



Quality by design in the optimization of the ultrasonic assisted solvent extraction for the GC-MS determination of plasticizers in sediments and shells

Elise Lorre^a, Nicolò Riboni^b, Federica Bianchi^{b,c,*}, Serena Orlandini^d, Sandra Furlanetto^d, Maria Careri^b, Mindaugas Zilius^{a,b}

^a Marine Research Institute, Klaipeda University, Klaipeda 92295, Lithuania

^b Department of Chemistry, Life Science and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, Parma 43124, Italy

^c Interdepartmental Center for Energy and Environment (CIDEA), University of Parma, Parco Area delle Scienze, Parma 43124, Italy

^d Department of Chemistry "U. Schiff", University of Florence, Via U. Schiff 6, Sesto Fiorentino, Florence 50019, Italy

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ABSTRACT

Phthalate esters (PAEs) are the most widely used plasticizers worldwide and are considered as ubiquitous environmental contaminants. Due to both their ubiquity and potential health and environmental risks, their determination is a matter of worldwide concern. In the present study, an efficient method based on ultrasonic-assisted solvent extraction followed by gas chromatography-mass spectrometry is proposed for the simultaneous determination of PAEs and di(2-ethylhexyl) adipate in estuarine sediments and shells. Method optimization was successfully carried out according to Quality by Design principles. Shaking time, ultrasonic time, extractant volume, and solvent type were selected as Critical Method Parameters. Validation proved method reliability for the determination of the investigated analytes, achieving detection limits in the 0.1–0.7 ng g⁻¹ and 0.1–0.5 ng g⁻¹ range for sediments and shells, respectively. A good precision was obtained with RSD < 20% and trueness (recovery) in the 73(±7)–120(±10)% and 70(±10)–111(±3)% range, for sediments and shells, respectively. Finally, the method was applied to analyze sediment samples and mollusc shells, collected from the Curonian Lagoon (Southeast Baltic Sea). Di(2-ethylhexyl) phthalate was present in all the analyzed samples, thus pointing out its ubiquity in estuarine environment. PAEs were also found in shell debris or living mussels, highlighting them as a hotspot of organic contaminants, especially in transitional environments, where accumulation of organic rich deposits is limited.

1. Introduction

Phthalate esters (PAEs) are the most widely used plasticizers worldwide. Their presence as additives in plastics, cosmetics, wall covering, floors, and packaging materials make them ubiquitous compounds in daily life [1,2]. Being classified as endocrine disruptors, these compounds could exert adverse health effects on both humans and wildlife in all environments [3].

In aquatic environments, owing to their relative high hydrophobicity, PAEs are adsorbed onto suspended particles and settled down to surface sediment [4]. Consequently, sediments can act as a temporal storage for PAEs. In Europe, the concentration of PAEs in aquatic

systems has decreased over the last decades due to the introduction of strict regulations on their use in industry [5,6]. Nevertheless, the level of PAEs observed in some areas is still high: for example in the Baltic region, concentrations over 3900 and 800 ng g⁻¹ were determined in settling deposits and sediments, respectively [6,7]. To overcome the restrictions on the use of PAEs, many manufacturers have replaced them with phthalate-free alternatives such as adipates, among which di(2-ethylhexyl) adipate (DEHA), thus contributing to increase their presence in the plasticiser market and, ultimately, in the environment [6].

The quantification of PAEs in sediments can be a real challenge since it requires the use of reliable analytical methods to avoid overestimation

* Corresponding author at: Department of Chemistry, Life Science and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, Parma 43124, Italy.

E-mail address: federica.bianchi@unipr.it (F. Bianchi).

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[8]. The extraction of PAEs from solid matrices such as sediments has been reported using a large variety of methods, like Soxhlet, microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), ultrasonic-assisted extraction (UAE) or solid-phase micro-extraction (SPME) [8–15]. The major advantage of the UAE approach relies on its ease of implementation in every analytical lab, requiring simple devices.

In recent years, Quality by Design (QbD) principles have been introduced by US Food and Drug Administration [16] for assuring quality in pharmaceutical products and processes. This quality paradigm has been outlined also in International Council for harmonisation (ICH) Pharmaceutical Development guideline Q8 [17] and its use has been implemented in Analytical Method Development ICH Q14 guideline [18]. Analytical Quality by Design (AQbD) consists of a systematic framework in analytical method development, and represents an important ally of the analytical researcher, in particular for the set-up of separation procedures [19,20]. Even if the most important field of AQbD application is still pharmaceutical analysis [21–23], recently few applications have been reported in food [24,25] and environmental analysis [26,27], confirming the great potential of this sound-science based approach. QbD principles are founded on Design of Experiments (DoE) and Risk Analysis, and their application makes it possible to efficiently drive method development, both clarifying the effects of multiple parameters on the analytical output and identifying the method operable design region (MODR). The latter is defined as the multivariate zone where it is assured that the analytical target is achieved with a selected level of probability [21].

In this study, for the first time in the literature, QbD was applied to the development of an extraction procedure in environmental analysis. Despite DoE optimization of UAE extraction has been already proposed in the literature [9,25,28–30], the application of QbD to extraction techniques is still limited to one example in food analysis, associated with the extraction of polyphenols from a vegetable matrix [31].

In this study, QbD was applied to optimize the UAE conditions for the GC-MS determination of several PAEs and DEHA in estuarine sediments. Validation was carried out to assess method reliability for the determination of the analytes at trace levels in samples collected from the Curonian Lagoon (Southeast Baltic Sea). The obtained results will provide a better understanding of the pollution levels in one of the most important lagoons of the Baltic region, since the compositional profiles of PAEs in the sediment of the area are still understudied and rarely considered in the state monitoring programs.

2. Material and methods

2.1. Chemicals and materials

Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Dibutyl phthalate (DBP), ButylBenzyl phthalate (BBzP), Di(2-ethylhexyl) phthalate (DEHP), Di-*n*-octyl phthalate (DOP), Diisobutyl phthalate (DiBP), and Di(2-ethylhexyl) adipate (DEHA) standards (all purity grade > 98%) were purchased from HPC Standard GmbH (Cunnersdorf, Germany).

Internal standards D4-ring deuterated dibutyl phthalate (D4-DBP, 98%) and D4-ring deuterated di-*n*-octyl phthalate (D4-DOP, 98%) were purchased from LGC Standard Sp. Z O.O. (Lomianki, Poland). Capillary grade ethyl acetate (EA), supratrace grade dichloromethane (DCM), acetone (>99.8% purity) (AC) and supergradient grade methanol (MeOH) were purchased from VWR International GmbH (Vienna, Austria). Both the stock (1000 mg L⁻¹) and the working (10 mg L⁻¹) solutions were prepared in EA and stored in the dark at -20 °C until analysis. To avoid background pollution, the laboratory equipment used for sampling and analysis was exclusively made of glass or stainless steel. Prior to use, all glass apparatus was soaked in 10% HCl for 12 h, rinsed with distilled water, dried, burned at 500 °C for 6 h, and finally pre-rinsed with MeOH.

2.2. Optimization of the ultrasonic assisted extraction

The Critical Method Attributes (CMAs) [19,22] corresponding to the responses to be investigated and maximized, were chosen as the chromatographic areas of DiBP, DBP, BBzP, DEHP. The Critical Method Parameters (CMPs) of the extraction [19,22], namely the factors that could potentially impact the CMAs, were represented by three quantitative factors, i.e. shaking time (*SHA time*), ultrasonic time (*US time*) and extraction volume (*VOL*), and one qualitative factor, namely solvent type (*SOLV type*). The knowledge space, that is the experimental domain of the CMPs explored by DoE, was the following: *SHA time*, 10.0–20.0 min; *US time*, 10.0–20.0 min; *VOL*, 10.0–20.0 mL (Table A1). The investigated solvents were DCM and the solvent mixtures DCM/AC and DCM/MeOH, both in 1:1 ratio. The final optimized conditions for the ultrasonic assisted extraction parameters, together with the MODR interval in brackets, are the following: *SHA time*, 15.0 min, *US time*, 12.0 min (10.5–15.0 min), *SOLV type*, DCM/AC; *VOL*, 17.5 mL (15.1–20.0 mL).

The extraction procedure was as follows: dry sediment (2.5 g) was weighed and transferred to a 100 mL borosilicate glass bottle, then the extraction solvent (optimized condition: 17.5 mL of DCM/AC) was added. The bottles were closed, shaken (optimized condition: 15.0 min) on a horizontal shaking table (KS 4000 ic control, IKA, Germany) and sonicated (optimized condition: 12.0 min) at room temperature in an ultrasonic bath (setting power: 100%; Sonorex Digiplus DL 512 H, Bandelin, Germany). Supernatant was separated from sediment, evaporated under a gentle stream of nitrogen gas, and dissolved in 1.5 mL of EA. Prior to GC-MS analysis, the extracts were filtered through 0.22 µm polytetrafluoroethylene filters (Frisenette, Denmark).

NemrodW software (NemrodW, LPAI sarl, Marseille, France) was used for planning the symmetric screening matrix and for related data treatment. MODDE 13 software (Sartorius Data Analytics AB, Göttingen, Germany) was used to generate the Central Composite Design (CCD) in Response Surface Methodology (RSM), to perform related data treatment and to identify the MODR through Monte-Carlo simulations [32]. When performing risk analysis, the target for defect per million opportunities (DPMO) [33] was set at 100,000, matching with a risk of error of 10%.

2.3. GC-MS analysis

A Shimadzu GC-2010 Plus gas chromatograph equipped with a GCMS-TQ8040 mass spectrometer (Shimadzu Corporation, Japan) was used for GC-MS analyzes. Helium (99.9999%) was used as the carrier gas at a constant flow rate of 1 mL/min; the GC injector was operated in splitless mode at 250 °C, whereas chromatographic separation was performed on a Rxi-5Sil MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Restek®, Bellefonte, USA) using the following temperature program: 60 °C, held for 2 min, to 240 °C at 25 °C/min, held for 2 min, to 300 °C at 10 °C/min, held for 3 min. Transfer line and ion source were maintained at 280 °C and 230 °C, respectively. The mass spectrometer was operated in time scheduled single-ion monitoring mode (SIM) by recording the current of the ions reported in Table A2. Signal acquisition and data handling were performed using the LabSolutions (Shimadzu Corporation) software.

2.4. Method validation

A blank analysis was performed prior to validation to establish the absence of contamination in the matrices used as reference (shells and sediments). Then method validation was performed according to EUR-ACHEM guidelines [34] under the optimized conditions using the blank matrices. Detection (yD) and quantitation limits (yQ) expressed in terms of signals were obtained by the analysis of blank sediments spiked with each analyte at 0.2 ng g⁻¹. Both the mean (y_b) and the standard deviation (s_b) of 10 replicated measurements were calculated. Finally, detection

(LOD) and quantitation (LOQ) limits were obtained by projection of the corresponding signals y_D and y_Q through a calibration plot $y = f(x)$ onto the concentration axis. Linearity was assessed using a matrix-matching calibration (sediment and shells) on 6 concentration levels: LOQ, 2, 10, 50, 100, 150 ng g⁻¹ for all the analytes, with the only exception of BBzP (LOQ, 4, 10, 50, 100, 150 ng g⁻¹), by performing 3 replicated measurements per level. D4-DBP and D4-DOP were used as internal standard at the concentration of 10 ng g⁻¹. Homoscedasticity was verified by applying the Hartley test. Lack of fit and Mandel's fitting tests [35] were also performed to assess the goodness of fit and linearity ($\alpha = 0.01$). The significance of the intercept ($\alpha = 0.05$) was evaluated by performing a Student's *t*-test. Repeatability and intermediate precision were evaluated as relative standard deviations (RSD%) on 3 concentration levels: LOQ, 25 and 150 ng g⁻¹, performing four replicated measurements per level. Intermediate precision was estimated over three days, verifying homoscedasticity of data and performing the analysis of variance (ANOVA) at the 95% confidence level. Trueness was calculated for each analyte in terms of recovery rate (RR%) by performing 8 replicated measurements at LOQ, 25 and 150 ng g⁻¹. RR% was calculated as follows: $RR\% = (C_{exp}/C_{spk}) \cdot 100$ where C_{exp} is the observed concentration and C_{spk} is the concentration of the fortified matrix. Finally, the matrix effect (ME) was calculated at LOQ, 25 and 150 ng g⁻¹ using the following formula:

$$ME(\%) = A_1 / A_2 \times 100$$

where A_1 is average peak area of each analyte/internal standard in matrix extract and A_2 is that in DCM/AC (1:1, v/v) at the same concentration.

2.5. Real sample analysis

Sediment samples from the oligohaline Curonian Lagoon (salinity ≤ 0.5 [36]), which is located along the southeast coast of the Baltic Sea, were collected in August 2021 (Site A and B) and in February 2022 (Site C) from two principal sedimentary environments: deeper confined (3.5 m depth) and shallow transitional (~1.5 m depth). Confined area (Site A, 55°17'14.3" N/ 21°01'17.4"E) has a longer water renewal time and organic-rich deposits (organic carbon content (C_{org}) = 12.0%, median grain size = 0.042 mm). Shallower transitional area (Site B, 55°26'40.1" N/ 21°10'57.8"E; Site C 55°20'25.9"N/21°11'24.4"E) is characterized by shorter water renewal time, and sandy sediments with low organic matter content (C_{org} = 0.1–0.3%, median grain size = 0.209–0.223 mm). Sediments were collected using a hand corer with stainless-steel tubes (i. d. 8 cm, length 30 cm) at each of the three sites. The upper 0–5 cm layer was subsampled, transferred to glass jars and kept at -20 °C. Prior to analysis, sediments were freeze-dried for 48 h, grinded using agate mortar and pestle, and homogenized by sieving through a stainless-steel sieve (0.5 mm, Retsch GmbH, Germany). At stations with fine sand deposits (Site B and C), large debris of shells (> 0.5 mm) were also separated and mashed prior to analysis. In total, four types of shells (only Site C): i) a mixture of 95% gastropods + mussels together and 5% of unionid, ii) unionid, iii) gastropods, and iv) mussels were submitted to analysis.

3. Results and discussion

Phthalate esters can be categorized into two main groups: low molecular weight (LMW; 3–7 carbon atoms in their chemical backbone) and high molecular weight compounds (HMW; 7–13 carbon atoms in their chemical backbone). These two groups have different applications, toxicological properties, and legal requirements. In the present study, our focus was on LMW PAEs, as HMW PAEs are not associated with adverse health effects and do not cause endocrine disruption [2,37]. Among the LMW PAEs, only four compounds, namely DBP, BBzP, DEHP and DIBP are classified as very dangerous substances by REACH [2],

suggesting their use as model compounds for the optimization of the extraction conditions.

The choice of the extraction solvent is always a critical step in the development of a novel extraction procedure. A wide variety of organic solvents have been proposed to extract PAEs from sediment, including hexane/AC mixtures (1:1, v:v) [9,12], pure DCM or mixture of solvents, such as DCM/MeOH (1:1, v:v) or DCM/AC (1:1, v:v) [13,38,39]. Unfortunately, most organic solvents are potentially dangerous, and extra care must be taken during sample handling, recycling or disposal [40].

Preliminary results (data not shown) showed that the use of hexane, both pure and in mixture with AC was able to produce lower GC-MS responses compared to DCM, so only the performance of DCM (pure or in mixture with MeOH) or AC was evaluated. DCM has been used to extract PAEs from different matrices including water, sediment, plastic toys, food, and other biological matrices [41,42]. However, the addition of water-miscible solvents, such as AC or MeOH, could increase the extraction capability of the mixture due to both increased hydrophilicity and water/organic solvent partitioning. As previously reported, residual water present in the sample can reduce the extraction efficiency [43] by reducing the wettability of the surface, thus limiting the extraction of the most apolar compounds. By contrast, the use of a water-miscible organic modifier facilitates PAEs extraction by allowing a better penetration of the solvents into the sample [44]. In this context, the extraction of PAEs relies on the establishment of weak London dispersion forces, dipole-based interactions, and hydrogen bonding with carboxylic groups of the analytes.

In this study, following the Quality by Design framework, the optimization of the UAE extraction conditions was carried out in subsequent steps, consisting of a screening phase for obtaining preliminary information on the effects of the investigated CMPs on the CMA, and a subsequent RSM to obtain a map of the predicted CMA values throughout the experimental domain. The final step was represented by the definition of the MODR.

3.1. Screening phase

Preliminary experiments were carried out to identify the CMPs, i.e. the factors potentially affecting the effectiveness of UAE, and to select their experimental domain for the screening phase (Table A1). To reduce environmental impact, cost and time, the range of factors, such as extraction time (shaking and ultrasonic) and solvent volume, was selected based on the minimum value reported in the literature (10/15 min extraction time [13,38] and 5/10 ml of solvent [10,39]).

A Free-Wilson model was postulated to investigate the relationship between the CMPs and the CMA [45]. This includes one constant term A_0 and a number of coefficients for each factor equal to the number of considered levels minus one. Each factor was investigated at three levels, so the model comprised two coefficients for each factor:

$$y = A_0 + A_1A + A_2A + B_1B + B_2B + C_1C + C_2C + D_1D + D_2D$$

In this model, y is the response (CMA), namely the area of each of the considered compounds, A is *SHA time*, B is *US time*, C is *SOLV type* and D is *VOL* (see Table A1). A 3⁴/9 symmetric screening matrix was selected to estimate the coefficients, thus highlighting the effect of the considered factors for each CMA. This type of matrix is very useful to achieve preliminary information on both the significant factors to be considered in the study and on the experimental domain to be studied in subsequent RSM [45,46]. The experimental domain investigated and the 3⁴/9 symmetric screening matrix used in this study are reported in Table A1.

Graphical analysis of effects was performed, and the results obtained from the different models are plotted in Fig. 1 referring to DiBP (Fig. 1a, b), DBP (Fig. 1c,d), BBzP (Fig. 1e,f) and DEHP (Fig. 1g,h). In the plots on the left (Fig. 1a,c,e,g) the bar length is related to the effect of the change of level of each CMP on the CMA; each bar is related to a specific pair of levels. Hence, the bar named as b1/2-1 shows the entity of the effect

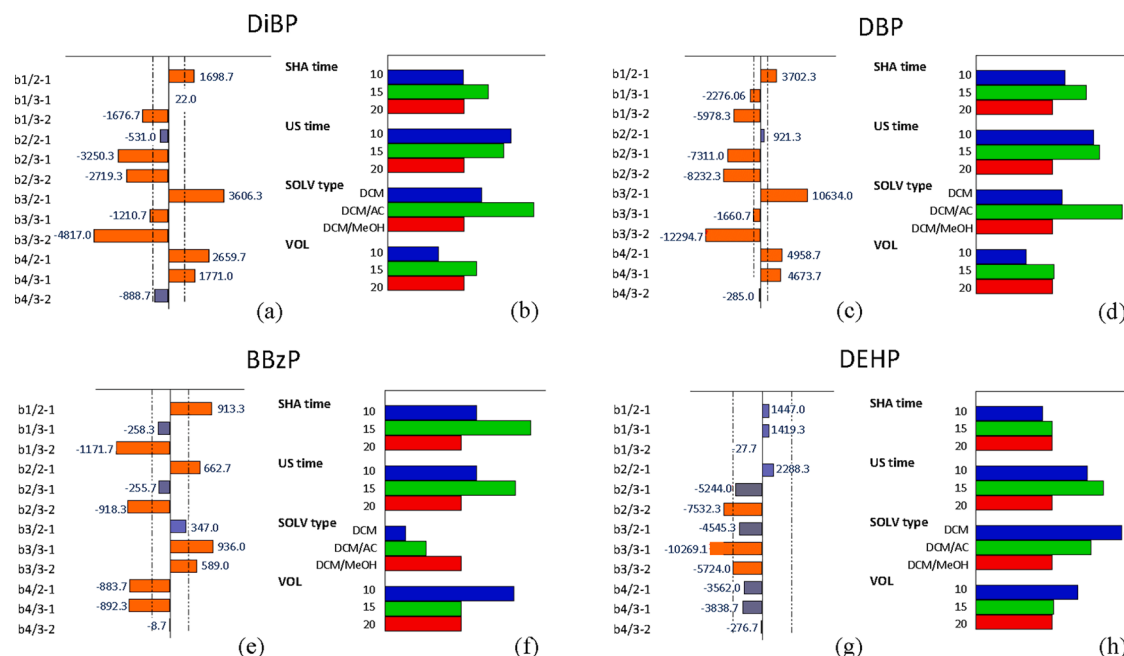


Fig. 1. Screening graphic analysis of effects for DiBP (a, b), DBP (c, d), BBzP (e, f), DEHP (g, h). In (a, c, e, g) plots the length of the bar is related to the entity of the effect produced by a change of level of the factors; the statistically significant effects are coloured in orange. In (b, d, f, h) plots the bar length is related to the effect of the single level on the response.

when changing level between the medium one (2) and the low one (1) of the first considered CMP, i.e. shaking time. In the same way, b1/3-1 refers to the effect observed when moving from high level (3) to low level (1) of the same CMP. In the plots on the right (Fig. 1b,d,f,h) the length of the bar is related to the effect of each single level of the CMPs (blue bar, low level; green bar, medium level; red bar, high level) on the CMA.

The analysis of the results reported in Fig. 1 allowed for the identification of the CMPs levels leading to a maximization of the CMAs. As for *SHA time*, the best level was the medium one for all the analytes, apart from DEHP, for which no significant effect was observed. Thus, this factor was set at 15 min (medium level) for RSM. As concerns *US time*, the graphs for all the analytes evidenced that the maximization of all the four CMAs was in general obtained at low-medium levels, consequently the new experimental domain to be studied in the RSM phase was moved towards low levels, corresponding to 8–15 min. As for *SOLV type*, it was observed that for DiBP and DBP the best results were obtained by using DCM/AC, whereas for DEHP the best solvents were either DCM or DCM/AC. Finally, the highest extraction for BBzP was obtained by using the DCM/MeOH mixture. On the basis of these findings, considering that DCM/AC was able to effectively extract the majority of the analytes, the DCM/AC mixture was selected as the best compromise for the simultaneous extraction of the investigated PAEs. As for *VOL*, increased peak areas were obtained at medium and high level for both DiBP and DBP, whereas low levels were required for BBzP extraction. The effect of this factor was not significant on DEHP. Since no common trend was observed for this CMP, the same experimental domain as in the screening was studied in the RSM phase. The experimental conditions to be investigated through RSM are summarized in Table A1.

3.2. Response surface methodology

To obtain detailed information on the effects of both *US time* and *VOL*, RSM was carried out. RSM makes use of optimization designs to generate tridimensional response surfaces or bidimensional contour plots which allow the dataset to be described and previsions of the response values to be made with the purpose of selecting the optimal conditions [33,47].

A second-order polynomial model was postulated linking the CMPs to the CMAs and investigating the presence of curvature. The following equation was hypothesized relating the response y (CMA) to the independent factors x_i (CMPs), where linear (β_i), interaction (β_{ij}) and quadratic (β_{ii}) coefficients are included, β_0 is the intercept and ε is the experimental error.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon$$

A CCD was used to estimate the coefficients of the model. This design is composed by 2^k Full Factorial Design points, $2k$ star points and n experiments at the center of the design. In this case, the factorial and the star points lied equidistant from the center, obtaining a circumscribed CCD [45,46]. The factors were studied at five levels ($-\alpha, -1, 0, +1, +\alpha$), where $\alpha=1.41$. Due to the low number of factors involved, the design was replicated for obtaining a reliable estimate of the experimental variance, for a total of eighteen experiments including two experiments at the center of the experimental domain. No mathematical transformation of the responses was required. Model refining led to negligible improvements in the quality parameters of the model, thus all the coefficients were retained, regardless of their statistical significance. All the four models resulted valid and significant by ANOVA ($p < 0.05$), apart from DiPB, whose model was not valid due the high reproducibility observed (DiPB reproducibility = 0.935). However, for DiPB, as for all the other responses, the quality parameters of the model (Table A3) were good [33]. The values of coefficient of determination R^2 ranged from 0.716 to 0.914, whereas the values of coefficient of predicted variation Q^2 were in the 0.39–0.814 range. Therefore, all the models could be used for drawing and investigating the contour plots.

The coefficient plots, which provide a graphical representation of both the weight and the significance of the model terms, are represented in Fig. A1. *US time* exerted a significant positive effect on DiBP and DBP, whereas *VOL* exerted a significant positive effect on all the responses, with a higher weight (longer bar) with respect to *US time*. Negative quadratic effects were found for DiBP (both for time and volume) and for DBP (volume). There was a significant *US time***VOL* positive interaction only for DEHP.

The contour plots obtained plotting *VOL* vs. *US time* are reported in Fig. 2. The best results for each CMA corresponded to the red-coloured

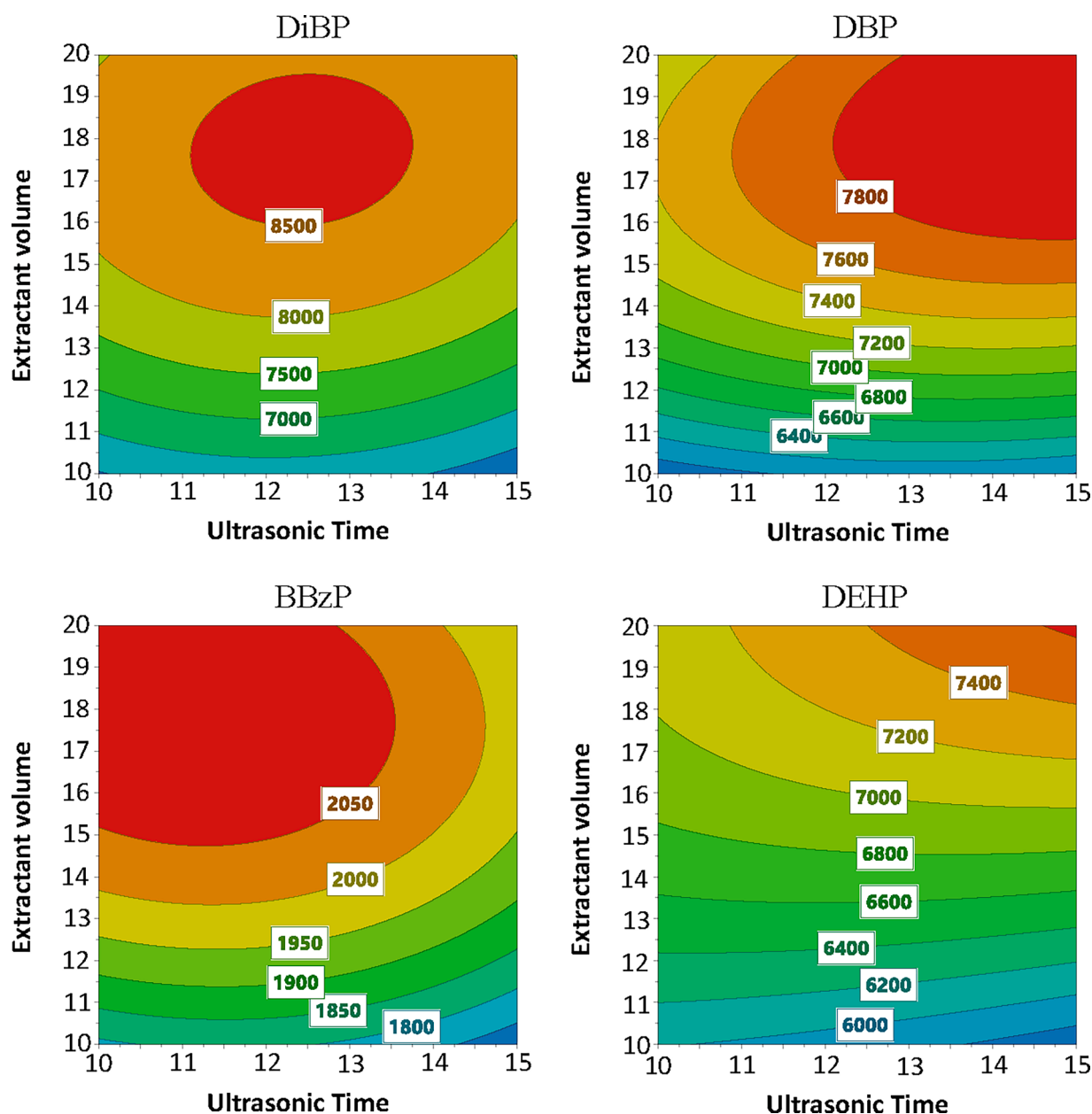


Fig. 2. RSM contour plots of the CMA obtained plotting volume of extractant vs. ultrasonic time.

zones, where the maximization of the responses was achieved. For all the CMA high values of VOL were preferred, whereas for *US time* different behaviors were observed, even if a medium value of this CMP could represent a good compromise among the different CMA.

This aspect was confirmed by observing the sweet spot plot shown in Fig. A2. This plot is built by overlaying all contour plots for individual CMA to define the area where all the required response criteria are met. The color scale, reported in the legend, makes it possible to distinguish the zone where all the CMA requirements are satisfied, highlighted in bright green and corresponding to the sweet spot, as well as the zones where three, two, one, or no CMA requirements are satisfied, coloured in green, teal, blue and white, respectively. The following minimum and target values were set for the CMA: DiBP, 7800–8000; DPB, 7300–7500; BBzP, 1900–2000; DEHP, 6800–7000. These values were fixed by considering the responses measured when running the CCD, in particular, the target values were approximately between the median and the 75% quartile of the measured results, to obtain a good compromise for all the CMA.

The MODR was established considering not only the predicted average values of the CMA but also the probability of fulfilling the CMA requirements, combining the information of calculated models and Monte-Carlo simulations [32]. The desired level of probability that the requirements for the CMA are met was set to 90%, corresponding to 10% risk of failure, thus meaning a target value for DPMO equal to 100,000 [32]. The model error was included in the predictions of response distributions and the risk of failure was plotted in the probability map shown in Fig. 3. The MODR was calculated from a robust set-point which corresponded to 13.1 min and 18.0 min for *US time* and VOL, respectively, and was represented by the green zone, included in the 10% isoprobability line. The MODR interval, reported in Table A1, was calculated as: *US time*, 10.5–15.0 min; VOL, 15.1–20.0 min. The MODR was validated by testing four verification points spanning the interval and verifying the satisfaction of the CMA targets. The working point was selected to reduce both the time required for the extraction and the amount of solvent used, limiting costs and environmental pollution. The following conditions were set: *US time*, 12.0 min; VOL, 17.5 mL.

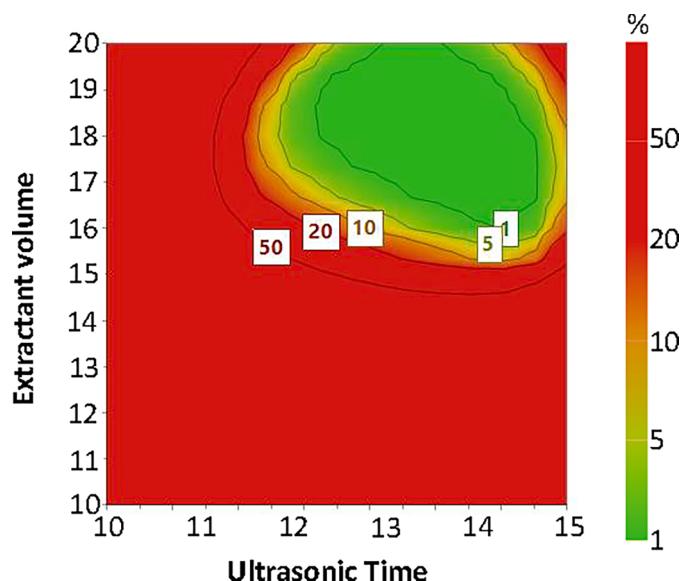


Fig. 3. Probability map by plotting ultrasonic time vs. volume of extractant. The risk of failure is represented; the MODR is coloured in green and is included in the 10% isoprobability line.

3.3. Method validation

The method was validated by operating under the optimized conditions. Validation was carried out considering some of the most used and harmful PAEs as target analytes: as previously stated, all the selected compounds are present in lists of substances of very high concern and widely found in the environment [48,49]. In addition to their inclusion in the US EPA priority pollutant list [50], DEHP, DBP, DIBP and BBzP have been recognized as compounds having endocrine disrupting effects to human health according to Regulation (EC) No 2021/2045 [51]. DEHP has been also identified as having endocrine disrupting properties for which there is scientific evidence of probable serious effects to the environment. Among the target analytes, DEHP, DBP, DOP, and BBzP were prohibited in childcare products; in addition, the use of BBzP, DBP, DEHP in the electrical and electronic equipment was banned since 2019 [52]. To extend the potential applications of the optimized method, DMP and DEP were also included into the validation study. Although these PAEs are not classified as carcinogenic, mutagenic, endocrine disruptors, or reproductive toxicant substances by REACH, they are present in the US EPA priority pollutant list [50]. Finally, due to the increasing use of phthalate-free alternatives like adipate in the plasticizer market and environment [6], DEHA was also investigated for potential environmental discharge due to its widespread use as a low-toxic phthalate-free alternative [53].

LOD and LOQ values in the 0.1–0.7 and 0.4–2 ng g⁻¹ range for

sediments, and in the 0.1–0.5 and 0.4–2 range for shells, were achieved (Table 1 and Table 2).

Linearity was assessed in the LOQ–150 ng g⁻¹ range for each analyte (Tables 1 and 2). As for precision, good results were obtained in terms of intermediate precision, with RSDs always lower than 20%. No significant difference among the mean values obtained over 3 days was observed by applying ANOVA ($p > 0.05$). Since no certificated reference material was available for PAEs in sediment, the trueness of the method was evaluated by spiking sediment and shells samples. Recovery rates -RR% in the 73(±7)–120(±10)% range and 70(±10)–111(±3)% range in sediment and shells respectively, were calculated at LOQ, 25 and 150 ng g⁻¹, thus assessing the efficiency of the developed method. A matrix effect in the 12–25% range was observed, thus confirming that matrix-matched calibrations have to be performed. Finally, a preconcentration factor of 12 was calculated. An example of a GC-MS chromatogram of a sediment sample spiked with the investigated analytes is shown in Fig. A3.

Overall, it can be stated that the LOD values achieved by the proposed method were either better or comparable with those reported in previous studies (Table 3). It has to be highlighted that the reported LODs and LOQs are lower compared to other UAE-based methods, obtaining limits similar to those achieved by using miniaturized sample extraction techniques (Table 3). Despite the preconcentration factor may not be remarkable, the proposed method offers several additional advantages including low costs, speed, and the availability of the instrumentation in all analytical labs. With respect to the traditional Soxhlet extraction, which is time- and solvent-consuming, UAE, MAE, and ASE can be considered as greener approaches, i.e. more time- and solvent-efficient techniques. As reported in Table 3, the proposed method is based on the use of 17.5 ml of extraction solvent, which is lower than those used in previous UAE-based methods for PAEs extraction [9,12,41,54]. Although MAE and ASE require lower solvent consumption with respect to UAE, concerns related to the cleaning of sample cells need to be considered, since potential cross contamination can occur, thus affecting the achievement of reliable results especially when trace analytes are performed [8,11]. UAE can be also considered an efficient technology in terms of energy consumption, since it does not require high temperature and pressure conditions. Another key point of this technique relies on its high versatility, allowing for the optimization of many extraction variables depending on the matrix and the targeted analytes.

3.4. Analysis of real sample

To demonstrate the feasibility and applicability of the proposed method, solid matrices (lithogenic particles + shells debris or live mollusc individuals, $n = 27$) from 9 different sedimentary environments of the Curonian Lagoon were analyzed. Samples and the obtained results are given in Tables 4 and A4.

Plasticizers were detected in all samples, indicating that PAEs are

Table 1

LODs, LOQs, regression coefficients, precision (RSDs%) and trueness (RR%) for sediment samples.

Analyte	LODs (ng g ⁻¹)	LOQs (ng g ⁻¹)	Regression coefficient* b(±s _b) Sediment	Repeatability (RSD%) Level (ng g ⁻¹)			Intermediate precision (RSD%) Level (ng g ⁻¹)			Trueness (RR%±SD) Level (ng g ⁻¹)		
				LOQ	25	150	LOQ	25	150	LOQ	25	150
DMP	0.2	0.8	1.72 (±0.02)	7	4	2	20	10	10	90±20	73±8	80±10
DEP	0.1	0.4	1.55 (±0.02)	8	2	2	20	20	10	100±20	80±10	78±9
DiBP	0.3	0.8	0.76 (±0.03)	3	3	2	9	9	5	120±10	92±8	85±4
DBP	0.2	0.7	0.76 (±0.02)	6	2	0.8	10	6	2	120±10	83±6	82±2
BBzP	0.7	2	1.90 (±0.04)	9	5	3	9	7	2	100±10	90±7	84±2
DEHA	0.1	0.5	1.96 (±0.04)	7	5	2	20	10	10	100±20	90±10	86±9
DEHP	0.2	0.6	0.71 (±0.01)	7	2	1	20	9	3	110±20	100±10	89±2
DOP	0.2	0.7	0.71 (±0.01)	6	4	4	10	3	5	100±10	87±3	88±4

* Regression equation: $y = b x$. Intercept not significant for all the analytes.

Table 2

LODs, LOQs, regression coefficients, precision (RSDs%) and trueness (RR%) for shell samples.

Analyte	LODs (ng g ⁻¹)	LOQs (ng g ⁻¹)	Regression coefficient* b(±s _b) Shell	Repeatability (RSD%) Level (ng g ⁻¹)			Intermediate precision (RSD%) Level (ng g ⁻¹)			Trueness (RR%±SD) Level (ng g ⁻¹)		
				LOQ	25	150	LOQ	25	150	LOQ	25	150
DMP	0.2	0.5	1.45 (±0.01)	10	6	1	20	10	2	70±10	84±5	78.0 ± 0.7
DEP	0.1	0.4	1.328 (±0.007)	20	9	2	10	7	2	90±20	90±20	87±2
DiBP	0.2	0.6	0.62 (±0.01)	10	4	4	10	3	2	77±8	90±4	96±4
DBP	0.1	0.4	0.667 (±0.003)	10	2	2	9	2	2	90±10	97±2	98±2
BBzP	0.5	2	1.82 (±0.01)	20	1	2	10	2	3	90±10	108±1	109±2
DEHA	0.4	1	1.77 (±0.01)	10	5	2	10	3	1	90±10	104±5	111±3
DEHP	0.1	0.4	0.693 (±0.006)	10	10	7	10	8	4	96±9	100±10	102±7
DOP	0.2	0.7	0.695 (±0.004)	3	2	0.3	3	2	1	89±2	98±2	100.9 ± 0.3

* Regression equation: $y = b x$. Intercept not significant for all the analytes.**Table 3**

Comparison of LOD values achieved in this work (sediment samples) with previous studies for determination of PAEs.

Refs.	Extraction method	Compounds	Solvent	Solvent volume (ml)*	LOD (ng g ⁻¹)
<i>This study</i>	UAE	7 PAEs + DEHA	DCM/AC (1:1)	17.5	0.1–0.7
[11] Reid et al., 2009	ASE	4 PAEs	DCM/AC (1:1)	NS	15
[13] Xu et al., 2008	UAE	DBP, DEHP	DCM/AC (1:1)	NS	10
[55] Huang et al., 2008	ASE	6 PAEs	Ethyl acetate	6	6–11
[56] Ramirez et al., 2019	MAE	6 PAEs + DEHA	MeOH	15	15
[10] Fernández-González et al., 2017	UAE + HS-SPME	6 PAEs	MeOH	5	1–79
[57] Liu et al., 2014	Soxhlet	16 PAEs	DCM	300	1.12–8.59
[58] Hassanzadeh et al., 2014	Soxhlet	DBP, DEHP	DCM	300	1 (DBP); 8 (DEHP)
[41] Zhang et al., 2017	UAE	8 PAEs	DCM	50	0.12–1.60
[12] Souaf et al. 2023	UAE	4 PAEs + DEHT	hexane/AC (1;1)	30	10–80
[9] Cao et al. 2022	UAE	15 PAEs	AC/hexane (10:1)	20	0.27–5.85
[54] Jiménez-Skrzypek et al., 2020	UAE + dSPE*	10 PAEs + DEHA	AC+DCM	22	LOQ: 0.020–4.0

* m-dSPE: micro-dispersive solid-phase extraction;

MAE: Microwave assisted extraction

ASE: accelerated solvent extraction

HS-SPME: headspace solid phase extraction

dSPE: dispersive solid phase extraction

NS: Not specified.

ubiquitous pollutants in the Curonian Lagoon. Regarding the compositional profiles of sediments, only 4 plasticizers were detected in the samples collected from the Curonian Lagoon. DEHP was the most predominant PAE and was observed in all samples, accounting for 66–93% of the total plasticizer amount. DiBP and DBP were the other predominant PAEs and were detected in 94% and 77% of the samples, respectively. However, their relative contribution to the total concentration of plasticizers ranged only from 0 to 26% and 0 to 8%, respectively, which is in line with the values observed in other contaminated areas [59]. These findings can be explained considering that DEHP is characterized by a high octanol-water partitioning coefficient (K_{ow}) compared to other PAEs (Table A2), thus indicating its tendency to be bound with particles

[4]. In previous years, PAEs, particularly DEHP, accounted for the vast majority of plasticizers used. However, due to health concerns and legal restrictions, numerous phthalate plasticizer manufacturers have converted to phthalate-free alternative compounds such as adipates or citrates, which now account for a significant part of the EU plasticizer market [6,60]. DEHA was detected only at Station A (Table 4) at a concentration level (6.5 ± 1.6 ng g⁻¹) lower than those of other PAEs identified at this station. However, its detection in the Curonian Lagoon sediments encourages the development of new monitoring programs including the evaluation of alternatives free-phthalate plasticizers.

Hydrophobic organic compounds such as PAEs are mainly accumulated in the organic matter fractions of particles [61], explaining the

Table 4Average concentrations (ng g⁻¹, $n = 3$) of PAEs in sediment samples collected in the Curonian Lagoon (SE Baltic Sea).

Station	Sediment Type	C _{org} (%)	Concentration ± SD (ng g ⁻¹)					DEHA	DEHP	DOP	Total
			DMP	DEP	DiBP	DBP	BBzP				
A	Sediment	15.10±0.09	<LOD	<LOD	12±3	< LOD	<LOD	6 ± 2	60±20	<LOD	80±20
B	Fine sediment	0.41±0.01	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	6 ± 2	< LOD	6 ± 2
B	Shells debris mix ^a	–	< LOD	< LOD	2.3 ± 0.5	0.7 ± 0.3	< LOD	< LOD	6 ± 1	< LOD	9 ± 2
C	Fine sediment	0.44±0.01	< LOD	< LOD	2.5 ± 0.1	1.5 ± 0.3	< LOD	< LOD	36±1	< LOD	40±2
C	Shells debris mix ^b	1.76±0.02	< LOD	< LOD	3.1 ± 0.4	1.9 ± 0.3	< LOD	< LOD	27.0 ± 0.7	< LOD	32±1
C	Gastropod shells debris	2.0 ± 0.1	< LOD	< LOD	2.3 ± 0.6	2.0 ± 0.8	< LOD	< LOD	20±1	< LOD	25±3
C	Unionid shells debris	4.61±0.03	< LOD	< LOD	2.6 ± 0.3	1.6 ± 0.4	< LOD	< LOD	40±6	< LOD	44±7
C	Zebra mussel shells debris	2.34±0.06	< LOD	< LOD	2.1 ± 0.3	1.5 ± 0.2	< LOD	< LOD	22.6 ± 0.5	< LOD	26.3 ± 0.9
C	Mussel shells ^c	9.16±0.03	< LOD	< LOD	3.1 ± 0.4	2.7 ± 0.5	< LOD	< LOD	73±9	< LOD	80±10

^a Mix of gastropod and mussel shells debris.^b Mix of gastropods (~50%), mussels (~45%), and unionid (~5%).^c Alive individuals; C_{org} – organic carbon content.

highest concentrations of PAEs in most organic loaded sediments (Station A, $C_{org} = 15.10 \pm 0.09\%$; PAEs: $80 \pm 20 \text{ ng g}^{-1}$). However, other factors such as the presence of shells of mollusc can facilitate the accumulation of organic pollutants in the benthic compartment. At Station C, a significant difference ($p < 0.001$) in concentration levels among the type of sediment and shells was observed. The shells of live zebra mussels had a significantly ($p < 0.001$) higher concentration of PAEs than the rest of the samples (sediments and shell debris), which may be explained by considering both the higher organic content of the shells, and the particle filtration of live mussels. Mussels, and in general benthic filter-feeders, are largely used for monitoring water quality as they can bioaccumulate the contaminants dissolved in water, sequestered in surficial sediment, or bound to particulates in the water column [62,63]. Therefore, the surface of shells can be an important factor for determining the accumulation of organic pollutants. As shown in Table 4, results showed that larger unionid shells had a significantly ($p < 0.05$) higher amount of PAEs ($44 \pm 7 \text{ ng g}^{-1}$) compared to smaller zebra mussels and gastropods ($26.3 \pm 0.9 \text{ ng g}^{-1}$ and $25 \pm 3 \text{ ng g}^{-1}$, respectively). As for the shell debris mix, it has to be noticed that unionid shells represented a small portion of the mixture ($\sim 5\%$ of the total weight), thus explaining the similar concentration between the shell debris mixture ($32 \pm 1 \text{ ng g}^{-1}$) and gastropod and mussel shells, which represented the 95% of remaining debris (Table A4). These findings suggest that the presence of shells debris or living molluscs in organic poor sediments can increase the potential retention of organic pollutants in environment depending on the type of shells.

In general, the total concentration of plasticizers in the analyzed samples ranged from $6 \pm 2 \text{ ng g}^{-1}$ to $80 \pm 20 \text{ ng g}^{-1}$. This range of concentration is relatively lower compared to that found in other regions of the world, especially in China, where concentrations in sediment often exceeds 500 ng g^{-1} and can reach over 2400 ng g^{-1} [10,64]. Although collected sediment samples are not necessarily representative of pollution level in the Baltic area, the ubiquity of PAEs in all samples encourage the development of further monitoring studies in estuarine systems such as the Curonian Lagoon.

4. Conclusions

The results obtained in this study demonstrate that a novel reliable UAE-GC-MS method is applicable for the simultaneous determination of different PAEs in sediment samples, with good precision, recovery and detection limits. The optimization of the UAE parameters was efficiently carried out by Quality by Design principles, making it possible to identify the MODR. For the first time in the literature QbD is applied for developing an extraction procedure in environmental analysis. The developed method allowed the simultaneous determination of 8 plasticizers that are rarely considered all together in monitoring programs. Among the investigated analytes, DEHP was present in all the samples collected from estuarine system. PAEs were also found attached to the shell debris or live mussels, highlighting them as a hotspot in organic poor sandy sediments. An important future perspective is the application of the developed methodology for the determination of PAEs in complex environmental matrices, including sediments with a different level of organic matter or shell debris, to improve results deriving from environmental monitoring strategies.

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.

Data availability

Data will be made available on request.

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Supplementary materials

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