 mL, 1:1 (v/v)	500	20–25	5
Extraction conditions$^{\diamond}$	Methanol 1 mL	500	60–70	60
Desorption conditions§	Acetonitrile 0.2 mL	1000	20–25	15

$^{\varnothing}$This procedure is repeated two times; $^{\diamond}$For a 100 mL sample volume; § Using 300 µL conical glass inserts

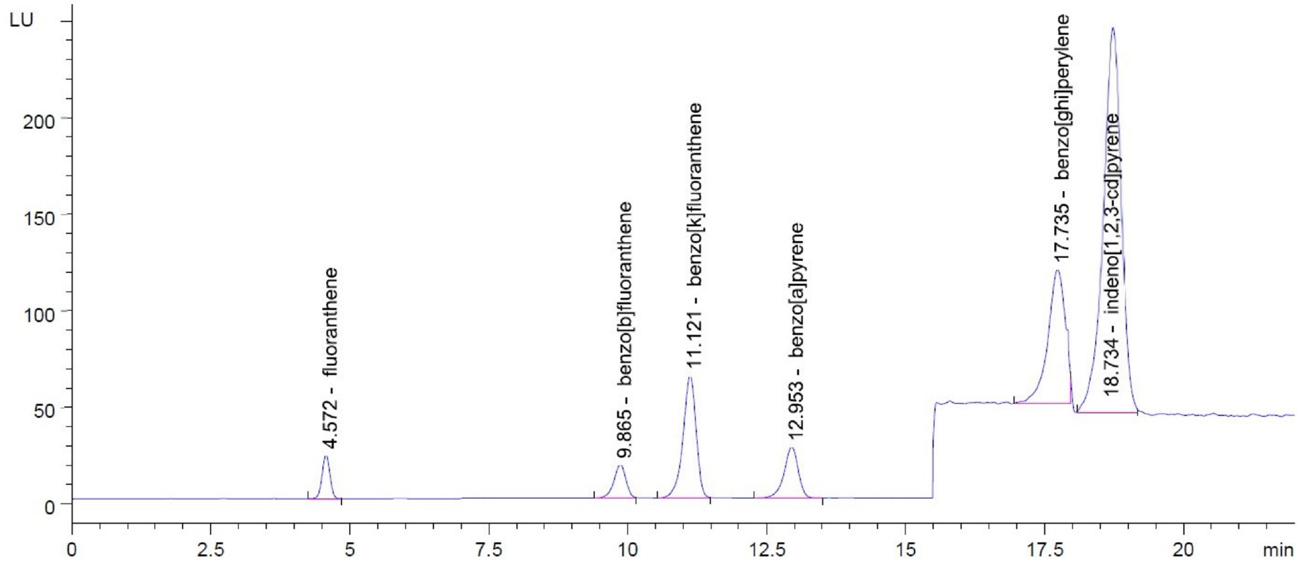

Fig. 1 Example of an SBSE–HPLC–FLD chromatogram for the target PAHs (spiked concentration: 25 ng L^{-1}

of the retention times of standards injected on that day (Bourdat-Deschamps et al. 2007). In the separation and quantification, according to the selected HPLC-FLD conditions and calibration curves (n = 12), the following average retention times (min) where obtain: 4.6 (FLU), 9.8 (BBF), 11.1 (BKF), 12.9 (BAP), 17.6 (BPE) and 18.6 (IND). The RSD of retention time for each analyte was below 1.27% (obtained for fluoranthene), demonstrating the good repeatability achieved by the proposed procedure.

The performance of the HPLC-FLD method was evaluated by quality parameters (such as selectivity, quantification limits, detection limits, working range, analytical sensitivity, and linearity), which were determined using certified standard mix solutions. For each compound, the linearity of the calibration curve was obtained with a maximum of eight to ten calibration points (according to Table 1). This was accomplished by plotting analyte height versus concentration (with a minimum of 5 concentration levels) (Relacre 1996), without ever rejecting the upper and lower standards that define the analyte working range. The observed linearity (correlation coefficient, r) ranged between 0.99941 and 0.99952 for all six PAH compounds, showing an excellent correlation. The calibration curves (n = 12) for the target PAHs were validated in accordance with the ISO 8466-1 and ISO 8466-2 standards. The presented variation coefficients were less than 25% (the highest value was 24% obtained for benzo[ghi]perylene), and the residual values calculated with the linear regression model remained less than 15%, indicating a good homogeneity of variances. The analytical sensitivity interval limits were evaluated by considering the slope mean for each PAH and twice the obtained standard deviation.

Analyte quantification limits were determined to meet the fitness-for-purpose criteria specified in the Portuguese national water legislation (outlined in Table 2). In the case of drinking water, as per the applicable legislation (Decree-law nº 152/2017), the benzo[a]pyrene concentration should not exceed 10 ng L^{-1}, while the concentrations of the other four PAHs (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene) should remain below 100 ng L^{-1}. Therefore, an acceptable approach is to set the quantification limits for the target PAHs at three-tenths of the legislated parametric value for each analyte (or sum of analytes) (Magnusson et al. 2018). However, the working range limits repeatability ($n = 10$) were also evaluated to validate the experimental approach. In this assessment, all the RSD values remained below 15.7% (the limit of quantification for benzo[k]fluoranthene), and all the relative biases were below 19.4% (the limit of quantification for fluoranthene), indicating good repeatability. Regarding the limits of detection, they were estimated considering the LOQ standard deviation obtained for each analyte in repeatability terms (Relacre 2000).

In validation studies, the reference material used for calibration should not be employed to assess bias (Magnusson 2014; Relacre 2018). Thus, in this work, different PAH standard mix solutions batches were used, and all the evaluated quality parameters are summarized in Table 5.

Trueness evaluation

Trueness is the closeness of agreement between the average of an infinite number of replicates of measured quantity values and a reference quantity value, and this assessment is

Table 5 Summary of the evaluated quality parameters results

PAH	LOD (ng nL^{-1})	Working range (ng L)$^{-1}$	RT window (min)	Analytical sensitivity (average slope ± 2δ)$^{\oplus}$	*r* mean
Fluoranthene	1.1	7.5–150	4.4–4.8	0.18144 ± 0.04839	0.99946
Benzo[b]fluoranthene	0.5	3.0–60	9.7–10.0	0.34924 ± 0.10936	0.99949
Benzo[k]fluoranthene	0.7	1.5–60	10.9–11.3	1.21698 ± 0.38391	0.99941
Benzo[a]pyrene	0.4	1.5–60	12.7–13.2	0.55542 ± 0.24122	0.99943
Benzo[ghi]perylene	1.8	6.0–60	17.3–18.0	1.75324 ± 0.85760	0.99952
Indeno[1,2,3-cd]pyrene	1.7	6.0–120	18.2–19.1	1.82761 ± 0.73959	0.99944

$^{\oplus}$The δ represents the standard deviation of the calibration curves slopes

Table 6 Concentration levels used in spiked water samples for the six investigated PAHs

Amount added	FLU (ng L^{-1})	BBF (ng L^{-1})	BKF (ng L^{-1})	BAP (ng L^{-1})	BPE (ng L^{-1})	IND (ng L^{-1})
LOQ	7.5	3	1.5	1.5	6	6
IQL	25	10	10	10	10	20

expressed quantitatively in terms of "bias"(ISO 2012; Magnusson 2014; Ellison and Williams 2012).

The trueness evaluation in this experimental work was determined by recovery tests, which estimated the recovery of a known amount of analyte added to a previously analysed water sample and can be used as an indication of the expected level of bias.

When there are no significant analytical restrictions between water matrices, validation studies may include more than one water matrix (IPAC 2017). Therefore, the following validation studies for drinking water and groundwater were carried out in conjunction, apart from the surface water matrix.

In different water matrices, by measuring the relative spike recovery it was possible to assess the deviation from the mean recovery. Considering ten to nineteen analytical results (a minimum of ten tests are required (Magnusson 2014), it was possible to calculate the root mean square of the deviations from the recovery experiments (b_{rms}). Yet, for trueness assessment, water samples were also spiked with analytes at both the LOQ and an intermediate quantification level (IQL), as specified in Table 6. This aimed to determine whether the added quantity or the water matrix could influence PAH recoveries (Magnusson 2014).

The experimental findings from drinking water and groundwater samples indicated a mean recovery of 97.7% at the limit of quantification (LOQ), ranging from 90.3% (benzo[k]fluoranthene) to 109.9% (benzo[b]fluoranthene). When different quantities of reference material were introduced, the mean recovery for the six PAHs remained at 102.3%. In this scenario, the lowest and highest mean recovery values were seen with indeno[1,2,3-cd]pyrene (98.4%) and fluoranthene (107.7%), respectively. As a result, it was evident that for both matrices and various concentration levels, no matrix effects were observed. All recovery experiments at different concentration levels were below 110%, indicating the high trueness achieved through this experimental approach. The RSD of mean recoveries, considering the two concentration levels, for the individual analytes, was under 14.2% (attained with fluoranthene), and the lowest value of 0.6% was achieved by indeno[1,2,3-cd]pyrene, demonstrating good method robustness at different concentrations. Furthermore, the b_{rms} in the LOQ, for drinking water and groundwater samples, ranged between 0.0136 and 0.1134, obtained for fluoranthene and benzo[a]pyrene, respectively. At a higher concentration level, the determined b_{rms} oscillated from 0.0387 to 0.0820, achieved for benzo[b]fluoranthene and fluoranthene. Hence, the accuracy of the methodology was confirmed across various levels of spiked samples, with lower bias indicating higher trueness.

In terms of the surface water matrix, the recovery experiments at the limit of quantification (LOQ) indicated a notable loss in mean recovery for fluoranthene (63.3%), benzo[k]fluoranthene (66.5%), and indeno[1,2,3-cd]pyrene (65.4%) compared to other water matrices. The highest recovery was observed for benzo[b]fluoranthene (98.8%), resulting in an overall mean recovery of 79.0% for all analytes. Increasing the added amount revealed a consistent pattern of lower recoveries in this water matrix, with indeno[1,2,3-cd]pyrene achieving a recovery of 65.3%. Fluoranthene (97.6%) and benzo[a]pyrene (97.1%) exhibited the highest mean recoveries. Overall, the six PAHs achieved a mean recovery of 85.8% in this assessment. Thus, both at the LOQ and higher concentration levels, the mean recovery across all water matrices decreased from 99.9 to 102.3% (observed in drinking water and groundwater, respectively) to 79.0% and 85.8% (observed in surface water), respectively. The relative standard deviation (RSD) of mean recoveries for individual analytes in surface water varied from 42.6% (fluoranthene) to 0.1% (indeno[1,2,3-cd]pyrene) at the two concentration levels. These divergent outcomes highlight the impact of the water matrix and varying quantities. For indeno[1,2,3-cd]pyrene, the matrix effect is evident (seen in the consistent RSD within the added range). In contrast, fluoranthene and benzo[k]fluoranthene displayed RSD values of 42.6% and 25.0% (between concentration levels), indicating a pronounced amount-added effect. At the higher concentration level, mean recoveries for these two analytes were 97.6% and 85.5%, respectively. Finally, the RSD between concentration levels for benzo[b]fluoranthene, benzo[a]pyrene, and benzo[ghi]perylene ranged from 8.2% (benzo[a]pyrene) to 12.9% (benzo[ghi]perylene). This observation confirms the absence of matrix or added amount effects for these specific polycyclic aromatic hydrocarbons (PAHs). Notably, no matrix or added amount effects were observed in surface water samples for benzo[b]fluoranthene, benzo[a]pyrene, and benzo[ghi]perylene. For these PAHs, the mean recoveries between the LOQ and a higher concentration level, oscillated from 89.1% (benzo[a]pyrene) to 98.8% (for benzo[b] fluoranthene), and from 79.3% (benzo[ghi]perylene) to 97.1% (benzo[a]pyrene), respectively, and allow to assess the good trueness achieved for these PAHs. The RSD, in this case, was less than 13% between amounts added, demonstrating for the specified analytes, a good method robustness. Figure 2 illustrates these mean recoveries outcomes, for the investigated water matrices.

Furthermore, the b_{rms} in the LOQ, for surface water, varied from 0.0698 to 0.1593, acquired with benzo[a] pyrene and indeno[1,2,3-cd]pyrene, respectively. At a higher concentration level, the determined b_{rms} alternated between 0.0574 and 0.1474, achieved for fluoranthene and benzo[ghi]perylene. Despite the observed effects of matrix and added amount in surface water (shown in Fig. 3), the methodology demonstrated strong bias performance for both matrices across varied spiked amounts.

The matrix effect, possibly caused by higher total suspended solids and organic matter content (Mukhopadhyay et al. 2020), makes interactions complex between higher molecular weight PAHs, such as indeno[1,2,3-cd]pyrene (shown in Table 1), and the PDMS stir bar adsorbent. Future applications could benefit from determining bulk physicochemical parameters (e.g., pH, TSP, OC, DOC, conductivity) to support a more comprehensive interpretation It's crucial to account for the obtained mean recovery when reporting the LOQ. Concerning the added amount effect, higher analyte concentrations can induce interactions between PAHs and the adsorbent phase (Hsieh and Chang 1997), resulting in improved mean recoveries, as seen with fluoranthene and benzo[k]fluoranthene. Consequently, depending on the validation's water matrix, it may be necessary to determine the LOQ based on actual experimental mean recovery outcomes. In some cases, reevaluating the initial target LOQ to a higher concentration level may be necessary to address this concern, provided it aligns with the relevant parameter

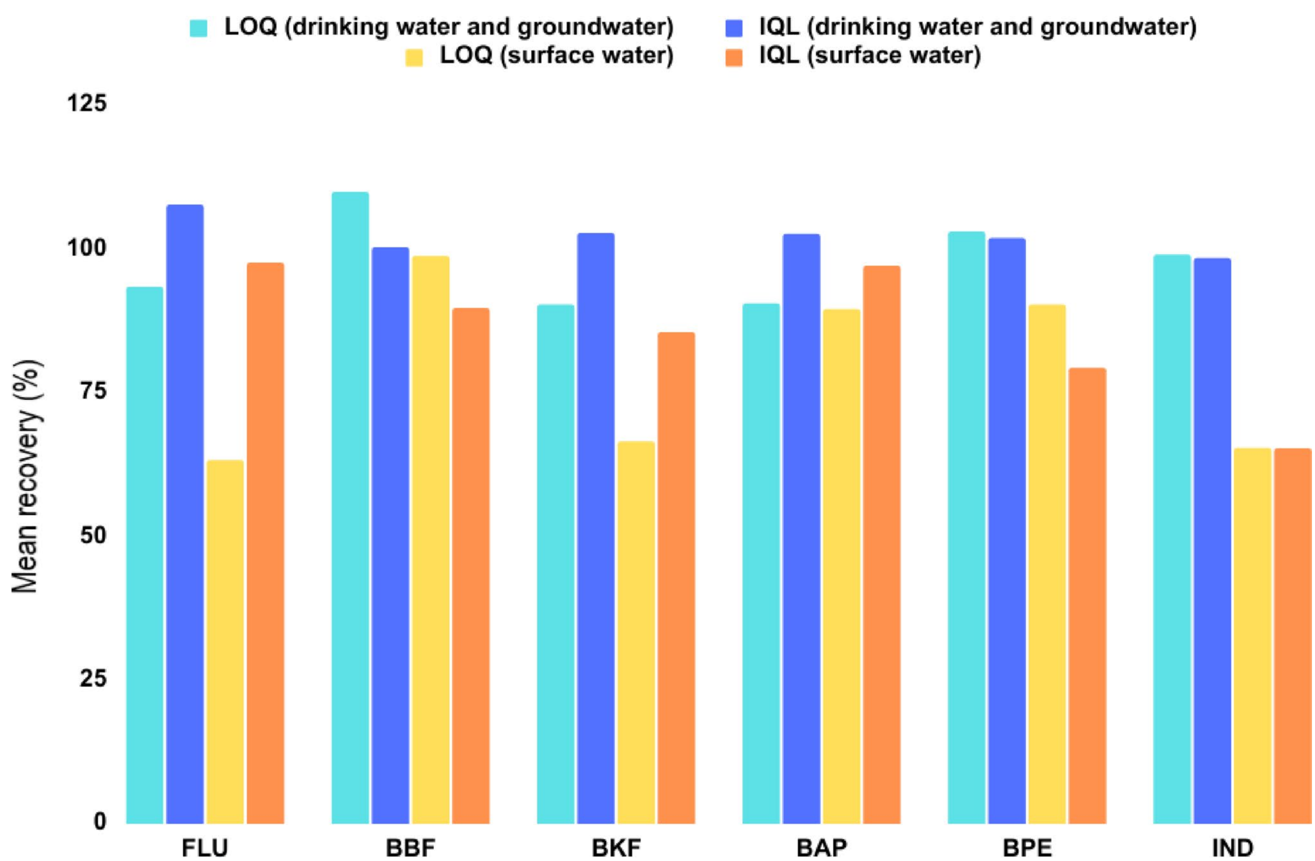

Fig. 2 Mean recoveries achieved for the six PAHs in the investigated water matrices, with the proposed method

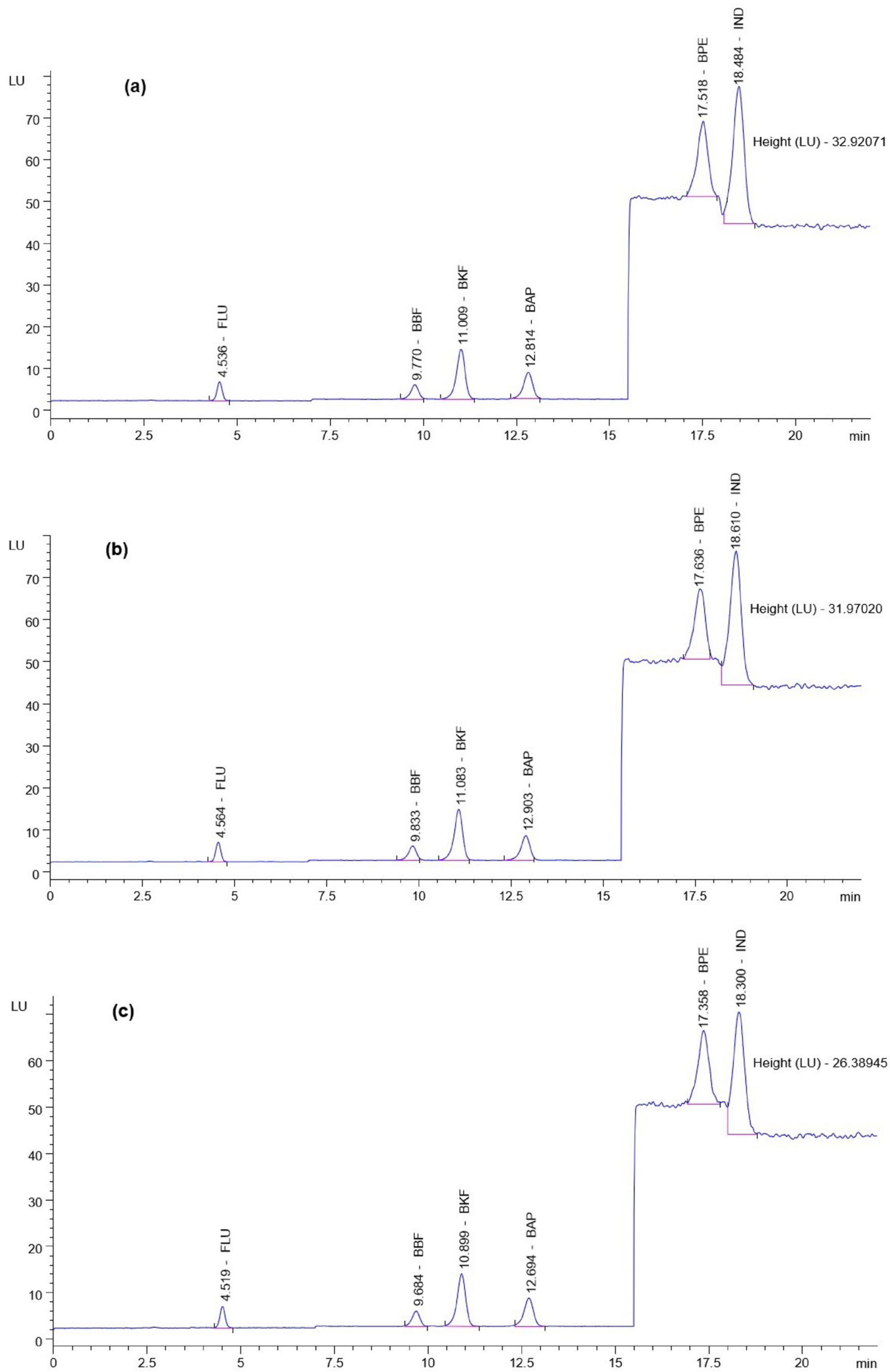

Fig. 3 Example of the water matrix effect observed in indeno[1,2,3-cd] pyrene peak height when adding an IQL amount. **a** QC sample: IQL standard prepared in UPW; **b** Recovery: IQL standard prepared in drinking water and groundwater; **c** Recovery: IQL standard prepared in surface water

value as per applicable legislation. As a result, after conducting recovery tests on various water matrices, a Student's t-test was performed on spiked water samples with an added limit of quantification (LOQ) amount. This was prompted by the higher RSD value observed at this concentration level (at intermediate precision), compared to higher concentration levels. The significance test was assessed, which compares the calculated t result with the corresponding $t_{critical}$ value. This comparison was made using a two-way Student's t-table, with the number of degrees of freedom associated with Rec and u_{Rec}, at a 95% confidence level, as described in Table 7.

During this evaluation, it became evident that results correction was necessary for all analytes, except for benzo[a]

Table 7 Student's t-test data achieved for the different water matrices

PAH	Drinking water and groundwater					Surface water				
	df[∅]	Rec[⊤]	u_{Rec}[¥]	t	$t_{critical}$	df[∅]	Rec[⊤]	u_{Rec}[¥]	t	$t_{critical}$
Fluoranthene	9	0.9056	0.0041	23.027	2.262	12	0.8582	0.0242	5.863	2.179
Benzo[b]fluoranthene	9	1.0372	0.0146	2.543	2.262	12	0.9325	0.0257	2.631	2.179
Benzo[k]fluoranthene	9	0.7398	0.0214	12.148	2.262	18	0.5447	0.0169	27.011	2.101
Benzo[a]pyrene	9	0.9580	0.0218	1.930	2.262	18	0.9479	0.0156	3.341	2.101
Benzo[ghi]perylene	9	0.9286	0.0254	2.811	2.262	12	0.8137	0.0236	7.891	2.179
Indeno[1,2,3-cd]pyrene	9	0.8631	0.0154	10.668	2.262	12	0.5527	0.0254	17.605	2.179

[∅] The df stands for degree of freedom (n-1, where n represents the number of observations); [⊤] Rec represents the relative recovery; [¥] u_{Rec} is the standard uncertainty, considering the available relative recovery data of the reference value

Table 8 Summary of obtained results for the trueness assessment

PAH	Spiked level (ng L^{-1})	Drinking water and groundwater			Surface water		
		MR[□] (%)	b_{rms}	u_b (%)	MR[□] (%)	b_{rms}	u_b (%)
Fluoranthene	7.5	93.4	0.0136	1.4	63.3	0.1177	11.8
	25	107.7	0.0820	8.2	97.6	0.0574	5.7
Benzo[b]fluoranthene	3	109.9	0.0423	4.2	98.8	0.0954	9.5
	10	100.3	0.0387	3.9	89.7	0.0833	8.3
Benzo[k]fluoranthene	1.5	90.3	0.0868	8.7	66.5	0.1312	13.1
	10	102.8	0.0588	5.9	85.5	0.0876	8.8
Benzo[a]pyrene	1.5	90.5	0.1134	11.3	89.5	0.0698	7.0
	10	102.6	0.0591	5.9	97.1	0.0606	6.1
Benzo[ghi]perylene	6	103.0	0.0821	8.2	90.3	0.1005	10.0
	10	101.9	0.0751	7.5	79.3	0.1474	14.7
Indeno[1,2,3-cd]pyrene	6	99.0	0.0555	5.5	65.4	0.1593	15.9
	20	98.4	0.0487	4.9	65.3	0.1458	14.6

[□] Stands for mean recovery

pyrene in drinking water and groundwater matrices. This determination was based on relative recovery (Rec). For the determination of the standard uncertainty estimated from these recovery experiments (u_b), the uncertainty in the concentration of the analyte added (u_{add}) also needs to be assessed. However, through careful selection of certified standards (e.g., as high-purity reference materials), and the volumetric equipment involved (e.g., the use of high-quality micro syringes) in fortifying the sample, the contributions of the uncertainty in the concentration and volume added (u_{conc} and u_v, respectively) are negligible(Relacre 2018; Ellison and Williams 2012), so u_{add} was not accounted. Moreover, the reference standards were continuously weighted before opening and after used for the intermediate solutions preparation, with a criterion of (±) 10 mg between consecutive uses (to evaluate the reference standard stability), reducing even more the u_{add} contribution. Consequently, in this experimental work, the evaluated u_b (for each water matrix) corresponds to calculated b_{rms} (Eq. 5).

$$u_b = b_{rms} \quad (5)$$

In this method validation no relevant systematic errors were identified, and all the obtained results for the trueness evaluation and measuring are summarized in Table 8; Fig. 4.

Additionally, another strategy for acquiring information about method bias is to participate in proficiency testing schemes (ISO 2005). PT schemes additionally confirm ongoing method performance and offer pertinent information for uncertainty assessment. To obtain a reliable estimate, it is recommended to conduct a minimum of six different trials to determine the bias from the interlaboratory comparison results (ISO 2012; Ellison and Williams 2012).

During this method validation phase, participation in a PT scheme was feasible after optimizing the SBSE–HPLC–FLD experimental conditions. The chosen PT (round 576, sample 7 C) was sourced from LGC Standards (Aquacheck), and all results were satisfactory(Boley 2000). Excellent z-score values (all below one) were achieved for the six PAHs. Figure 5 summarizes the PT scheme outcomes, confirming strong method performance and reproducibility for the analysed analytes.

However, the acquired data cannot be used to assess the method bias, as only one satisfactory PT scheme was available for that purpose, and a minimum of six satisfactory trials are required.

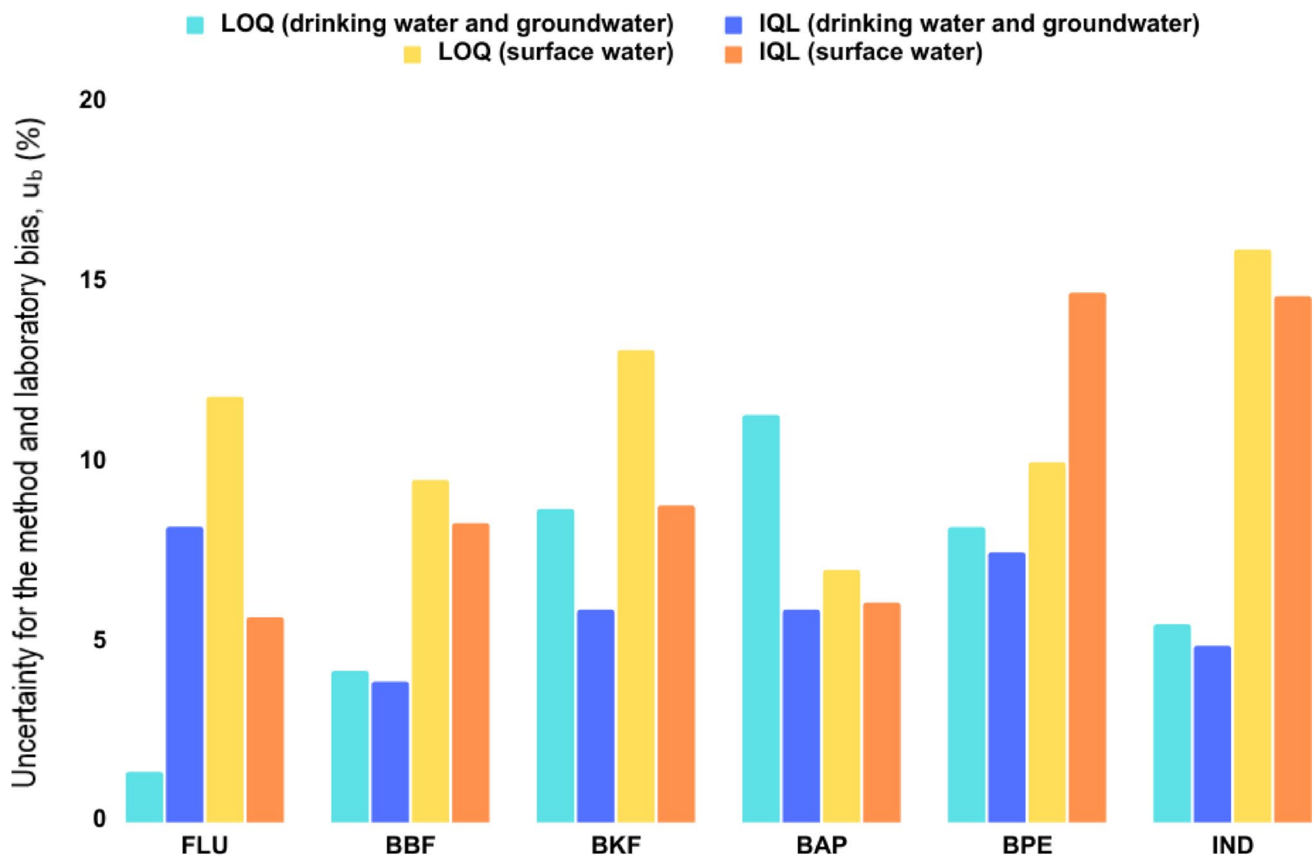

Fig. 4 Standard uncertainty estimated from the recovery experiments for the method and laboratory bias, in the different water matrices concentration levels

Scheme: Water Chemistry (AQUACHECK)

7C - Polycyclic Aromatic Hydrocarbons (2 Spikes)

Analyte	Analyst	Method	Result	Units	Z Score	Assigned Value	SDPA	Number of results
Fluoranthene	EL	Other	36.5	ng/L	-0.42	38.1	3.81	43
Benzo(b)fluoranthene	EL	Other	20.55	ng/L	-0.52	21.68	2.168	56
Benzo(k)fluoranthene	EL	Other	17.16	ng/L	0.02	17.12	2.0	57
Benz(a)pyrene	EL	Other	9.38	ng/L	-0.63	10.01	1.001	58
Benzo(ghi)perylene	EL	Other	7.79	ng/L	-0.34	8.47	2.0	57
Indeno(1,2,3-cd)pyrene	EL	Other	9.11	ng/L	-0.69	10.48	2.0	58

Fig. 5 PT scheme results obtained for the six PAHs using this SBSE–HPLC–FLD methodology (figure adapted from Aquacheck laboratory individual report), where SDPA represents the standard deviation for proficiency assessment

Precision assessment

Precision refers to the agreement among signals or measured quantity values obtained through repeated measurements conducted on the same or comparable objects(ISO 2012). It is typically expressed using statistical parameters that describe the spread of results, such as the standard deviation (SD) or the relative standard deviation (RSD) (Magnusson 2014).

During this method validation, the uncertainty component for within-laboratory reproducibility (u_{Rw}), was determined from the standard deviation of quality control results. This involved combining the uncertainty component from routine QC sample RSD ($u_{Rw,\ stand}$), and the uncertainty component from range control charts ($u_{r,\ range}$), established using mean range $\bar{R}$ from duplicates of proposed water matrices, to provide a consistent u_{Rw}. Estimating both uncertainty factors demands at least eight measurements, with more measurements leading to higher estimation confidence (ISO 2012). This validation employed between eight and twenty-six measurements for precision assessment.

In routine analysis, a 20% RSD for internal QC is common, and in certain instances, complex prosome-like chromatography techniques may even require a 50% RSD (Magnusson 2014). During this validation procedure, a pre-established criterion of 25% RSD was chosen for internal quality control parameters (such as QC samples and water matrix duplicates), with every twentieth sample analysed being a QC sample.

The QC samples analysed under intermediate precision conditions (e.g., different days), and concentrated at the working range extremes with the highest RSD value, were used to assess $u_{Rw,stand}$ for each PAH. The achieved results (from eighteen to twenty-one measurements) revealed benzo[k]fluoranthene and benzo[b]fluoranthene with the highest and lowest $u_{Rw,}$ standing at 16.9% and 6.8%, respectively. These results indicate strong within-laboratory reproducibility, with an average relative standard deviation (RSD) of 13.1% across the six PAHs. The additional uncertainty component due to inhomogeneity (ISO 2012), $u_{r,\ range}$, was estimated for the different water matrices from range control charts outcomes, through the mean range of eight quantifiable sample duplicates, spiked at different concentration levels (due to the analyzed water being absent of target analytes). For drinking water and groundwater, the obtained $u_{r,\ range}$ oscillated between 11.6% and 5.2%, achieved for indeno[1,2,3-cd]pyrene and benzo[ghi]perylene, respectively. The average $u_{r,\ range}$ value of 7.8%, considering all analytes, demonstrated the good repeatability achieved for these water matrices with this methodology.

Relative to the surface water, the assessed $u_{r,\ range}$ remained below 15.4% achieved for benzo[ghi]perylene, and the lowest value of 7.6% was accomplished by benzo[a]pyrene. The achieved $u_{r,\ range}$ mean of 10.2%, contemplating all PAHs, discloses good repeatability in this water matrix, as through the investigated spiked water samples, the $u_{r,\ range}$ stayed below 15.4%, considering all the water matrices.

With both contributions evaluated ($u_{Rw,\ stand}$ and $u_{r,\ range}$), the estimated uncertainty component for the within-laboratory reproducibility, u_{Rw}, for drinking water and groundwater, ranged from 19.1% (for benzo[k]fluoranthene) to 9.1% (for benzo[b]fluoranthene), with an average result of 15.3% for the target PAHs. In surface water, the u_{Rw} remained under 20.6% (achieved for benzo[ghi]perylene), and the mean value of this uncertainty component stayed at 16.7%.

Hence, the investigated water matrices have generally demonstrated good within-laboratory reproducibility achieved in different concentrations (above the LOQ for some PAHs), and no significant random errors were identified. All the precision assessment results are described in Table 9; Fig. 6.

Measurement uncertainty

Measurement uncertainty can be interpreted as a quantitative estimate of accuracy, normally studied as two components: trueness and precision(Magnusson et al. 2018; Magnusson 2014). Accuracy is a measure of how closely a single result reflects the true value. Consequently, accuracy encompasses both systematic error and random error that affect the outcomes, and a method with less random error and a smaller bias is considered more accurate(Magnusson 2014). As no further uncertainty components are evaluated beyond u_{Rw} and u_b, the combined standard uncertainty, u_c, is calculated according to Eq. 6.

$$u_C = \sqrt{u_{Rw}^2 + u_b^2} \tag{6}$$

Considering drinking water and groundwater matrices, the u_c values ranged between 10.0% and 19.2%, attained for benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene, respectively. The average u_c result of 17.0% is representative of the exemplary method accuracy when combining all the uncertainty components.

Regarding the surface water matrix, the accomplished u_c results were very similar comparing to other matrices, except for benzo[b]fluoranthene (14.6%), benzo[ghi]perylene (22.9%) and indeno[1,2,3-cd]pyrene (23.6%). For those PAHs, the highest values may result from a matrix effect, such as increased dissolved organic matter

Table 9 Summary of uncertainties achieved results to precision evaluation

PAH	$u_{Rw,\ stand}$ (%)	Drinking water and groundwater		Surface water	
		$u_{r,\ range}$ (%)	u_{Rw} (%)	$u_{r,\ range}$ (%)	u_{Rw} (%)
Fluoranthene	15.2	8.7	17.5	9.8	18.1
Benzo[b]fluoranthene	6.8	6.0	9.1	8.7	11.0
Benzo[k]fluoranthene	16.9	8.9	19.1	9.6	19.4
Benzo[a]pyrene	11.5	6.2	13.1	7.6	13.8
Benzo[ghi]perylene	13.7	5.2	14.7	15.4	20.6
Indeno[1,2,3-cd]pyrene	14.2	11.6	18.3	10.0	17.4

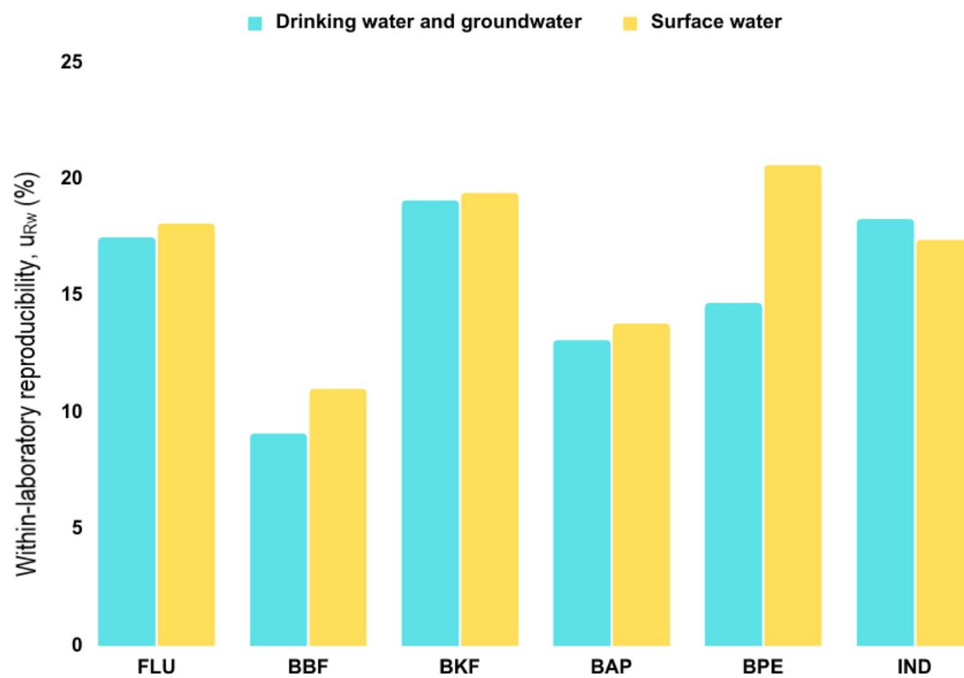

Fig. 6 Summarized within-laboratory reproducibility achieved with this validation method in different water matrices

Table 10 PAHs combined and expanded uncertainties results obtained in the investigated water matrices

PAH	Drinking water and groundwater		Surface water	
	u_c (%)	U (%), k=2	u_c (%)	U (%), k=2
Fluoranthene	17.5	35.1	21.5	43.1
Benzo[b]fluoranthene	10.0	20.1	14.6	29.1
Benzo[k]fluoranthene	21.0	42.0	23.5	46.9
Benzo[a]pyrene	17.3	34.6	15.5	31.0
Benzo[ghi]perylene	16.8	33.7	22.9	45.9
Indeno[1,2,3-cd]pyrene	19.2	38.3	23.6	47.1

(Bourdat-Deschamps et al. 2007), which diminishes the desired homogeneity between duplicates and alters the stir bar analyte adsorption. Nonetheless, in this water matrix, the lowest result, 14.6%, was observed for benzo[b]fluoranthene. The average UC of 20.3% also indicates good accuracy for this matrix. Considering all the target PAHs and water matrices, the calculated results remained below the pre-established value of 25%.

Therefore, for the investigated water matrices, a significant contribution of random errors was observed for the UC assessment, compared to systematic errors. However, this component of error varies unpredictably and can diminish as the number of observations (Ellison and Williams 2012) increases.

The expanded uncertainty, U, is necessary to provide an interval that can be expected to cover a significant portion of the distribution of values that can be reasonably attributed to the measurand(Ellison and Williams 2012). Hence, U was calculated using a coverage factor (k) of two, based on the desired 95% level of confidence(Ellison and Williams 2012; ISO 2012; Magnusson 2014). The obtained results for the six PAHs in different water matrices are summarized in Table 10 and illustrated in Fig. 7.

All the calculated expanded uncertainties, U, for a 95% level of confidence, were below 42.0% and 47.1%, obtained for drinking water and groundwater (with benzo[k]fluoranthene) and for surface water (by indeno[1,2,3-cd]pyrene), respectively.

These results are an essential element of the analytical requirement, to evaluate the whether method is suitable for its intended purpose (Magnusson 2014), according to the applicable Portuguese legislation (described in Table 2). Therefore, for all water matrices, since all the validation results (in trueness, precision, and uncertainty assessment) remained below the established requisites, it is possible to confirm that the proposed method is in concordance with the mandatory legal requirements for the analysis of the target PAHs.

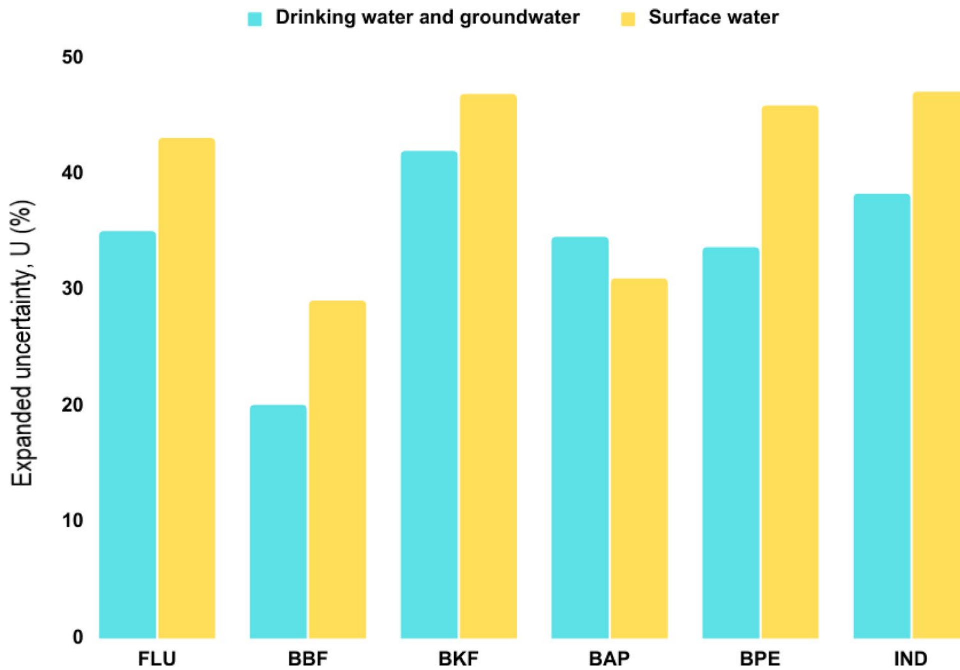

Fig. 7 Achieved expanded uncertainties results (k = 2) for the investigated water matrices

Analytical performance: analysis of PAHs in water samples

Numerous studies have been conducted to determine the presence of PAHs in water samples, and a variety of analytical techniques have been employed. For their identification, the most common methods are gas chromatography (GC) with a mass spectrometer (MS) or a flame ionization detector (FID), and high-performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence detection (FLD (Mojiri et al. 2019; Bing-Huei et al. 2022; Díaz-Moroles et al. 2007; Aygun and Bagcevan 2019). Although both techniques are adequate for analyzing PAHs in water samples, HPLC-FLD has higher sensitivity and a lower relative standard deviation percentage value (Aygun and Bagcevan 2019; Díaz-Moroles et al. 2007), and for that reason, it is the most commonly employed technique to evaluate these EOCs. The different extraction, desorption, chromatographic, and detection conditions can be optimized to reduce time and solvent costs, decrease quantification levels in smaller sample amounts, and increase the analytes' mean recoveries in various water matrices (Hu et al. 2014; Jaworek 2018).

As PAHs are usually present in trace amounts in water samples (Abiodun Olagoke et al. 2017), it is necessary to extract and concentrate the analytes so they can be identified and quantified (Jaworek 2018), to meet the stricter legislative requirements (e.g., uncertainty and ng L^{-1} quantification levels). The SBSE, a microextraction technique, has gained widespread acceptance as a highly effective sample preparation method for enriching solutes from aqueous samples and has been used extensively for PAH analysis (Mollahosseini et al. 2016; Foan et al. 2015). The complexity of the matrices involved can significantly impact SBSE efficiency, just as with many other sample preparation methods for trace analysis. For instance, significant amounts of dissolved or suspended inorganic or organic matter present in environmental matrices (e.g., surface water) can affect the extraction of target compounds by PDMS or other phases, causing the extraction yield to vary significantly from sample to sample (Prieto et al. 2010). This problem was identified during this research, as the obtained mean recoveries for the highest molecular PAH (indeno[1,2,3-cd]pyrene) in surface water, decreased at both concentration levels with 65.4% and 65.3%, in contrast with the good mean recoveries of 99.9% and 98.4% observed for cleaner waters such as drinking water and groundwater, at the LOQ and IQL, respectively. Further studies incorporating physico-chemical characterization of the water matrices could enhance understanding of how these properties influence SBSE efficiency. Fluoranthene and benzo[k]fluoranthene, also showed considerably low mean recoveries of 63.3% and 66.5%, respectively, in spiked surface water matrix when a small analyte amount (in the LOQ) was added, with less than 30.1% and 23.8% of total mean recoveries, comparing with both other matrices. Therefore, considering the achieved mean recoveries, it may be essential to evaluate the sample turbidity factor and the method's LOQ in more contaminated water matrices (Gachanja 2019), to predict and enhance this methodology's performance and applicability. However, in this validation work, the SBSE–HPLC–FLD optimization steps generally yielded good mean recoveries, ranging from 63.3% to

Table 11 PAHs theoretical and method limits of quantification, according to the calculated relative recoveries and the student's *t*-test conclusions, for the proposed water matrices

PAH	Theoretical LOQ (ng L^{-1})	Drinking water and groundwater		Surface water	
		Rec	Method LOQ (ng L^{-1})	Rec	Method LOQ (ng L^{-1})
Fluoranthene	7.5	0.9056	8.3	0.8582	8.7
Benzo[b]fluoranthene	3.0	1.0372	2.9	0.9325	3.2
Benzo[k]fluoranthene	1.5	0.7401	2.0	0.5447	2.8
Benzo[a]pyrene	1.5	n/a	1.5	0.9479	1.6
Benzo[ghi]perylene	6.0	0.9286	6.5	0.8137	7.4
Indeno[1,2,3-cd]pyrene	6.0	0.8361	7.2	0.5527	10.9

109.9% (Hu et al. 2014; Zuin et al. 2005), considering all PAHs and water matrices.

The Student's t-test also revealed the necessity of results correction for all analytes (except for benzo[a]pyrene in drinking water and groundwater matrices), as the calculated t-values are higher than the corresponding $t_{critical}$ values for the proposed water matrices. Consequently, the calculated method LOQ values were higher for most of the investigated PAHs (with benzo[b]fluoranthene exception), in comparison to the theoretical LOQ, as shown in Table 11.

The good selectivity, sensibility, linearity ($R \geq 0.99941$), LOD (0.4–1.8, ng L^{-1}), method LOQ (1.5–10.9 ng L^{-1}), trueness ($u_b \leq 15.9\%$), precision ($u_{Rw} \leq 20.6\%$), expanded uncertainty ($U \leq 47.1\%$, $k=2$), and the satisfactory PT scheme, are indicators of the exemplary method performance and reproducibility, for PAHs quantification in drinking water, groundwater, and surface water. Representative SBSE–HPLC–FLD chromatograms of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene at their LOQ spike levels are shown in Fig. 8, illustrating baseline separation and the signal-to-noise ratios achieved at 1.5 ng L^{-1} (BKF and BAP), 3 ng L^{-1} (BBF) and 6 ng L^{-1} (BPE and IND). No trends were noticed in the obtained x-charts and range charts for the six PAHs, indicating that the method is statistically under control.

The method demonstrated fitness for purpose as defined by Magnusson (2014), the selected optimization conditions permitted a complete chromatographic separation within 22 min (26 min at the end of the post time), for a total volume of 39 mL of eluents spent per sample run (according to Table 2), and the analysis of 20 samples per day. This optimization reduced both the overall processing time and solvent consumption compared with similar analytical procedures (Aygun and Bagcevan 2019; Hu et al. 2014; Karyab et al. 2013; Foan et al. 2015). Furthermore, the SBSE technique is uses very minimal solvent and relatively simple to us (Niehus et al. 2002), and the reutilization of stir bars after the conditioning step reduces the analytical procedure costs, thereby enhancing the economic advantages.

Conclusion

A sensitive and accurate SBSE–HPLC–FLD method was optimized for the determination of priority pollutants PAHs (FLU, BBF, BKF, BAP, BPE, and IND) in drinking water, groundwater, and surface water samples. The optimization of the SBSE step resulted in reduced solvent use and disposal, as well as an increased speed of the entire procedure. In contrast, the preference for HPLC-FLD allowed for a speed-up in the separation and quantification of the target PAHs, achieving high selectivity and sensitivity. The improvements introduced throughout the method significantly enhanced its sensitivity, enabling the determination of low concentration levels in such samples. This allowed the method to meet the detection limits for the respective parameter values and fulfill the minimum performance characteristics of uncertainty of measurement, as required by applicable Portuguese national legislation. Furthermore, this method validation follows the fitness-for-purpose requirements within an accredited laboratory, as outlined in NP EN ISO 17025:2018, which is routinely applied for the analysis of PAHs in the proposed water matrices. This approach meets both internal (e.g., QC samples) and external (e.g., proficiency testing schemes) quality control requirements.

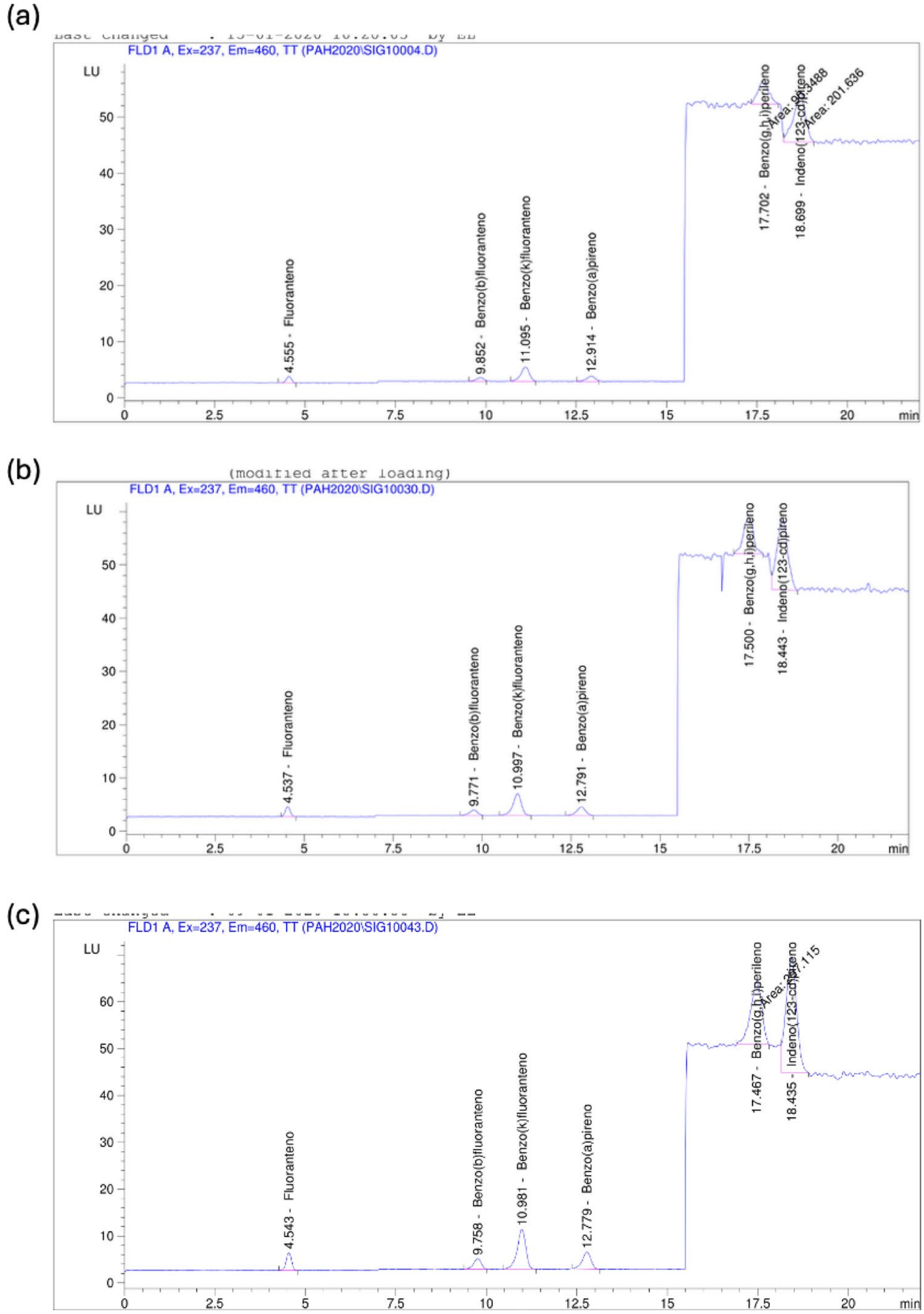

Fig. 8 SBSE–HPLC–FLD chromatogram of a drinking/groundwater sample spiked at the LOQ levels for the six target PAHs: **a** benzo[a]pyrene (1.5 ng L^{-1}), benzo[k]fluoranthene (1.5 ng L^{-1}), **b** benzo[b]fluoranthene (3 ng L^{-1}), **c** benzo[ghi]perylene (6 ng L^{-1}) and indeno[1,2,3-cd]pyrene (6 ng L^{-1}). The chromatogram illustrates baseline separation and the signal-to-noise ratios obtained at the lowest validated concentration for each analyte

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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