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Ultrasound assisted *in-situ* formation of switchable deep eutectic solvent for extraction of polychlorinated biphenyls in water

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ABSTRACT

A novel *in-situ* method for creating a CO_2-responsive switchable deep eutectic solvent (SDES) was developed for the homogeneous liquid-liquid microextraction (HLLME) of 18 polychlorinated biphenyls (PCBs) compounds from water samples. Following extraction, the compounds were separated and analyzed by gas chromatography-triple quadrupole tandem mass spectrometry (GC–MS/MS). Diethanolamine (DEA) was employed as the hydrogen bond acceptor (HBA), while 3-methoxyphenol (3-MP) served as the hydrogen bond donor (HBD). The *in-situ* formed CO_2-responsive SDES was used as the extractant, representing a green alternative to traditional toxic organic reagents. Under ideal extraction conditions, a strong linear relationship was established over the concentration range of 0.1–100 ng/mL. The limits of detection (LODs) and quantification (LOQs) were found to be between 0.001–0.040 ng/mL and 0.004–0.134 ng/mL. The preconcentration factors (PFs) varied from 49 to 76, while both intra-day and inter-day precision (RSDs) remained under 6.8 %. Ultimately, the method proved successful in detecting 18 PCBs in environmental water samples, with satisfactory recovery rates. These results demonstrate that the method is not only green and sustainable, but also exhibits excellent methodological performance, rendering it highly promising for applications in the field of HLLME.

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With the continuous development of industrialized societies, the use of chemical substances is also increasing, leading to increasingly serious contamination of environmental water samples [1]. Polychlorinated biphenyls (PCBs) are a widely recognized group of organic contaminants noted for their environmental persistence. Their substantial environmental release and the resulting adverse impacts on human health underscore their significance as a key pollutant class [2,3]. Widely produced since the beginning of the 20th century, it was not until the 1970s that attention was paid to PCBs, and their production was restricted globally [4] and included in the list of banned persistent organic pollutants (POPs) under the Stockholm Convention in the United States [5,6]. It is necessary to analyze organic pollutants in the environment, and pretreatment is an essential step in the analysis process [7–11]. Widely used pretreatment techniques encompass liquid-liquid extraction (LLE) [12], solid-phase extraction (SPE) [13], accelerated solvent extraction (ASE) [14], liquid-liquid microextraction (LLME) [15], solid-phase microextraction (SPME) [16], QuEChERS method [17] and so on.

Currently, homogeneous liquid-liquid microextraction (HLLME) has been widely utilized in various sample pretreatment techniques, distinguished by the infinite contact area between the organic and aqueous phases, thereby enhancing mass transfer [18–20]. However, the HLLME process needs to be accomplished by inducing reversible switching between homogeneous and heterogeneous systems. Materials and reagents that respond to external conditions are expected [21–23]. For instance, switchable deep eutectic solvents (SDESs), formed by selecting specific precursors, can subsequently alter their properties between hydrophilic and hydrophobic through the introduction of external driving forces (*e.g.*, temperature [24], pH [25], or CO_2/N_2 gases [26]). Due to its excellent characteristics, it has attracted widespread attention in the field of sample pretreatment [27,28].

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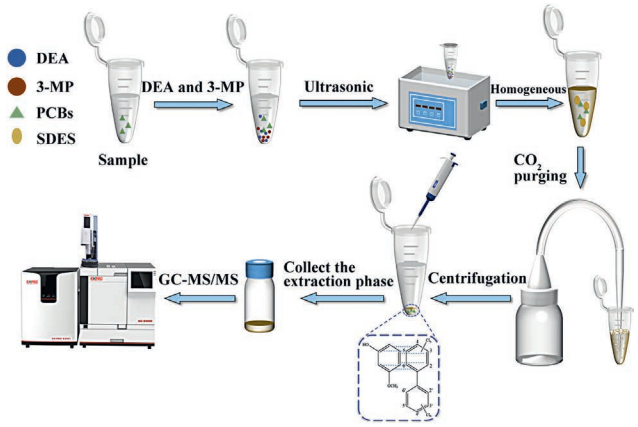


Fig. 1. HLLME procedure for extraction of polychlorinated biphenyl.

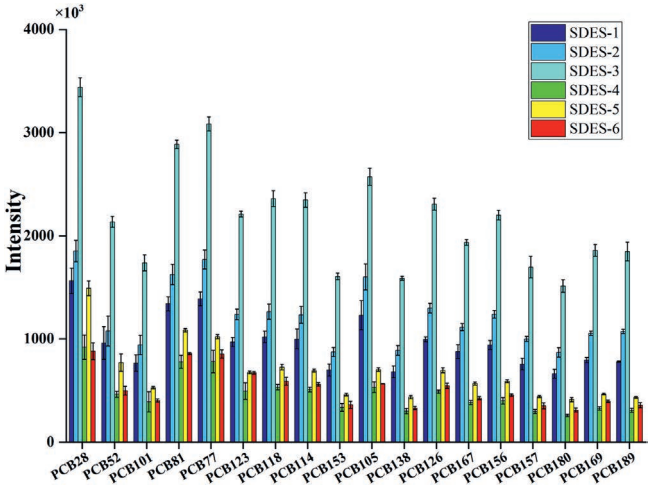


Fig. 2. Effect of the type of SDES on the extraction of PCBs by the HLLME method.

In general, it is necessary to realize the mixing of SDESs precursors by stirring at a certain molar ratio, heating and other processes in order to form a clarified and transparent liquids before the extraction [29]. Nevertheless, due to the presence of specific precursors in some SDESs, which makes it impossible to preserve these SDESs for a long period of time, it is necessary to synthesize new SDESs prior to each pre-treatment, which increases the experimental time. On the contrary, the HLLME process based on *in-situ* formation of SDES eliminates the need for prior formation. Here, the hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) are directly introduced into the preprocessed solution during the extraction process, enabling the simultaneous formation of SDES and extraction of the target compound in solution [30,31]. In comparison to conventional HLLME, this method offers benefits such as reduced pretreatment time, ease of operation, and improved accuracy.

This study introduced a simple, rapid, and accurate method termed ultrasound-assisted *in-situ* SDES homogeneous liquid-liquid microextraction (*in-situ* SDES-HLLME) for the determination of PCBs in water using gas chromatography-triple quadrupole tandem mass spectrometer (GC–MS/MS). Instead of using toxic volatile organic extractants or extra formation step, the method involves the direct *in-situ* formation of SDES as the extractant in the solution to achieve rapid and efficient extraction.

The HLLME process was illustrated in Fig. 1. Firstly, 0.5 mL of sample solution containing 18 PCBs was poured into a 1.5 mL centrifuge tube. 18.6 μL of DEA and 21.4 μL of 3-MP were added and settled at the bottom of the tube without dissolving. The centrifuge tube was then processed in a water bath sonicator at 40 °C. After 10.0 s, it was observed that DEA and 3-MP remained at the bottom and formed a transparent phase resulting in clear stratification, indicating the *in-situ* formation of SDES. And homogeneous system can be achieved through slight shaking due to the hydrophilic of SDES. Following this, 1.0 g of citric acid monohydrate, 1.2 g of sodium bicarbonate, and 2.0 mL of water were sequentially added to a homemade CO_2 vial. A spinal needle connected through tubing was inserted into the centrifuge tube containing the preprocessed solution, allowing the generated CO_2 to bubble through the solution for 3.0 min. During the bubbling, homogeneous solution gradually became turbid, indicating the phase transition of SDES. Subsequently, centrifugation was carried out at a rotational speed of 4000 rpm for 3.0 min, resulting the thorough phase separation at the bottom. The lower extract phase, approximately 4.0 μL in volume, was collected and diluted with ethyl acetate at a ratio of 1:5 (v/v) before being employed for GC–MS/MS analysis (Table S1 in Supporting information) [32].

The selection of extraction solvents played a pivotal role in achieving successful extraction and separation of the target substances. Wan *et al.* [33] identified various SDESs that involved alkanolamine compounds as HBAs and phenolic compounds as HBDs. These compounds can form hydrophilic DES, but they become hydrophobic after exposure to a certain amount of CO_2. Because the introduction of CO_2 produces H_2CO_3 that reacts with HBA to form ammonium salts, breaking the hydrogen bonds between HBA and HBD and causing phase separation of HBD that is insoluble in water. Thus, it was speculated that the π-π force between the target compound PCBs and HBD can separate them from the aqueous phase simultaneously. In this paper, six SDESs were formed based on two alkanolamine compounds and two phenolic compounds with different molar ratios, as shown in Table S2 (Supporting information).

The extraction efficiencies of six SDESs were compared based on the peak areas of 18 PCBs. As illustrated in Fig. 2, it was found that among these SDESs, the extraction effect of SDES with 3-MP as HBD was overall better than that of SDES with 4-MP as HBD. This is due to the placement of the $-OCH_3$ group on the benzene ring. In the case of 3-MP, the $-OCH_3$ group is located in the ortho position, acting as an electron-withdrawing group, which reduces the electron cloud density on the benzene ring, leading to easier generation of DES with the HBA and thereby enhancing the extraction efficiency. Conversely, in the case of 4-MP, where the $-OCH_3$ is in the *para* position, it acts as an electron-donating group, resulting in reduced extraction efficiency. Furthermore, a comparison of the pK_a values of HBA reveals that MEA (9.50) > DEA (8.82), and DEA with smaller pK_a values is more stable after the formation of SDES [33], and therefore more capable of enriching the target. In summary, the response of 18 PCBs obtained by SDES-3 consisting of DEA and 3-MP was the highest.

The Fourier transform infrared (FT-IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectras of DEA, 3-MP, and SDES-3 were utilized to verify the formation of hydrogen bond. As shown in Fig. S1 (Supporting information), the stretching vibrational peak of N–H in SDES-3 shifted from 3385 cm^{-1} to 3410 cm^{-1} compared to DEA, and the stretching vibrational peak of the O–H bond of 3-MP was at 3440 cm^{-1} while it shifted to 3410 cm^{-1} in spectra of SDES-3. This indicates that the O–H bond in 3-MP engages in hydrogen bonding, causing electrons to migrate from the oxygen atom towards the hydrogen bond. Consequently, the force constant of the O–H bond decreases, resulting in a red-shift phenomenon. In the spectra of ^{1}H NMR, the hydrogen signals at 2.73 ppm and 3.65 ppm in DEA were attributed to the hydrogen atoms on the carbon chain. The hydrogen signals at 6.42, 6.46, 6.52, and 7.13 ppm in 3-MP corresponded to the hydrogen atoms on the benzene ring. Additionally, the sin-

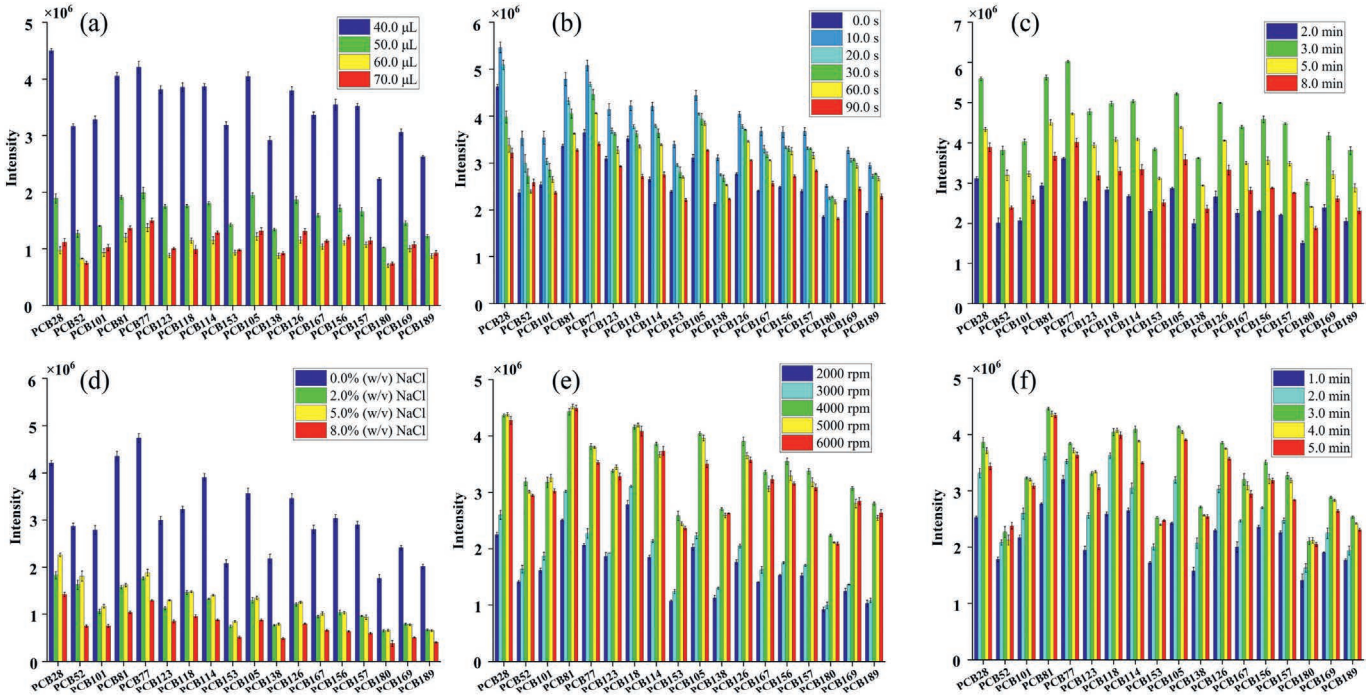


Fig. 3. Effect of the volume of SDES (a), ultrasound time (b), CO_2 bubbling time (c), salt concentration (d), centrifuge rate (e), and centrifuge time (f) on the extraction of PCBs by the HLLME method.

gular peak at 3.78 ppm was attributed to the hydrogen of the methoxy group (–OCH_3). All of these hydrogen signals were characteristic peaks observed in the SDES-3 spectrum, confirming the presence of DEA and 3-MP in SDES-3 without chemical reaction.

The volume amount of SDES is crucial in HLLME, and different volumes have different extraction efficiencies for PCBs in water, so the volume of the extractant SDES was varied from 40 μL to 70 μL (Table S3 in Supporting information). Fig. 3a illustrates the impact of SDES volume on extraction efficiency, showing that higher extraction efficiency was achieved with smaller volumes of SDES. This phenomenon occurred because, while a larger volume of SDES enhanced the size of the extraction phase, the concentration of target compounds in it was diluted, ultimately reducing the response intensity [34]. When the volume of the SDES precursor added was too small, no visible phase separation and therefore HLLME process could not be performed. Thus, the volume of SDES was 40 μL in the subsequent experiment.

For the optimization of ultrasound time, six ultrasound times (0, 10, 20, 30, 60, and 90 s) were selected and the results were illustrated in Fig. 3b. Firstly, it was evident that with an ultrasound time of 0 s, efficiencies for PCBs were lower compared to when ultrasound-assisted extraction was applied. Hence, it can be demonstrated that the ultrasound-assisted extraction technique yields superior results for extracting PCBs in water. By increasing the sonication time from 10 s to 90 s, the peak areas of PCBs extracted by HLLME showed a tendency to decrease. When the sonication time was too long, the interaction between H_2O and SDES led to a weakening of hydrogen bonding between SDES components, which consequently affected the extraction efficiency of PCBs. Oliver *et al.* [35] demonstrated that with the introduction of water, the interaction between the SDES components decreases due to hydration. Therefore, the highest extraction efficiency of the target was achieved at a sonication time of 10 s.

The bubbling of CO_2 is critical for switching the polarity of SDES, and the duration of the CO_2 pass directly impacts the extraction efficiency. In our experiments, we focused on optimizing the duration of CO_2 flow into the sample solution, varying it between 2.0 min and 8.0 min. Notably, it was observed that insufficient duration (<2 min) did not induce SDES switching and failed to produce a turbid solution. As shown in Fig. 3c, the extraction of PCBs showed an increasing and then decreasing trend with increasing gas purge time. This phenomenon could be explained by the fact that during brief CO_2 bubbling periods, the SDES in the solution was not adequately separated from the aqueous phase, resulting in insufficient enrichment of the target compound in the extractant. Conversely, with excessive bubbling time, the extraction phase was separated for too long, causing the PCBs in the sample solution to volatilize and thus leading to a decrease in the extraction efficiency. Therefore, optimum efficiency was achieved with a CO_2 bubbling time of 3.0 min.

To assess the impact of salt content on extraction efficiency, experiments were carried out with different amounts of NaCl, ranging from 0.0 % to 8.0 % (w/v). As illustrated in Fig. 3d, it was observed that the extraction efficiency demonstrated a declining trend as the amount of NaCl increased. After adding a specific amount of salt in the experiments, 3-MP was separated more effectively due to the synergistic disruptive effect of the salt on the hydrogen bonding formed between DEA and 3-MP [36,37]. However, an increase in salt content enhanced the interaction forces between the molecules in the solution, resulting in higher viscosity and consequently reducing the extraction efficiency. Therefore, the best extraction efficiency was obtained without the addition of NaCl.

A centrifuge was employed to improve the separation of the extractive phase from the aqueous phase. Therefore, investigating the optimal rate and duration of centrifugation was crucial. The impact of centrifugation rates ranging from 2000 rpm to 6000 rpm on the extraction efficiency of PCBs was assessed, as illustrated in Fig. 3e. It was observed that improved separation occurred as the response increased with higher centrifugation speeds. Optimal extraction efficiency was achieved at 4000 rpm. Similarly, the extraction efficiency increased with longer centrifugation times, with the peak extraction efficiency observed at 3 min of centrifugation (Fig. 3f).

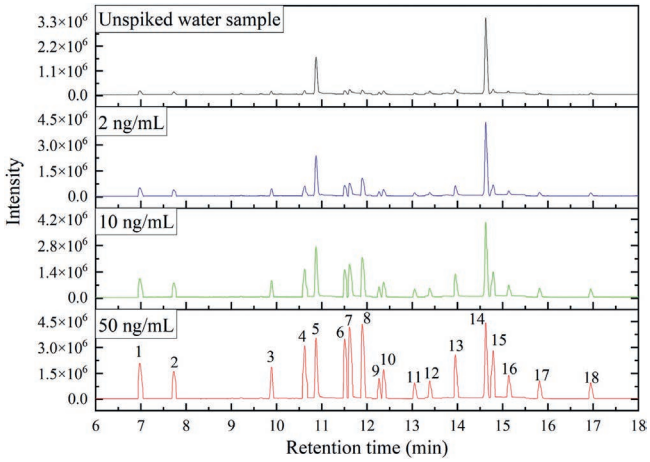


Fig. 4. Representative chromatograms of sewage samples and corresponding spiked water samples. The chromatographic peaks corresponding to PCBs are as follows: 1-PCB28; 2-PCB52; 3-PCB101; 4-PCB81; 5-PCB77, PCB77-*d*6; 6-PCB123; 7-PCB118; 8-PCB114; 9-PCB153; 10-PCB105; 11-PCB138; 12-PCB126; 13-PCB167; 14-PCB156, PCB156-d3; 15-PCB157; 16-PCB180; 17-PCB169; 18-PCB189, respectively.

The performance of the proposed SDES-HLLME method was assessed with linear regression equations, linear range (LR), correlation coefficient (R^2), limit of detection (LOD), limit of quantification (LOQ), preconcentration factor (PF), and relative standard deviation (RSD) of the target analytes under optimal conditions. Calibration curves for the internal standard were created by incorporating two PCB deuterated compounds (PCB77-d6, PCB156-d3) at a concentration of 50 ng/mL into various blank water samples containing different PCB levels. The results showed a linear function across concentrations from 0.1 ng/mL to 100 ng/mL, with the R^2 varying between 0.9947 and 0.9997. Additionally, LODs (S/N = 3) were in the range of 0.001–0.040 ng/mL, LOQs (S/N = 10) were in the range of 0.004–0.134 ng/mL, and the PFs for the target analytes varied from 49 to 76. The intra-day RSD (%) and inter-day RSD (%) ranged from 1.1 % to 4.1 % and 1.7 % to 6.8 %, respectively, indicating that the method had satisfactory repeatability (Table S4 in Supporting information).

In this work, three kinds of water including sewage, lake water and river water were used as samples to determine the PCBs concentrations and the spiked recoveries (%) to validate the feasibility of the method. Three blank water samples were spiked with low, medium and high concentration gradients (2, 10 and 50 ng/mL) to obtain the spiked recoveries of 18 PCBs, which were determined and calculated (Table S5 in Supporting information), the representative chromatograms of both the blank water samples and the spiked water samples at three concentrations are presented in Fig. 4. The analysis began with examining blank real water samples, revealing the presence of all target compounds in sewage and the detection of some target compounds in lake and river water. The spiked recoveries (%) of the 18 PCBs in sewage, lake, and river water ranged from 86.24 % to 116.8 %, 93.46 % to 112.5 %, and 90.51 % to 112.9 %, respectively, indicating that the method's extraction performance for real water samples and accuracy are satisfactory.

The proposed method was compared with the method described in a previously published article, and a summary of the analytical parameters is presented in Table 1 [38–45]. The results showed that the method is simple, rapid, environmentally friendly, and with better recovery and precision. Notably, it requires only a very short extraction time to achieve an enhanced extraction effect, representing a substantial improvement compared to extraction times of several minutes or even tens of minutes required by other methods. Additionally, the method demonstrated a wide linear range and an impressive detection limit. Overall, it offers the advantages of being environmentally friendly, rapid, simple, cost-effective, and delivering superior extraction performance.

This work investigated a novel pretreatment method for homogeneous liquid-liquid microextraction (HLLME) of polychlorinated biphenyls (PCBs) by employing *in-situ* formation of CO_2-responsive switchable deep eutectic solvents (SDESs) comprising diethanolamine (DEA) and 3-methoxyphenol (3-MP) precursors, as an alternative to conventional organic extractants. SDES was directly formed *in situ* in aqueous solution, and the extractant was rapidly separated from the aqueous solution after a brief passage of CO_2 to realize the enrichment and extraction of PCBs. Furthermore, the method was combined with gas chromatography-triple quadrupole tandem mass spectrometer (GC–MS/MS) with excellent analytical properties, including strong linearity, low limits of detection and quantification, as well as consistent precision and recovery rates. As a result, the present study presents a simple, efficient, environmentally friendly, and cost-effective sample pretreatment process for PCBs in water, with promising applications.

CRediT authorship contribution statement

Jiaqin Jiang: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Lingqi Shen:** Writing – review & editing, Investigation. **Fangxi Xu:** Writing – review & editing. **Lipeng Liu:** Writing – review & editing, Conceptualization. **Jian Li:** Supervision, Investigation. **Qiao Xu:** Resources, Investigation. **Zuguang Li:** Writing – review & editing, Conceptualization. **Hongdeng Qiu:** Writing – review & editing, Conceptualization.

Table 1
Comparison of the proposed method with other methods for the determination of PCBs.

Method	Sample	Extraction material	Extraction time (min)	LR	LOD	Recovery (%)	RSD (%)	Ref.
SE-PMSS-GC–MS	Diapers	DES (carvone/camphor)	16	0.4–40 (μg/g)	0.024–0.17 (μg/g)	84–116	5–14	[38]
IPS-GC–MS/MS	Food	DES (thymol/camphor)	10	0.5–100 (ng/mL)	0.12–0.23 (ng/mL)	80.1–111.4	0.4–8.2	[39]
SPME-HPLC	Water	MCFs-coated SPME fiber	40	5–3000 (ng/mL)	0.07–0.28 (ng/mL)	86.4–97.2	3.5–8.3	[40]
DLLME-GC–MS	Marine sediments	Acetone-isooctane	4	0.25–8 (ng/mL)	0.021–0.057 (ng/g)	90.07–100.4	<7.6	[41]
DI–SPME-HPLC-UV	Water	ZnO nanoflakes	25	0.1–200 (ng/mL)	0.017–0.02 (ng/mL)	95.9–104	4.1–10.9	[42]
HF-LPME-GC-μECD	Human breast milk	Toluene	40	0.03–1.4 (ng/mL)	0.007–0.014 (ng/mL)	70–117	4–9	[43]
SHS-HLLME-GC–MS/MS	Beverages	Heptanoic acid	5	0.01–20 (ng/mL)	0.002–0.005 (ng/mL)	93.2–114.3	1.9–4.2	[44]
LLE-dSPE-GC–MS	Edible oils	*N*-Hexane/acetonitrile	22	20–1000 (ng/mL)	100 (ng/g)	70.9–110	<20	[45]
SDES–HLLME-GC–MS/MS	Water	SDES (DEA/3-MP)	3	0.1–100 (ng/mL)	0.001–0.040 (ng/mL)	86.24–116.8	1.1–6.8	This work

Declaration of 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.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2026.112412.

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