

Application of dispersive liquid-liquid microextraction as a simple assisted clean-up and preconcentration technique for GC/MS determination of selected PAHs extracted from sewage sludge by Soxhlet and ultrasound assisted extraction method

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ABSTRACT

Herein, two different extraction methods (Soxhlet and ultrasound assisted extraction) along with dispersive liquid-liquid microextraction (as an assisted clean-up and preconcentration technique) were applied for the measurement of selected polycyclic aromatic hydrocarbons (PAHs) in sewage sludge. The measurements were made using gas chromatography-mass spectrometry (GC-MS). The extraction efficiencies of the above-mentioned methods were investigated and subsequently compared. Briefly, high amounts of sludge were gently heat-dried and sieved and the resulting solids (5 g for the Soxhlet extraction and ultrasound assisted extraction each) were then subjected to the corresponding extraction method. To clean-up, after completion of the extraction phase, the extract was dried under a gentle stream of nitrogen and then diluted with acetone (1.5 ml) and finally passed through a PTFE syringe filter. Further on, tetrachloroethylene (C₂Cl₄, 12 µl) was added to the acetone solution and the resulting mixture was rapidly added to a vessel containing deionized water (5.0 ml) for assisting the clean-up process and further preconcentration. Afterwards, the sedimented organic phase (5 ± 0.5 µl) was withdrawn and injected into the GC/MS (1.0 µl) for identification and quantification of the PAHs. Some differences between the two methods were observed for reproducibility and limits of quantification and were determined to be insignificant and significant, respectively. Overall, Soxhlet extraction had better extraction efficiency compared to that of the Ultrasound assisted extraction for the PAHs.

Keywords: Soxhlet extraction, Ultrasound assisted extraction, Dispersive liquid-liquid microextraction, Sewage sludge

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of highly toxic compounds [1,2]. They are

formed and released into the environment from various sources [3,4]. PAHs are deposited on urban soil and enter the wastewater treatment station as sewage. They can also migrate and be absorbed through the food chain. From the sludge treatment point of view, high PAH levels have a negative effect on aerobic and anaerobic digestion [5,7]. Additionally, the accumulation of PAHs in sewage sludge,

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used for many years as an organic fertilizer in agriculture, is harmful to humans and animals and can have potentially adverse effects on the environment [8,10].

Due to the heterogeneous characteristics of sewage sludge, pretreatment of sludge is required to ensure good contact between the extraction solvent and the matrix in the extraction process. Such a pretreatment usually comprises of several steps. A key step is the drying of the sludge sample by water elimination using air-drying or heating followed by homogenization through grinding and sieving to achieve a final solid sample [11,12]. Solvent selection is arguably the most influential aspect in the development of an extraction method for solid samples. Despite the multitude of modern extraction methods, Soxhlet extraction is still the standard method for the extraction of nonvolatile organics from solid samples [13,18].

Although Soxhlet extraction is time-consuming and labor-intensive and requires the use of large volumes of organic solvents, it continues to be the method of choice in organic compound extraction from solid matrices due to its high extraction efficiency which is due to the sample being repeatedly brought into contact with fresh portions of extracting solvent [19,20].

As an alternative to Soxhlet extraction, ultrasonic solvent extraction (USE) has been widely applied for the leaching of organic compounds from solid samples. In this extraction method, ultrasonic vibrations ensure a close contact between the sample and the extraction solvent. USE is relatively quick, but the extraction efficiency is not as high as for other methods since enough time is not given to the sample. Further progress was made by inserting the ultrasound assisted extraction directly into the vial containing the extraction solvent mixture and the solid sample in order to reduce the extraction time [19,21,22].

A range of extraction methods for PAHs from sewage sludge matrices have been compared within the literature [22–26].

In the present study, almost high amounts of sludge were gently heat-dried and sieved and the resulting solids obtained from each extraction method were then subjected to the corresponding method. To reach an optimum extraction condition, several parameters such as type of extraction solvent and extraction time, etc. were evaluated in detail. We systematically compared the extraction efficiency of two methods (Soxhlet extraction and ultrasound assisted extraction) for the extraction of selected PAHs from a wide variety of sewage sludge samples. After which, the samples from each extraction method underwent a simple filtering clean-up and a further highly efficient novel assisted clean-up preconcentration method known as dispersive liquid-liquid microextraction (DLLME) method [27,28]. The above-mentioned methods were also compared for relative recovery, reproducibility, detection limit and chromatogram.

2. Experimental

2.1. Chemicals and samples

The selected PAHs (acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene and chrysene) were purchased from Aldrich (Milwaukee, WI, USA). Analytical grade n-hexane and acetone, acetonitrile

were all supplied by Merck (Darmstadt, Germany). Deionized water was provided by a home-made deionizer. Stock standard solutions of PAHs (100 mg/l) were prepared in acetonitrile and were then diluted with acetone (as working solutions) and stored at $-18 \pm 2^\circ\text{C}$. Highly dried cooled sludge samples (1000 ml) were collected twice a week during one month in order to obtain a truly representative set of samples. Upon their arrival to the laboratory, the samples were centrifuged (4000 rpm, 10 min) to remove particulate matter and maintained at 4°C until the time of analysis. Blank sewage sludge samples—later confirmed to be PAHs free—were spiked with a number of known concentration levels of the tested PAHs. The calibration curve was constructed of ten calibration standard solutions (1–200 ng/g; CS) were prepared by spiking the diluted working solution into the blank samples. The validation tests were carried out using two quality controls (QC, 5 and 10 ng/g). Both CS and QC samples were stored at $-18 \pm 2^\circ\text{C}$ until analysis. Suspected sewage sludge samples (hereafter, called real samples) were taken from five different wastewater locations in Tehran province (Tehran, Iran)¹. Before analysis, the sludge (blank, spiked or real sample) were dried in an oven and sieved in order to obtain a fraction of <1 mm. This fraction was stored in cold desiccators until subsequent analysis. As PAHs are sensitive to photo degradation [29,30], exposure to light was avoided during all steps of sample preparation.

2.2. Instrumentation

The gas chromatographic system consisted of an Agilent (Centerville Road, Wilmington, USA) series 7890A GC coupled to an Agilent MSD 5975C quadrupole mass spectrometer. The GC was fitted with HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25- μm film thickness) from Agilent J&W Scientific (Folsom, CA, USA). Helium (99.999%) was used as the carrier gas at a flow rate of 1.0 ml/min. The following temperature program was employed for the separation: 80°C for 1 min, increased to 280°C at $8^\circ\text{C}/\text{min}$, and held for 6 min; finally increased to 300°C at $50^\circ\text{C}/\text{min}$ and held for 3 min. The MS quadrupole and the MS source temperatures were set at 150 and 230°C , respectively. Data acquisition was performed in the full scan (50–400 m/z) and selected ion monitoring (SIM) mode was used for quantitative determination of PAHs. A dwell time of 100 ms was used for each mass (SIM mode) with high resolution. The filament delay time was set at 3 min.

A vortex-homogenizer was applied to homogenize both the blank and real dried sludge samples prior to each extraction. A home-made Soxhlet extraction apparatus and an Ultrasound assisted extraction probe (UP-500 ultrasound homogenizer) from ECHROM Company (Avagene, Taiwan) were used for the extraction of the tested PAHs from dried sludge matrices.

3. Methodologies

3.1. Soxhlet extraction

A Soxhlet extraction apparatus fitted with a 250 ml flask was used for the extraction of the tested PAHs from portions of the dried sludge (5.0 g) to which n-hexane and ace-

¹South, Ekbatan, Shoush, Ghods and Mahallati treatment plants.

tone (1:1, v/v, 100 ml) had been added. The whole system was heated for 8 h. After the extraction, the sample underwent the clean-up procedure [31,32].

3.2. Ultrasound assisted extraction

The dried sludge (5.0 g) was placed into an Ultrasound assisted extraction to which n-hexane and acetone (1:1, v/v, 10 ml) was added. The tested PAHs were extracted via sonication in different time ranges between 1–5 min. with the output control knob set to full. Afterwards, the sample underwent the clean-up procedure [32,33].

3.3. Clean-up

The extracts, either from Soxhlet extraction or ultrasound assisted extraction, were evaporated to nearly dryness under a gentle stream of nitrogen. It is worth noting that in the Soxhlet extraction case, due to the high amounts of post-extraction remaining solvents, a rotary evaporator (Heidolph; Kelheim, Germany) was employed to reduce the volume of the solvent. Then, to the resulting concentrated extract from the Soxhlet extraction, acetone was added (10 ml) and swiftly homogenized. The resulting mixture was withdrawn and put in an Eppendorf plastic vial to reach total dryness under the gentle stream of nitrogen, while the concentrated extract from the ultrasound assisted extraction had already been put in another Eppendorf vial and dried totally. In the end, further 1.5 ml of acetone was added to the above-mentioned Eppendorf vials to reconstitute the tested PAHs. In each case, the recovered acetone was finally passed through a PTFE syringe filter (0.45 μm) to obtain 1.0 ml of clear filtrate [32,34].

3.4. Assisted clean-up and further preconcentration

Within the current study, a highly efficient method known as DLLME was applied for assisting the clean-up process followed by further preconcentration of the tested PAHs. For this reason, deionized water (5.0 ml) was placed in a 10-ml screw cap glass test tube with conical bottom. To the clear filtrate (1.0 ml) obtained from the clean-up process, different amounts of highly apolar organic solvents, which should be denser than water, were added in order to determine the optimum conditions. It should be noted that the sedimented phase, resulting from the above-mentioned solvents must fall within $5 \pm 0.5 \mu\text{l}$. The resulting mixture was rapidly injected to the glass test tube. A cloudy solution (a mixture of water, acetone, and organic solvent) was instantly present within the tube. This cloudy solution was then centrifuged (5 min, 4000 rpm). As a result, the fine particles of organic solvent were dispersed and sedimented at the bottom of conical test tube which was about ($5 \pm 0.5 \mu\text{l}$). Of which, 1.0 μl was injected to the GC/MS for identification and quantification of the tested PAHs.

4. Results and discussion

4.1. Optimization of Soxhlet extraction parameters

In order to gain the highest recovery applying the Soxhlet extraction procedure, the main regarding parameters were taken into considerations, which are the type of extraction solvent and extraction time [35]. To find the opti-

mal conditions, three ratios (n-hexane: acetone; 1:1, 2:1, 1:2 v/v; with the total volume of 100 ml) were employed. Of the mentioned three, the ratio of 1:1 yielded best results.

It is worth noting that the polarity of n-hexane was raised with acetone, so that the recovery of PAHs with fewer aromatic rings was increased which is in agreement with other reports [36,37].

Meanwhile, the extraction time was investigated through the submission of the sample solution under three different periods of time (4, 8, 12 h). The results confirmed that 8 hr was enough to extract sufficient amounts of PAHs from the samples. Therefore, 8 h was taken as an optimal time extraction in the Soxhlet extraction procedure.

4.2. Optimization of ultrasound assisted extraction parameters

Ultrasound assisted extraction leaching is commonly applied for rapid, inexpensive isolation prior to the determination of PAHs in sewage sludge samples, and quantitative extraction usually requires high-intensity assisted extraction sonication [38].

For the ultrasound assisted extraction procedure, the variables optimized were those of extraction time and the power. The extraction time and the power were varied in the range of 1, 3, 5 min and 25, 50, 75 W, respectively. The results showed the highest extraction efficiency was obtained under the condition of 3 min and 75 W.

It should be noted that for gaining better results regarding the comparison of the two procedures (i.e., the ultrasound assisted extraction and Soxhlet extraction) the composition of extraction solvent was taken the same as the one applied in the Soxhlet extraction procedure.

4.3. Optimization of DLLME parameters

In DLLME, the most important parameters to attain high sensitivity following the two above-mentioned procedures (i.e., the ultrasound assisted extraction and Soxhlet extraction) are as follows: type of extraction solvent and disperser solvents as well as their volumes and salt addition which were all optimized in detail.

Acetone was selected as the reconstitution solvent (see section 3.4) due to its effectiveness for the extraction of apolar PAHs from a diverse range of matrices [39]. While, the other advantages include low toxicity and cost, miscibility with water to be used as disperser solvent in the DLLME procedure. It seems to be important to mention that acetone is not only an extraction solvent in conventional small-scale liquid solid extraction (e.g., Soxhlet extraction and ultrasound assisted extraction), but also acts as a disperser solvent in DLLME (herein, as an assisted clean up preconcentration procedure) [40].

Also, different amounts of apolar organic extraction solvents commonly used in DLLME (i.e., C_2Cl_4 , CCl_4 , CHCl_3) were initially chosen to reach the highest sensitivity. The results showed that amongst the volumes of 12, 14 and 16 μl ; 12 μl of C_2Cl_4 was the most suitable conditions for organic extraction solvent (see Figs. 1 and 2). It is noted that due to application of highly apolar organic extraction solvent; polar and semi-polar interferences associated with the sludge are repelled from the sedimented organic phase and remain in the aqueous phase, which assists the clean-up process [41].

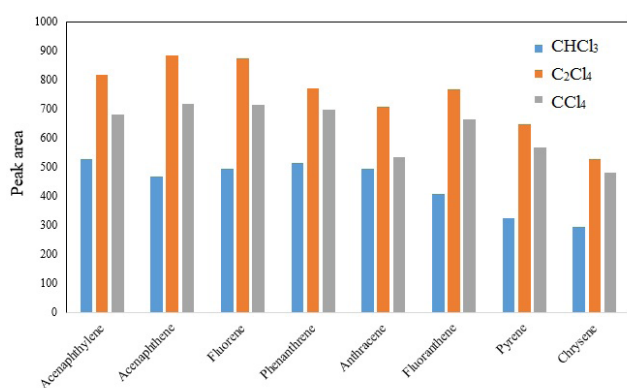


Fig. 1.

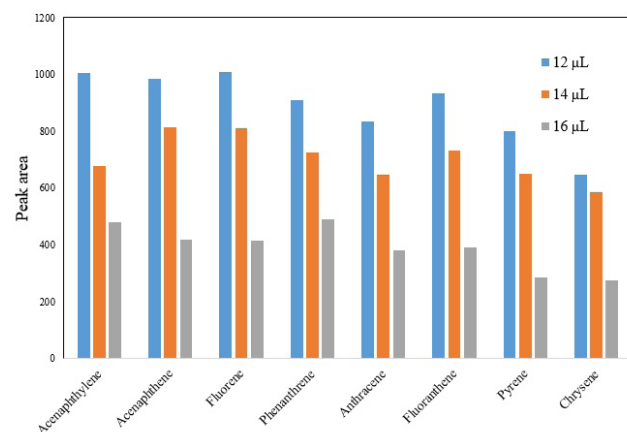


Fig. 2.

Meanwhile, the effect of salt addition (NaCl) was investigated on the extraction efficiency in the range of 0–4 (w/v %). The results showed that by increasing the salt content the extraction efficiency dramatically fell down (data not shown). This could be explained due to the fact that with an increase in the salt concentration the viscosity of the sample solution becomes higher, and, as a result, the mass transfer of PAHs is sharply hampered. Therefore, no salt was added to the sample solutions. All in all, the optimal conditions of DLLME were found out to be as follows: Disperser solvent (1 ml, acetone), (12 μl, C₂Cl₄), no addition of salt.

4.4. Evaluation of analytical parameters

The analytical parameters used for comparison of the two procedures were selectivity, relative recovery (RR), reproducibility (RSD), linearity and lower limit of quantification (LLOQ). They were all evaluated for each method using spiked and non-spiked dried sludge. The blank dried sludge was spiked with the working solutions as described earlier.

4.4.1. Selectivity

The selectivity of the method was determined by injecting the diluted working solution of the tested PAHs. In

addition, 8 blank samples were analyzed to check if there were any additional peaks in the chromatogram that could potentially interfere with the analysis. The results clearly showed however that there were no interfering peaks, originating from the blank sample matrix or the chemicals and reagents used in any of the 8 blank samples studied in the selectivity experiments (data not shown).

4.4.2. Relative recovery and reproducibility

Relative recovery (RR) was determined using the following equation:

$$\%RR = 100 \times \frac{(C_{found} - C_{real})}{C_{added}} \quad (1)$$

where C_{found} and C_{added} are the concentrations of the corresponding PAH in the real/blank sample after and before the addition of the working solutions. For each method, recovery tests were performed three times at each QC level, and the recovery value considered was the average of the three measurements. As can be seen in Tables 1 and 2, the relative recoveries varied between 74.6 and 96.3%. Reproducibility (RSD) of the methods was investigated on four replicate experiments which varied from 5.9 to 17.1% (see also Tables 1 and 2).

Table 1
Analytical data obtained by the Soxhlet/ DLLME method

PAHs	LLOQ	RSD% (n = 4)		RR%	
		5	10	5	10
Acenaphthylene	1.8	13.6	8.5	80.5	87.8
Acenaphthene	1.2	11.8	6.8	88.2	90.8
Fluorene	1.2	11.1	10.2	81.4	89.7
Phenanthrene	1.2	10.0	5.9	80.2	85.1
Anthracene	1.2	8.7	9.2	79.2	88.6
Fluoranthene	1.2	13.2	8.4	81.1	96.3
Pyrene	1.2	12.5	9.2	82.5	82.4
Chrysene	1.4	8.8	7.1	84.3	86.8

Unit of concentration (ng/g); RR%: RRecovery%

Table 2
Analytical data obtained by the Ultrasound probe/ DLLME method

PAHs	LLOQ	RSD% (n = 4)		RR%	
		5	10	5	10
Acenaphthylene	2.4	17.1	9.3	77.4	80.0
Acenaphthene	1.8	12.9	10.5	83.3	89.5
Fluorene	1.6	13.4	7.2	79.0	88.1
Phenanthrene	1.6	11.2	7.6	88.2	74.6
Anthracene	1.8	10.9	8.2	79.7	84.0
Fluoranthene	1.8	8.9	6.8	82.2	80.6
Pyrene	1.6	14.7	10.0	80.0	90.2
Chrysene	2.0	15.2	11.1	78.9	88.5

Unit of concentration (ng/g); RR%: RRecovery%

4.4.3. Linearity and evaluation of LLOQ

Linearity of the methods was tested by spiking the blank samples at ten concentration levels over the range of 1–200 ng/g for the tested PAHs. Calibration curves were constructed by plotting the PAH signal obtained, which was the average of three measurements, against the respective concentration of the test PAH. The results demonstrated a fair linearity for the tested PAHs within the above test range with a minimum correlation coefficient of 0.988. The LLOQs were found to be in the range of 1.2–2.4 ng/g based on the following definition (see Tables 1 and 2): The lowest concentration at which the error falls between 20% and –20% with the maximum RSD of 20% obtained under three measurements.

4.5. Analysis of real samples

Application of the two methods along with DLLME to the sewage sludge from five different waste water locations was assessed. Of the five sewage sludge samples analyzed, four were found to be contaminated with the tested PAHs. Then, the methods were successfully applied to the determination of the tested PAHs in the above-mentioned samples spiked at each QC level to investigate the extraction efficiency of the methods combined with DLLME (see Tables 3 and 4). The RSD results based on four similar measurements were within the range of 7.3–17.5%, as presented in the mentioned Tables. Also according to the Tables, good relative recoveries in the range of 76.4–96.1% were achieved, indicating that the applied methods combined with DLLME are highly efficient for the measurement of the tested PAHs in various sewage sludge samples. Fig. 3 represents the SIM chromatograms of the real samples undergone the joint method of Soxhlet extraction-DLLME (Pane A–E) as well as the standard solution of the tested PAHs (bottom pane). It is noted that the chromatograms obtained from the Soxhlet

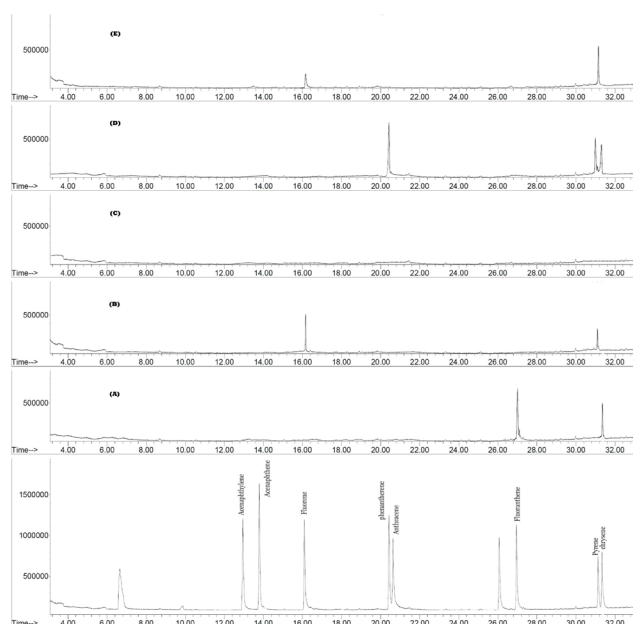


Fig. 3.

extraction and Ultrasound assisted extraction were mostly comparable due to the application of DLLME.

4.6. Comparison of the extraction efficiency of the methods

Comparison of the main parameter relating to the extraction efficiency of the methods (i.e., LLOQs) was made using a *t*-test statistical approach (95% confidence level interval with the *t*-value of 2.78) for the tested PAHs. From this test, the LLOQ differences between the two methods

Table 3
Analysis of real samples by Soxhlet – DLLME method (5.0 ppb added)

PAHs	1			2			3			4			5		
	^a RC	Found ^b	RR(%)	RC	Found	RR(%)	RC	Found	RR(%)	RC	Found	RR(%)	RC	Found	RR(%)
Acenaphthylene	ND	^d 4.2 ± 0.6	84	ND	4.1 ± 0.6	82	ND	4.4 ± 0.4	87	ND	4.7 ± 0.5	94	ND	4.5 ± 0.6	90
Acenaphthene	ND	4.6 ± 0.5	92	ND	4.8 ± 0.7	96	ND	4.1 ± 0.6	82	ND	4.4 ± 0.6	87	ND	4.7 ± 0.5	94
Fluorene	ND	4.3 ± 0.4	86	2.0	6.0 ± 0.8	85	ND	4.0 ± 0.5	81	ND	4.3 ± 0.5	85	2.7	6.8 ± 0.5	88
Phenanthrene	ND	4.8 ± 0.7	96	ND	4.4 ± 0.5	87	ND	4.3 ± 0.6	86	3.2	7.4 ± 0.8	90	ND	4.0 ± 0.3	80
Anthracene	ND	4.1 ± 0.5	83	ND	4.3 ± 0.6	86	ND	4.7 ± 0.5	93	ND	4.1 ± 0.6	82	ND	4.1 ± 0.4	81
Fluoranthene	4.2	8.7 ± 1.1	95	ND	4.1 ± 0.6	82	ND	4.2 ± 0.5	83	ND	4.7 ± 0.5	93	ND	4.3 ± 0.5	85
Pyrene	ND	4.4 ± 0.6	87	2.9	6.6 ± 0.5	83	ND	4.1 ± 0.6	82	2.2	6.7 ± 0.6	92	2.6	6.7 ± 0.8	88
Chrysene	2.7	7.3 ± 0.9	95	ND	4.0 ± 0.4	80	ND	4.2 ± 0.4	84	3.0	7.6 ± 0.7	95	ND	4.2 ± 0.5	84

a: Real concentration (ppb); b: ppb (*n* = 4); c: non-detected; d: mean ± sd (*n* = 4)

Table 4
Analysis of real samples by Ultrasound probe – DLLME method (5.0 ppb added)

PAHs	1			2			3			4			5		
	^a RC	Found ^b	RR(%)	RC	Found	RR(%)	RC	Found	RR(%)	RC	Found	RR(%)	RC	Found	RR(%)
Acenaphthylene	ND	^d 4.4 ± 0.6	87	ND	3.9 ± 0.4	78	ND	4.0 ± 0.7	89	ND	3.8 ± 0.5	76	ND	4.0 ± 0.7	88
Acenaphthene	ND	4.1 ± 0.7	81	ND	4.5 ± 0.5	90	ND	4.0 ± 0.4	90	ND	4.3 ± 0.4	85	ND	4.2 ± 0.4	84
Fluorene	ND	4.3 ± 0.4	86	2.1	6.3 ± 0.7	88	ND	3.9 ± 0.5	77	ND	4.2 ± 0.8	84	2.1	6.3 ± 0.7	88
Phenanthrene	ND	4.5 ± 0.6	91	ND	4.4 ± 0.6	87	ND	4.2 ± 0.7	84	3.2	7.1 ± 0.8	86	ND	4.0 ± 0.6	89
Anthracene	ND	4.2 ± 0.6	84	ND	4.4 ± 0.5	88	ND	4.6 ± 0.7	91	ND	4.2 ± 0.6	84	ND	4.1 ± 0.4	91
Fluoranthene	4.0	8.1 ± 1.0	90	ND	4.2 ± 0.7	84	ND	4.3 ± 0.6	87	ND	4.5 ± 0.6	90	ND	4.3 ± 0.6	86
Pyrene	ND	4.3 ± 0.8	87	2.5	6.8 ± 0.8	90	ND	4.1 ± 0.5	81	2.0	5.4 ± 0.7	77	2.6	6.9 ± 0.8	90
Chrysene	2.5	6.8 ± 0.9	90	ND	4.3 ± 0.7	86	ND	4.0 ± 0.7	79	2.8	6.3 ± 0.6	80	ND	4.0 ± 0.5	89

a: Real concentration (ppb); b: ppb ($n = 4$); c: non-detected; d: mean ± sd ($n = 4$)

Table 5
Comparison of the applied method with other related studies

Reference	RR (%)	LOQ (ng/g)	RSD (%)	Analytical method
[3]	69–110	14–41	8.1–15.4	Microwave /HPLC-FL
[43]	70	0.2–4.6	3.2–13.6	LLE and SPE
[25]	69–116	0.97–3.40	0.9–11.5	Soxhlet/GC-MS
[44]	29–82	0.07–2.5	6.0–47.1	Ultrasound/GC-MS
This work	80–96	1.2–1.8	5.9–14.6	Soxhlet/GC-MS

were found to be statistically significant. The Soxhlet extraction exhibited lower LLOQ values compared to that of the ultrasound assisted extraction. Additionally, the relative recoveries in the Soxhlet extraction were found to be better than those of the ultrasound assisted extraction.

An F-test at (95% confidence level interval with the F-value of 9.28) was used to compare the methods' reproducibility for the tested PAHs. As confirmed by the F-test, no significant differences were observed between the reproducibility of the methods. Considering all above-mentioned parameters, it was determined that the Soxhlet extraction exhibited the better extraction efficiency than the ultrasound assisted extraction. This result may be attributed to the fact that the matrix interferences play a vital role in the extraction efficiency of the methods. In the Soxhlet extraction case, owing to the longer extraction time and larger consumption of the extraction solvent, the efficiency of the method is less hampered by the interferences. On the other

hand, the main drawbacks of Soxhlet extraction approach (time-consuming, labor intensive, expensive and consumption of large volume of organic solvents) still remain a major challenge whilst, the extraction by the applied ultrasound assisted extraction proved to be faster, more cost-effective and required much less organic solvent [42].

4.7. Comparison of this study with other related studies

The results obtained from this study were compared with other related studies regarding the determination of PAHs in several sewage sludge/soil samples. As indicated in Table 5, the application of a simple clean-up and further preconcentration using DLLME has yielded comparable data to other related studies found in the Table 5 [3,25,43, 44].

5. Conclusions

Within the current study, the extraction efficiencies of the two extraction methods (Soxhlet extraction and ultrasound assisted extraction) were compared in detail. Then, a highly efficient method known as DLLME was applied as a clean-up process followed by the further preconcentration of some PAHs. No significant differences were observed between the extraction efficiencies of the two joint methods except for the lower limits of quantification. Finally, the two joint methods were also applied successfully to the analysis of dried real sewage sludge samples.

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