An improved method for measuring phthalates in seawater with blank contamination using GC-MS

Jeyakumar dhavamani[*a,b], Aaron J. Beck[a] , Martha Gledhill[a], , Mohammad I. Orif[e], Iqbal M.I. Ismail[b], Eric P. Achterberg[a], Mohammad S. El-Shahawi[c,d**]

[a]GEOMAR Helmholtz Center for Ocean Research Kiel, Wischhofstrasse 1-3, 24148 Kiel, Germany.

[b]Center of Excellence in Environmental Studies, King Abdulaziz University, P. O. Box 80200, Jeddah 21589, Saudi Arabia.

[c] Department of Chemistry, Faculty of Science King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia.

[d]Department of Chemistry, Faculty of Science, Damiatta University, New Damiatta, Damiatta, Egypt

[e]Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia.

***Corresponding authors:**

malsaeed@kau.edu.sa

[**c] Department of Chemistry, Faculty of Science King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia.

jdhavamani@geomar.de; jeyakumar.dhavamani@gmail.com

[*a]GEOMAR Helmholtz Center for Ocean Research Kiel, Wischhofstrasse 1-3, 24148 Kiel, Germany.

[b]Center of Excellence in Environmental Studies, King Abdulaziz University, P. O. Box 80200, Jeddah 21589, Saudi Arabia.

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Abstract

Quantification of phthalates or phthalic acid esters (PAEs) might be problematic due to matrix overlap, auto-self absorbance and background scattering noise by the plastic lab materials although plastics have been reported in the release of PAEs. These materials (ambient air, reagents bottles, sampling devices, and various analytical instruments), are ubiquitous in the laboratory environment, thereby making it more difficult to reliably analyze of trace concentration of PAEs. Thus, in the current study, a straight forward and reliable protocol has been established for the analysis of PAEs including control of blank contamination, and the experimental conditions such as extraction time and temperature were optimized. The mass of PAEs in blank tests of selected materials ranged from 3±0.7 to 35±6 ng for liquid-liquid extraction (LLE) and from 5±1.8 to 63±15 ng for solid-phase extraction (SPE). For both extraction methods, higher blank values were measured for dibutyl phthalate (DBP) (35±6 ng, 12±3 ng), and DEHP (63±12 ng, 23±5 ng) in LLE and SPE, respectively. Average recoveries of PAEs in LLE were 90-97% and obtained with successive aliquots of 2 mL, 1 mL, and 1 mL dichloromethane (DCM). For SPE, recoveries up to 86-90% were achieved with successive aliquots of 5, 3, and 2 mL DCM at a sample flow rate of 5 mL min^{-1}. Under the optimized conditions, the method quantification limits (MQL) for PAEs was 10-20 ng L^{-1} for LLE and 10-35 ng L^{-1} for SPE. Moreover, the dissolved concentrations of PAEs from LDPE measured by the LLE method ranged < 1.5 to 5.83 ng $cm^{-2,}$ and those measured by SPE ranged from 1.0 to 256 ng L^{-1}, in seawater samples of Sharm Obhur. The method has lower MQL values for LLE and SPE than average reported values of 10-100 ng L^{-1} and 30-100 ng L^{-1}, respectively. The method offers a reliable approach for quantifying blank contamination and the MQL values meets the

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requirements for PAEs analysis in seawater. The results suggest that LLE or SPE combined with GC-MC could be an easy and efficient method to quantify PAEs residue levels in seawater.

Keywords: Microplastics, Blank contamination, Liquid-liquid extraction, Solid-phase extraction, Phthalate esters; GC-MS

1. Introduction

Phthalic acid esters (PAEs) are the main group additives and are used as plasticizers to improve the flexibility and pliability of plastics. Phthalic acid esters account for about 92% of plasticizers produced and the most produced and consumed plasticizers worldwide [1, 2]. Some PAEs are considered endocrine-disrupting chemicals that can have significant effects on reproduction in marine animals and on the development of obesity and cancer in humans [3-5]. Given their potential environmental and health risks, six PAEs (dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di(2-Ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP)) have been identified as priority pollutants by U.S. Environmental Protection Agency (US-EPA) and the European Commission [6, 7].

The pre-treatment for analysis of PAEs in natural water samples is classically conducted by liquid-liquid extraction (LLE), solid-phase extraction (SPE), soxhlet extraction, and ultrasonication. The detection techniques for PAEs mainly concern gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) [8-10]. Generally, analysis with small sample volumes of 10-20 mL mainly used LLE to determine PAEs, with a relatively short extraction time (2-5 h). In analysis of PAEs, in environmental samples including freshwaters and seawaters, where sample volumes of 2-10 L are used, SPE has

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been employed. The extraction efficiency for SPE ranges between 65 to 95%, with extraction times between 5 and 8 h for a volume of 1-2 L [9].

Contamination by reagents and materials used as part of the analytical protocols of sampling, processing, and analysis of PAEs, forms an important challenge for PAE quantification with good precision and reliability. Most materials used in the field during sampling and in laboratories are sources of plastic contamination including samplers, lab coats, and other apparel worn by laboratory personnel, analytical instruments, water used to clean equipment before use, sponges or brushes used to clean equipment before use, synthetic polymer gloves and plastic sample containers. Extreme care must be taken to minimize contamination when collecting and analyzing water samples for microplastics. Contamination can significantly affect sample concentrations leading to overestimation. It is often not possible to confidently eliminate all contamination from samples during laboratory processing.

Nowadays, a recent literature survey has revealed that over the last 20 years, data on blank contamination appeared in 242 out of 552 articles (44%). In many cases, authors omit information regarding blank contamination. [11]. This may mean that no cross-contamination occurred in a given study, or it was sufficiently low to be considered negligible. Nonetheless, such information should be included in publications, because it may prove useful from the point of view of discussions regarding cross-contamination. Since the blank contamination is one of the major issues in PAE quantification, a blank measurement of each material and device was conducted individually to determine background contamination. Thus, the overall goal of the current study is aimed to develop a reliable method for PAE analysis. The work includes the evaluation of PAEs contamination in all materials involved in the experiments and analysis, as

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well as optimization of extraction conditions such as liquid-liquid extraction and solid-phase extraction.

2. Materials and methods

2.1. Reagents and materials

A standard mixture of PAEs, including three low molecular weight (LMW) compounds, DMP, DEP, DBP, and three high molecular weight (HMW) compounds, BBP, di(2-ethylhexyl) phthalate (DEHP), and DnOP at concentrations of 20,000 mg L^{-1} in methanol (purity 99.8%) was purchased from Sigma Aldrich (Germany). Some of the properties of the PAEs are listed in Table 1 [12]. Dichloromethane (DCM), MeOH, and ethyl acetate were purchased from Sigma Aldrich (HPLC grade). Working solutions of PAEs were prepared in isooctane and stored in the dark at 4°C for a maximum of two weeks. All experiments were performed in artificial seawater (ASW) prepared according to Sunda et al (2008) (3.0 mg L^{-1} NaF, 20 mg L^{-1} $SrCl_2$-$6H_2O$, 30 mg L^{-1} H_3BO_3, 100 mg L^{-1} KBr, 700 mg L^{-1} KCl, 1 mg L^{-1} $CaCl_2$-$2H_2O$, 4000 mg L^{-1} Na_2SO_4, 10.780 mg L^{-1} $MgCl_2$-$6H_2O$, 23,500 mg L^{-1} NaCl, 20 mg L^{-1} Na_2SiO_3-$9H_2O$, and 200 mg L^{-1} Na HCO_3) in ultrapure water (Milli-Q, resistivity 18.2 MΩ cm). Phthalate impurities in the ASW were removed by SPE with a polypropylene column (Chromabond Easy, 6 ml, 200 mg, Macherey-Nagel, Germany) at a flow rate of 5 ml min^{-1}; the ASW was sterilized in an autoclave (JASC-80JSR, Korea). All glass materials were baked at 400°C for 4 h.

2.2. Instrumentation

The concentrations of PAEs in the extracts were analyzed by GC-MS (QPplus-2010, Shimadzu, Japan) using electron ionization (EI) conditions. The initial temperature of the column oven was 250°C. A HP 5MS, 30 m capillary column was used (30 m × 0.25 mm i.d. × 0.25 m, 5% phenylmethyl siloxane, Agilent HP -5MS) with a temperature program of 60°C (2 min hold),

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ramp 5°C min^{-1} to 310, and 5 min hold. Helium was used as the carrier gas (2 mL min^{-1}). The ion source temperature was 220°C and the interface temperature was 250°C. The injection was performed in "splitless" mode, with a spitting time of 0.98 min and a purge flow of 30 mL min^{-1}. Target compounds were positively identified by comparing their retention times and target ions to specific reference ions. Instrument performance was calibrated using an eleven-point calibration curve. There were lower range calibration standards of 1, 2, 4, 8, 16, and 20 ng L^{-1} and higher range calibration standards of 20, 40, 80, 160, 200, and 400 ng L^{-1}.

2.3. Recommended procedures to avoid blank contamination

To avoid background contamination in experiments and standard solutions, as well as in the extraction procedures, all materials used were plastic-free except in cases where the material was not replaceable. To determine the method blank value, the contamination of materials and instruments was measured individually. The detailed information on the materials and testing procedures are given in the Supplementary information (Table S. 1). As a first step, instrument blank values analyses (without any injection) were performed and the signal intensity at characteristic mass to charge ration (m/z) values for quantization ions (Q) was obtained for each PAE. Then, the organic solvents DCM, MeOH, and isooctane were directly injected into GC-MS and the difference between their signal intensity and instrumental blank was measured to calculate the contamination of each solvent. Consumable materials including micropipette tips (autoclaved), GC-MS vials, syringes, syringe filters, solid-phase extraction cartridges, test tubes, test tube caps, sodium sulfate, and laboratory sample preparation instruments including nitrogen units, and vacuum units were treated separately with 5 mL of DCM and MeOH and the leached contaminants were GC-MS analyzed. The extracts were evaporated to near dryness at 25°C with N_2 gas using a TurboVap LV (Model N- EVAP 111, USA). The extracts were then reconstituted

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with isooctane (1.0 mL). The extracted samples were stored at -20 °C until further analysis. All the samples obtained were stored at -20 °C until analyzed by GC-MS. Based on the difference in m/z values at each step; the mass of PAEs from each material was calculated. The workstations were cleaned before using DCM: MeOH (80:20) at each step and a laboratory coat (cotton clothing) was worn at all times during the study.

2.4. Recommended pre-concentration procedures

2.4.1. Liquid-liquid extraction

The extraction efficiency of the target analyte (PAEs) from spiked ASW (10 mL) was performed by liquid-liquid extraction with successive aliquots of solvents (DCM, ethyl acetate, n-hexane). The combined extracted volume was pre-concentrated to 0.2 ml and stored as described above (Section 2.3). The extracted samples were stored at 4°C until analysis. We employed the standard addition method (SAM) as an efficient approach to correct for matrix effect and obtain an overall evaluation of the extraction efficiency at different concentrations. The method quantification limits were determined using the standard addition method (Frenna, Mazzola, et al. 2012). Known concentrations of standard PAEs (5, 10, 20, 40, 80, 160, and 200 ng L^{-1}) were spiked into 10 ml ASW and extracted by the same procedure.

2.4.2. Solid-phase extraction

Concentrations of dissolved 6PAEs in ASW (1L) were determined by SPE (Teflon column, Chromabond Easy, 6 ml, 200 mg, Macherey-Nagel, Germany). The samples were spiked with a mix of 6PAEs at 200 ng L^{-1}. A vacuum system (J.T. Baker, The Netherlands) was used for pre-concentration. The SPE cartridges were conditioned with methanol (5 mL), followed by 5 mL of DCM (5 mL) and 5 mL of ultrapure water, all at 5 mL min^{-1} flow rate. Samples were aspirated through the cartridges at a flow rate of 2-10 mL min^{-1}. The cartridge was finally rinsed with 5

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mL × 3 ultrapure water and dried under vacuum for 30 min. Elution was performed with 10 mL of DCM with successive aliquots of 5, 3, and 2 mL. The combined volume of the extract was pre-concentrated to 0.2 mL as and stored as described above (section 2.3). The quantification limits of methods were also performed using a range of concentrations of 5, 10 20, 40, 80, 166, and 200 ng L^{-1} that were dosed to 2 L of ASW and extracted by the described procedure.

3. Results and Discussion

3.1. Programming of GC-MS analysis

In the method development for gas chromatography-mass spectrometry, the infusion is the first step. Thus, the preparation process in MS involves the isolation of a single precursor ion and subsequent dissociation of the precursor ion into characteristic product ions. First, the individual standards were injected into the GC-MS in full scan mode (50-1500 m/z mass range). The ion with the highest abundances for each analyte was selected as the base peak. As shown in Table 2, a total of 6 different certified reference materials (CRM) were monitored by GC-MS to cover all target contaminants and the surrogate standards. To confirm the positive finding of the CRM, one or two molecular ions and their ratio shall be measured [13]. The transition with the highest intensity was selected as the quantitative transition (Q). The GC-MS spectra for PAEs were obtained by selecting the base peak at m/z 163 $[M\text{-}OCH_3]^+$ for dimethyl phthalate and m/z 149 $[C_9H_9O_2]^+$ for all other PAEs as the characteristic precursor ion. The main ion m/z 149 resulted from the fragmentation with the loss of the alkyl ester groups and a furan ring formation [1]. A similar fragmentation pattern is found for the other phthalates, except for DMP. In the mass spectrum of DMP, the molecular ion is detected at m/z 194. The most abundant ion is at m/z 163, corresponding to the loss of a methoxy group (M–31) [1].

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Parameters of GC, such as injector temperature (250°C), flow rate (2 mL min^{-1}), and temperature gradients were optimized for higher intensity elution of chromatographic peaks. The optimized GC parameters are shown in section 2.2. The intensity observed in this study ranged from 52-97 (Table 2). These conditions are typical for the analysis of phthalates by GC-MS and result in sufficient resolution of the most important phthalates. As previously reported, these conditions have a good compromise between resolution and speed of analysis [1, 10].

In the current study, good linearity responses were obtained for all target contaminants over the entire tested concentration range, and an eleven-point calibration was analyzed in triplicate at 1, 2, 4, 8, 16, 20 ng L^{-1} (R^2; 0.998) and 20, 40, 80, 160, 200, 400 ng L^{-1} (R^2; 0.996) for all target contaminants. Quantification was performed using the standard external method in the selected ion monitoring mode (SIM). The regression coefficient of linearity was greater than 0.99 with a relative standard deviation (RSD) of 18%. The instrumental detection limit (LOD) was calculated using the signal-to-noise ratio (S/N) of the lowest concentration used. The optimized parameters for GC-MS calibration and instrument detection limits are summarized in Table 3. The performance of this instruments was calibrated based on the European regulation, article number; 10/2011/EU [14].

3.2. Blank contamination levels

Plastic parts are a significant component of materials needed during this study. Such as rubber seals on GC-MS, nylon lining on N_2 and vacuum units, polypropylene housing on the SPE, and most salts are stored in polyethylene containers. In Table, S1 details are provided about all the materials and suspected plastic parts of each material. Each material was analyzed for contamination with PAEs by GC-MS. Table (S2-S7) shows the increasing GC-MS background

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contamination by each material introduced into the method. The various materials used in the method have different concentrations of PAEs. For example, the mass of DEP increased starting from the instrument blank sample by adding all the required materials sequentially as presented in Table 4. The mass value of DBPs in the instrument blank (GCMS) alone was 8±2 ng and increased to 96.3, 72.2, 110.0, and 57.0 ng using autoclaved micropipette tips (0.2 mL, polypropylene, CAAP, Denmark), syringes (5 mL, disposable, polyethylene, KSA), PTFE syringe filters (0.2 µm, polypropylene, Whatman, UK), and artificial seawater (ASW). After considering the pre-concentration, the given mass values were found equivalent to 0.008, 0.1, 0.8, 0.11, and 0.05 ng L^{-1}, respectively. Because of the sterilization process, such autoclaves affect the chemical stability of tips and this may lead to chemical leaching. DCM, leading to contamination, also affects PP housing and black rubbers in the syringe filter. The list of suspected plastic parts in the materials is summarized in Table S1. Due to high blank contamination, the use of sterile tips, syringes, syringe filters, and unfiltered ASW was avoided in the LLE and SPE extraction methods. Other selected materials listed in Tables 5 and 6 with DEP concentration below 40 ng (0.008 ng L^{-1}), were further used for extraction analysis in this study.

Overall, the mass of PAEs in the blanks ranged from 3±0.2 to 35±6 ng for LLE and 6±2 to 65 ±17 ng for SPE, as shown in Tables 5, and 6. The highest mass was found for DEP (35.0 ng) followed by DEHP (18 ng) (Table 5). Indeed, DBP and DEHP are the most commonly used plasticizers and account for half of the total PE production in Western Europe [15]. The mass of DMP, DBP, BBP, and DnOP was estimated to be 10, 12.7, 9.4, and 3.2 ng, respectively for LLE blank (Table 5). Due to the higher number of materials involved in SPE, the mass of DEP, DBP,

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and DEHP was measured as 63.1, 23.0, and 24.5 ng, respectively (Table 6). The mass of PAEs in the LLE blanks and SPE blanks was subtracted from the pre-concentrated samples.

3.3. Optimization of liquid-liquid extraction

As PAEs are weak polar compounds, weak and nonpolar solvents were better than medium and strong polar solvents for PAEs extraction. Following prior studies [9, 16], dichloromethane (DCM), ethyl acetate, and n-hexane were used as extraction solvents, and we evaluated their extraction efficiency. Good efficiency was achieved with DCM and hexane as nonpolar solvents, related to the fact that the dielectric, as well as the auto pyrolytic constants of both solvents, are very low (Liu et al., 2013; Gao et al., 2014; Net et al., 2014). The highest yield was obtained with DCM (80-90% yield), followed by n-hexane (75-89% yield), the mixture of DCM: ethyl acetate (80:20) (60-72% yield), and ethyl acetate (65-75% yield) (Fig. 2a), which might result from its larger polarity [17]. However, the recovery of DMP was low (65-80%), which might result from its lower partitioning coefficient of octanol and water (log Kow = 1.6), and its high solubility in water (4000 mg L^{-1}). In contrast, the log Kow of DnOP, DBP, and DEHP were 4.5, 7.6, and 8.4, and their solubility in water was 11.2, 0.27, and 0.09 mg L^{-1} (Christian, 2020).

The yield (%) increased with increasing the molecular weight of PAEs. Normally, the water solubility of the alkyl phthalate ester varies inversely with the length of the alkyl side chain [1]. To determine the recovery rate, the mean peak area of each analyte was determined for an ASW spiked with the analyte (n = 6). To improve the extraction efficiency with DCM, the recovery experiments were performed with a number of extraction cycles of 1-5. The reasonable

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extraction yield was obtained at 2-3 cycles, as shown in Fig. 2b. As the number of extraction cycles increased from 2 to 3, the recovery rate increased to 90-97%. Multiple extractions by DCM enhanced the mass transfer of the PAEs from the aqueous to the organic phase (Christian, 2020). Maximum PAEs extraction was achieved after 3 cycles and remained constant which is most likely attributed to their equilibrium concentrations. Thus, in the subsequent experiments, three extraction cycles were adopted.

Photolysis experiments were performed with compact fluorescent tubes (Philips CFL, 15W) for 120 hours. The irrigation cycle was 12 h and the distance between the test tube and the lamp was about 10 cm. The light intensity was measured with a light intensity meter and was about 165 lx. The dark experiment was performed without light and also by covering the tubes with aluminum foil. The PAE in the tubes placed in the dark were affected only by hydrolysis, while PAEs in light was affected by both hydrolysis and photolysis. The LMW-PAEs were degraded by 10-25% under light conditions, in contrast, HMW-PAEs the degradation rate ranged from 8-10% (Fig 2c). After 10 days, more than 80% of the original concentration was still present in tubes placed in sunlight. This behavior is most likely attributed to the hydrolysis of the ester group by cleavage of the C-O bond as reported (Balabanovich and Schnabel, 1998; Lau et al., 2005). The half-life ($t_{1/2)}$ of DEHP varied between 0.2 -2 days whereas for DMP it was 9.3 days) [18]. The degradation of HMW-PAEs (BBP, DEHP, and DnOP) in the dark was negligible and in good agreement with the data reported [19]. To estimate the stability and the limit of quantification of the LLE method, 10 mL of ASW was prepared with different concentrations (5, 10, 20, 40, 80, 160, 200 ng L^{-1}) of PAEs. The estimated value was compared with the mean value obtained with the same range (5-200 ng L^{-1}) of PAEs in 0.2 mL isooctane. The estimated recoveries were linear with a range from 10-200 and ng L^{-1} (Fig 2d). The minimum quantification limit of LLE

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obtained was 10-20 ng L^{-1} (Table 3). These results demonstrate the feasibility and reliability of the established method for PAE_S determination in water samples [20, 21].

3.4. Optimization of the solid-phase extraction

To optimize the eluting agent, different solvents were used to evaluate their effects on extraction efficiency, including 100% dichloromethane (DCM), MeOH, and the mixture of DCM: MeOH (50:50) and (80:20). The samples were initially spiked with PAEs to give a final PAEs concentration of 200 ng L^{-1}. At a low flow rate (1 mL min^{-1}), the sample volumes were then percolated over the SPE column and the concentration of each PAEs in the effluent was then measured. The results showed that the best extraction results were obtained with DCM: MeOH (80:20) as desorption solvent when the volume of desorption solvent exceeded 5 mL. In solid-phase extraction, maximum recovery (80-90%) of PAEs was achieved with DCM: MeOH (80:20), followed by DCM (75-89%), MeOH (60-72%), and DCM: MeOH (50:50) (65-75%) (Fig.3a). With DCM, the recovery percentage of PAEs also increased by raising the molecular weight of the PAEs. The solubility of HMW-PAEs in water is low thus it extracts easily in a non-polar solvent like DCM (5 mL) (Liu et al., 2013; Gao et al., 2014; Net et al., 2014). The color of the extract of the DCM; Methanol was deeper than dichloromethane, which is attributed to the increased polarity of the mixed solvent (DCM: Methanol) which dissolves the polar impurities [22]. So far only DCM was adopted in the next study as a proper eluting solvent.

To determine the recovery rate, the mean peak area of each analyte was determined for an ASW spiked with the analyte (n = 6). To improve the extraction efficiency with DCM, the recovery experiments were performed over a number of 1 to 5 elution times 1-5. As mentioned before

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(section 3.3) a good extraction yield (90-92%) was obtained at between 2 to 3 cycles, as shown in Fig. 3b. No further increase was measured at 3 to 5 extraction cycles. To reduce the extraction time, and the adsorption efficiency of the column the experiments were performed under different sample flow rates. PAEs were first spiked into the samples to give the final PAEs concentration of 200 ng L^{-1}. The sample solutions were percolated through the SPE-packed columns at various flow rates ranging from 2 to 20 mL min^{-1}. The adsorption efficiency of the column decreased on increasing sample flow rate, and a reasonable efficiency was observed between 5-10 mL min^{-1} flow rate. Generally at a low flow rate, good equilibration of the species with the solid phase packed column is high (Christian, 2020).

It is worth mentioning that, the adsorption efficiency was reduced by 8-12% at 15 mL min^{-1} and by 15-22% at 20 mL min^{-1} (Fig 3c). To estimate the limit of quantification of the SPE approach, the samples were initially spiked with PAEs to give various known final concentrations of 1, 5, 10, 20, 40, 80, 160, and 200 ng L^{-1} of ASW. The estimated value was compared with the mean value obtained with the same range (5-200 ng L^{-1}) of PAEs in 0.2 mL isooctane. The estimated average recoveries were linear with a wide range of concentrations (10 -200 ng L^{-1}) as shown in Figure 3d where minimum quantification limits in the range of 10 to 35 ng L^{-1} of SPE were achieved (Table 3).

3.5. Analytical performance and figures of merits

Most studies reported blank or background contamination without reporting target PAE [23]. Studies reported blank contamination of dimethyl phthalate (DMP), nonylphenol (NP), and nonylphenol monoethoxylate (NMP) of 0.07-0.8 ng L^{-1} [10, 24]. Although DEP, DBP, and occasionally DnBP have been detected in blank samples, their values were below 0.07 ± 0.02 ng

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L^{-1}, 0.8 ± 0.3 ng L^{-1}, and 0.7 ± 0.2 ng L^{-1}, respectively [24]. These values are higher than the blank contaminants determined in the current study. The highest measured mass of DBP and DEHP in the LLE blank (35±6 and 18.2 ng, respectively; Table 5) corresponded to 0.035 and 0.018 ng L^{-1}, respectively, after accounting for the pre-concentration. Similarly, the mass of DBP (63.0 ng) and DEHP (25 ng) measured in the SPE blanks corresponds to 0.06 and 0.02 ng L^{-1}, respectively. Blank contamination control and evaluation are particularly important for samples with low environmental concentrations.

A diversity of methods can be used for the extraction and quantification of PAEs from seawater. Limits of quantification vary depending on the extraction method (LLE, SPE, Soxhlet, ultrasound, etc.) and quantification technique (GC-MS, GC-MS/ MS, HPLC, UPLC, etc.). The quantification limits of PAEs in different techniques for the LLE and SPE extraction methods for published works are listed in Table 7. The average limits of quantification are in the range of (10-100 ng L^{-1}) for the LLE method and (10.0-180 ng L^{-1}) for the SPE method using GC-MS. In some studies, the limits of quantification obtained with GC-MS/MS were in a higher range (0.2.-0.53 ng L^{-1}), and UPLC with MS / MS detection was in the range of (3-8 ng L^{-1}). The MQL obtained by LLE and SPE were comparatively 5-10 higher than the MQL obtained by similar techniques (GCMS), which may be due to the careful selection of experimental materials. In conclusion, our analytical method provides reliable quantification of blank contamination and increases the MQL. It is clear that some of these published methods revealed high MQL with significant interference issues, whereas the MQL achieved by the developed protocol was much lower than the maximum permissible limit of PAEs (> 1.3 μg L^{-1}) in fresh and marine water as per the EU guideline [25, 26]. Our approach provided better MQL, and will contribute to assessments of blank contamination from different materials during LLE and SPE extraction and

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analysis, which help improve the method detection limits. Thus, it can be concluded that the established protocol can substitute other expensive analytical methods and be practiced in environmental laboratories for the direct determination of PAEs in water samples. Thus, this method meets the requirements for the analysis of PAEs in water samples.

3.6. Real sample analysis

The analytical utility of the established LLE and SPE methods to detect the leached PAEs from LDPE polymer, and PAEs in surface waters of the Red Sea, were critically tested. To detect the leached PAEs concentrations from LDPE, the polymer surface was rinsed with ultrapure water and cut into squares of 1.0×1.0 cm^2. For each experiment, ~100 - 150 mg of the polymer (17 cm^2) was added to 30 mL screw-cap tubes containing ASW (10 mL). The tubes were tightly sealed with polytetrafluoroethylene (PTFE) septa, covered with aluminum foil, and shaken continuously for 6 h at 85 pm. The dissolved concentrations of all PAEs from LDPE ranged from < 1.5 to 5.83 ng cm^2. The highest amounts of PAEs released were DBP (5.83 ± 1.4 ng cm^{-2}), DEHP (4.2 ± 0.8 ng cm^{-2}), and DBP (4.8 ± 1.3 ng cm^{-2}). Indeed, DBP (LMW) and DEHP (HMW) are the most commonly used plasticizers and account for half of the total PE production in Western Europe [15]. The absence of other target compounds (DMP, DEP, and BBP) is due to their low concentrations (< 4 ng mL^{-1}) in the polymer, low leaching rates, high affinity to the polymer, or losses during the production process [41]. This is consistent with the fact that plasticizer leaching is concentration-dependent, with the rate decreasing with decreasing concentration [42]. Similarly, the reported DBP levels from PE ranged from (1.2 ng cm^{-2} over 1-78 days; Suhrhoff and Scholz-Boettcher 2016) to (8 ng cm-^{2} over 1-12 weeks) (Paluselli, Fauvelle, et al. 2018).

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The concentrations of PAEs in real seawater were measured following SPE extraction. The water samples were collected from 11 sampling sites along the Bay of Sharm Obhuur (Fig S1). The individual PAEs concentrations ranged from 1.0-256 ng L^{-1}, while the arithmetic average for individual PAEs concentrations ranged from 188 ng L^{-1}. High levels of DEP, DBP, and BEP were detected in all samples and ranging from 143 -276, 8.9 -238, and 138 - 282 ng L^{-1}, respectively. In contrast, DMP (3.4-16.6 ng L^{-1}), BBP (1.6-4.7 ng L^{-1}), and DnOP (2.2- 49.1 ng L^{-1}) were generally lower in most samples, The compounds DEP, DBP, and BEP accounted for more than 87% of the sum of concentrations of $\sum_6$ PAEs, with DEP (34%), DBP (22%) and BEP (33%). The concentration of the other three compounds represented between 4 - 6% of the total concentration, with the proportion for DMP (3%), BBP (6%), and DnOP (3%) (Table 2). This concentration range is consistent with data reported for other regions e.g. Σ_6PAEs have been reported in the Mediterranean Sea: 17.4- 8442 ng L^{-1} [43], 130-1330 ng L^{-1} [24] and 168-689 ng L^{-1} [39] and northern Europe: 76-1440 ng L^{-1} [44].

4. Conclusion and future perspectives

This study demonstrates analytical utility and sensitivity of the established protocol with blank contamination for quantifying PAEs. Possible sources of blank contamination are highlighted with their impact on quantification limits in LLE and SPE extraction. A low level of blank contamination seems to be inevitable during experiments, despite many strict guidelines which do not necessarily guarantee the complete elimination of secondary contamination. Therefore, a detailed protocol is important to evaluate the control of blank contamination. The blank contamination with PAEs determined in the current study was 0.003- 0.04 and 0.005 – 0.06 ng L^{-1} for the materials used in the LLE and SPE methods, respectively. Failure to consider this could

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lead to an overestimation of the results obtained in the study. The MQL of PAEs obtained with the LLE and SPE methods are similar to the average reported MQL (0.3-20 ng L^{-1}). The current study provided lower MQL, and contributes to the assessment of blank contamination of different materials during LLE and SPE extraction and analysis, which helps to improve the MQL. In conclusion, this method is reliable in quantifying blank contamination and the MQL meets the requirements for the analysis of PAEs in seawater. We consider that it is important to standardize the methodology of sampling, separation, and identification of blank values with respect to PAE studies. This information is of prime importance in the analysis of PAEs, where it is of great value to achieve reliable determination of plastic additives in complex environmental samples. The eminence control of separable data is of great importance to assess the current environmental pressures associated with microplastic pollution.

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Appendix Supplementary information

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