Analytical Methods

Experimental design of ligandless sonication-assisted liquid- phases microextraction based on hydrophobic deep eutectic solvents for accurate determination of Pb(II) and Cd(II) from waters and food samples at trace levels

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ABSTRACT

A straightforward, accurate and efficient analytical procedure was developed by ligandless sonication-assisted liquid- phases microextraction based on hydrophobic deep eutectic solvents (SA-LPME-HDES) to trace toxic Pb(II) and Cd(II) in waters and foods. Optimization of the SA-LPME-HDES procedure was carried out by Box–Behnken design. Under optimum conditions, linear ranges for Pb(II) and Cd(II) were 0.8–350 (r^2:0.9962) and 1.5–500 µg L^{-1} (r^2: 0.9937), respectively. Relative standard deviations (N = 5, 10 µg L^{-1}) were 1.4% for Pb (II) and 1.6% for Cd(II), respectively. Limits of detection were 0.24, and 0.46 µg L^{-1}, respectively. The accuracy was evaluated by the analysis of two certified reference materials and the results were to be in agreement with the certified values. The SA-LPME-HDES method was successfully applied to tap water, mineral water, river water, well-water, sesame, peanut, eggplant, corn, wheat, soy and cucumber. The SA-LPME-HDES method allows operational simplicity, green, and low cost when compared with some microextraction procedure.

1. Introduction

Heavy metals are the most important factor causing environmental and soil pollution (Zwolak, Sarzyńska, Szpyrka, & Stawarczyk, 2019). Cadmium (Cd) is one of the most important of heavy metals that pose vital threats on the food chain and it causes serious problems in plants (Sarwar et al., 2010). Cd contaminates soils due to phosphorus fertilizers, industrial wastes, sewage wastes and even urban traffic (Yang et al., 2004). Extensive use of lead (Pb) in industrial and agricultural activities, it is a frequently encountered element in the environment (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Pb pollution in soil, water and air is increasing due to various anthropogenic activities (Saleem, Asghar, Zahir, & Shahid, 2018).

Different analytical techniques such as flame atomic absorption spectrometry (AAS) (Altunay, Hazer, Tuzen, & Elik, 2021), liquid chromatography with UV detection (Werner, 2018), inductively coupled plasma mass spectrometry (Chen, Yan, Li, & Lu, 2019), neutron activation analysis (Greenberg et al., 2011), energy dispersive X-ray fluorescence spectrometry (Meira, Almeida, Dias, & Teixeira, 2019),

graphite furnace atomic absorptions spectrometry (Maleki et al., 2019) and inductively coupled plasma optical emission spectroscopy (Li et al., 2018) have been used for the determination of Pb and Cd in various samples. Among these techniques, FAAS is an analytical device that can be found in many analytical laboratories due to its relatively simplicity, ease of use, cheapness and accuracy. Due to matrix interactions and low analyte concentrations in samples, direct determination of elements at trace levels with this technique is limited. In order to overcome these problems, many sample preparation procedures such as precipitation (Koosha, Shamsipur, Salimi, & Ramezani, 2020), solid-phase microectraction (Tuzen, Elik, Hazer, Şimşek, & Altunay, 2020), dispersive liquid–liquid microextraction (Şaylan, Zaman, Bakırdere, & Bakırdere, 2020), ultrasound-assisted ionic liquid microextraction (Hafez, Sheikh, Sayqal, AlMasoud, & Gouda, 2020) and cloud point extraction (Kasa, Sel, Chormey, & Bakırdere, 2019) have been reported before FAAS determination.

The recent trend in sample preparation studies has focused on the optimization of miniaturized and automated green methods that provide the desired analytical performance characteristics (Miró, & Hansen,

2012). In this context, sample preparation studies for the preparation of effective, green and cheap extraction solvents have become widespread. The new type of alternative solvent that has attracted considerable attention in recent years is deep eutectic solvents (DES). DESs are formed as a result of eutectic mixtures of two or three components, which are biodegradable and green, with high ability to form intermolecular hydrogen bonds (Safavi, Ahmadi, & Ramezani, 2018). DESs have important properties such as low vapor pressure, low toxicity, reusability, non-flammability, high thermal stability, and the ability to dissolve a wide variety of inorganic and organic compounds (Faraji, 2019). Unlike ionic liquids, surfactants and organic solvents, DESs exhibit easy synthesis and purification, biocompatibility and low cost. Therefore, in order to expand the application of DESs in aqueous solutions and increase the sensitivity of analytical procedures, the development of DESs can be achieved by the use of less DES volume and the elimination of dispersant or emulsifier. Because of these properties, DES have been used for the extraction of other heavy metals including As, Sb, Sn and Hg (Osowska, & Ruzik, 2019; Altunay, Elik, & Gürkan, 2019; Plastiras, Andreasidou, & Samanidou, 2020).

Recently, chemometric-assisted optimization of analytical methods provides a theoretical basis for processes and creates mathematical models that evaluate the importance and interactions of independent variables (Taşpınar, Elik, Kaya, & Altunay, 2021). This approach saves time, reagents and experimental study, identifies more important variables and determines the combination of variable levels that achieve the best analytical performance (Stalikas, Fiamegos, Sakkas, & Albanis, 2009).

The aim of the present investigation is to optimize the ligandless sonication-assisted liquid- phases microextraction based on hydrophobic deep eutectic solvents (SA-LPME-HDES) method with Box–Behnken design (BBD) design to ensure preconcentration of Pb (II) and Cd (II) ions from waters and foods, and then quantify analytes via FAAS. HDES-2 (L-Menthol/DoDecA) and sonication effect for analyte ions were used as extraction solvent and dispersing agent, respectively. After the validation studies, the optimized SA-LPME-HDES method was investigated in terms of some interfering ions and successfully applied in the determination of Pb (II) and Cd (II) in the selected sample. According to the other study, one of the most important innovations of the current study is that it does not require the use of ligands to ensure preconcentration. This is achieved by the DESs we used in this study.

2. Materials and methods

2.1. Reagents and solutions

All reagents used in the optimization step were of analytical reagent grade and were used without further purification. The standard solutions of Pb(II) and Cd(II) at 1000 mg L^{-1} were prepared by dissolving an appropriate amount of their nitrate salts (Merck, Darmstadt, Germany) in water. Working solutions were prepared by diluting the stock solution in water. DL-Menthol, (Sigma, St. Louis, Missouri, USA), L-Menthol (Sigma), decanoic acid (Merck, DecA), butyric acid (Merck, BuA), dodecanoic acid (Sigma, DoDecA), oleic acid (Merck, OleA), oxalic acid (Merck, OxaA), tetra-*n*-butylammonium bromide (Sigma, [N4444]Br), methyltrioctylammonium chloride (Sigma, [N8881]CI) and thymol (Merck) were used to prepare hydrophobic deep eutectic solvents. The pH of the aqueous solutions was adjusted in the range of 3.0–8.0 with Britton-Robinson universal buffer solution. All plastic and glass materials were kept permanently full of diluted HNO$_3$ solution (Sigma) before experimental studies.

2.2. Apparatus

A Shimadzu AAS-6300 model flame atomic absorption spectrometer (FAAS) (Kyoto, Japan), equipped with a D$_2$ lamp for background correction and air-acetylene flame was used for determination of Pb(II)

and Cd(II) ions. For lead and cadmium, hollow cathode lamps operating at 10 mA and 4 mA, respectively, were used as the radiation source. The other measurement parameters of FAAS have been set as recommended by the manufacturer. A SK5210LHC Kudos (Shanghai, China) ultrasonic bath was used for emulsion formation and the preparation of hydrophobic deep eutectic solvents. The pH-2005 model digital pH-meter (JP Selecta, Barcelona, Spain), centrifuge (320 model universal Hettich, London, England) and microwave system (Milestone Ethos, Italy) were used to adjust the pH of the solutions, achieve phase separation and digest the samples, respectively.

2.3. Sample collection and preparation

2.3.1. Water samples

Wastewaters was collected from the organized industrial zone in Sivas, Turkey. Tap water was collected in our laboratory, while river water was collected from the Kızılırmak river in Sivas. Mineral water and Bottled water were purchased from local markets in Sivas. All water samples were firstly filtered through a 0.45 μm filter and then kept at 4 °C prior to microextraction.

2.3.2. Food samples

In the study, soil taken from 0 to 20 cm depth was used and the soil is slightly alkaline (pH 7.51), low organic matter content (1.07%), sandy loam, calcareous (12.6%), low phosphorus concentration (322 kg ha^{-1}) and potassium concentration sufficient (1055 kg ha^{-1}). A plastic pot with a capacity of 3 kg was used in the study, which was carried out in 3 replicates according to the randomized plot design. In the study, sesame, peanut, eggplant, maize, wheat, soybean and cucumber were used as test plants. 100 N mg kg^{-1} (as CaNO$_3$·4H$_2$O) each pot for sesame, peanut, soybean plants, 200 N mg kg^{-1} (as CaNO$_3$·4H$_2$O) for each pot for eggplant, maize, wheat, cucumber plants, 100P mg kg^{-1} and 125 K mg kg^{-1} (as KH$_2$PO$_4$), 2.5 Zn mg kg^{-1} (as ZnSO$_4$·7H$_2$O) were applied to each pot for all plants. In the study, three different Cd and Pb doses were applied as 0 mg Cd kg^{-1}, 0.05 mg Cd kg^{-1}, 0.01 mg Cd kg^{-1} (in the form of CdSO$_4$), 0 mg Pb kg^{-1}, 0.05 mg Pb kg^{-1}, 0.01 mg Pb kg^{-1} (in the form of Pb(NO$_3$)$_2$) as respectively. Approximately 55 days after planting, plants were harvested. After the plants whose roots have been cut are washed in the laboratory with tap water and distilled water two times, and then they were dried at 70 °C for 48 h until they reached constant weight. Then, after grinding in an agate mill, 0.200 g were taken from the samples and burned in a microwave device (Milestone Ethos Easy Advanced Microwave Digestion System model, Italy) with 2 mL of 35% H$_2$O$_2$ and 5 mL of 65% HNO$_3$. Blank solutions were prepared exactly like samples, except that no analyte ions was added.

2.4. Preparation of HDESs

Generally, the most common procedure for preparing HDES involves mixing two or three ingredients and heating them until a homogeneous liquid is formed. These components consist of donor hydrogen compound (HBD), acceptor hydrogen compound (HBA) and, if necessary, water. In the preparation of HDES, components with low solubility in water are used. The method previously reported was used in the preparation of the HDES in this study (Makoś, Słupek, & Gębicki, 2020). DL-Menthol, L-Menthol, [N8881]CI, [N4444]Br and thymol were used as HBA, while DecA, DoDecA, BuA, OleA and OxaA were used as HBD. After mixing these components in an ultrasonic bath at the appropriate molar ratio, five different HDES were prepared by sonication application in range of 30–70 °C and 10–60 min. Three repetitive applications of the prepared HDESs were carried out for the recovery of the Pb(II) and Cd (II). Combination and codes of prepared HDESs and application results were given in Supplementary Data Table S1.

2.5. Experimental design

The optimization step and statistical analysis were carried out by a design of experiment (DOE) model with aid of Design-Expert® trial version 12.0.1. (Stat-Ease Inc., Minneapolis) to study the interaction between the important microextraction factors including HDES-2 vol (A), sonication time (B), extraction temperature (C), and pH (D). The DoE approach has reduced the time required to reach optimum conditions compared to the one factor approach at a time. Also, the model helped to predict microextraction factors and critical experimental variables in any condition. In this context, a five-factor and three-level Box–Behnken design (BBD) was established. The levels, units and symbols of the microextraction factors in the optimization model based on BBD were given in Supplementary Data Table S2.

2.6. Optimized SA-LPME-HDES procedure

According to the microextraction conditions optimized by the BBD, the SA-LPME-HDES procedure was carried out as follows: Food samples prepared (10 mL) by microwave digestion and filtered water samples (10 mL) were added to 15 mL-conical centrifuge tubes containing 50 µg L^{-1} Pb (II) and Cd (II) ions. The pH of the aqueous solution was then adjusted to pH 6.7 with BR buffer solution, and 375 µL of HDES-2 was added to the mixture solution. Subsequently, the tubes were placed in an ultrasonic bath and sonication was applied for 8 min at 35 °C to ensure the formation of microspheres, which were very useful for both the homogeneous distribution and the subsequent easy and rapid phase separation. At this stage, Pb(II) and Cd(II) ions were extracted into extraction solvent (HDES-2). Analytes of interest after centrifugation (3000 rpm 2 min) were collected into the enriched HDES-phase. After decantation of the aqueous portion, the analytes ions in the enriched HDES-phase were analyzed using FAAS. The experimental steps described were applied to the samples blank.

2.7. Calculation of extraction recovery

Extraction recovery (%ER) was evaluated as a reference to determine the appropriate values of extraction parameters in optimization studies. ER % was calculated according to the formula below.

$$ER\% = \left[C_{final} V_{final} / C_{Initial} V_{Initial} \right] \times 100 \tag{1}$$

where V_{final} and $V_{Initial}$ were the initial volume and final volume, respectively. C_{final} was the amount of Pb(II)/Cd(II) ions in the measurement phase after the optimized SA-LPME-HDES procedure, $C_{Initial}$ was the amount of Pb(II)/Cd(II) ions in the initial sample solution before the optimized SA-LPME-HDES procedure

2.8. Validation assays

Validation assays including calibration equation, linear dynamic range, correlation coefficient (r2), limit of detection (LOD), limit of quantification (LOQ), accuracy, precision (as relative standard deviation, RSD%), enhancement factor (EF), robustness and matrix effect were investigated using optimum conditions obtained via BBD design in replicate studies.

A linear regression analysis on the corresponding of the absorbance of Pb(II) and Cd(II) was performed using the triplicate injections of a series of the model solutions of Pb(II) and Cd(II) at different concentrations.

LOD and LOQ were calculated according to the formulas below.

$$LOD = 3 \times s_{blank} / m \tag{2}$$

$$LOQ = 10 \times s_{blank} / m \tag{3}$$

where s_{blank} was the standard deviation of the sample blank, and m was

the slope of the calibration curves. The numbers 3 and 10 were statistical constants at the 95% confidence level.

$$EF = m_1 / m_2 \tag{4}$$

where m_1 and m_2 were the slopes of calibration curves before and after the optimized SA-LPME-HDES procedure, respectively.

The precision of the method was investigated by repeatability and reproducibility studies. In this context, 3 different concentrations (5, 25 and 100 µg g^{-1}) of the Pb(II) and Cd(II) ions were added to the prepared samples. In the repeatability, five repeated extractions and analysis studies of each concentration were carried out in one day, whereas in the reproducibility, three repeated extractions and analysis studies of the same concentrations were carried out during consecutive five days.

$$RSD(\%) = X_{average} / s \times 100 \tag{5}$$

where, $X_{average}$ was the average of the values found in triplicate analysis, while s was the standard deviation of the analysis results.

The accuracy of an analytical method was expressed as the proximity of the values found experimentally with the reference value (accepted as conventionally true). These studies were usually done with reference materials. Therefore, the accuracy of the SA-LPME-HDES procedure was evaluated by the analysis of two certified reference materials (CRM) including INCT-TL-1-tea leaves and 1643e Trace elements in water. INCT-TL-1-tea leaves and 1643e Trace elements in water were purchased from Institute of Nuclear Chemistry and Technology and the National Institute of Standards and Technology, respectively.

Robustness of the method was investigated by measuring the effect of small and deliberate changes in the microextraction parameters on the extraction of Pb(II) and Cd(II) ions. The small changes parameters that were taken into consideration were pH, HDES-2 amount, sonication time, and extraction temperature. Other extraction parameters were kept constant while changing the controlled parameter throughout the experimental studies. Then, the RSD% of the extraction recovery of Pb (II) and Cd(II) ions were calculated for each parameter.

Since optimization studies are carried out on model solutions, different anionic or cationic species in real samples may affect microextraction studies. Therefore, to evaluate the matrix effect of the method, varying amounts of different chemical species were added to the model solutions and then the tolerance limit for the relevant chemical species was calculated using the SA-LPME-HDES procedure. An error of 5.0% in the absorbance reading was considered tolerance limit. The tolerance limit was found from the formula below.

$$\text{Tolerance limit} = [\text{interference ions amount}] / [\text{analyte amount}] \tag{6}$$

2.9. Statistical analysis

Student *t*-test and two-pair ANOVA analysis were applied for the statistical evaluation of validation studies. Calculations for the analytical data obtained were performed using the Excel 2016 (Microsoft Office®) computer program. The results obtained were expressed as mean ± SD, and were considered significantly different at p < 0.05.

3. Results and discussion

3.1. Selection of suitable HDES for experimental design

Before the optimization approach, the appropriate HDES should be selected. Some important features to be considered in the selection of HDES are as follows. (1) The HDES should effectively disperse the analyte ions in the sample solution. (2) The HDES must have a high dispersive strength and should not dissolve in sample solution. (3) It should be economical, environmentally friendly as possible, have low vapor pressure as well as high extraction coefficient. Based on these disclosures, HDES-1 (DL-Menthol/DecA), HDES-2 (L-Menthol/DoDecA),

HDES-3 ([N8881]CI/BuA), HDES-4 ([N4444]Br/OleA) and HDES-5 (Thymol/OxaA) were investigated as extraction solvent to effectively extract the Pb(II) and Cd(II) ions to the enriched phase. According to the results in Supplementary Data Table S1, the extraction efficiency of Pb (II) ions was HDES-2 (91.2%)>HDES- 1(87.5%)> HDES-3 (75.8 %)> HDES-5 (65.7 %)> HDES-4 (59.1 %), while extraction efficiency of Cd (II) was HDES-2 (94.7%)> HDES-4 (82.6 %)> HDES-1 (78.4 %)> HDES-3 (62.1 %)> HDES-5 (56.4 %). Based on these results, HDES-2 was chosen as the extraction solvent for optimization approach.

3.2. Box–Behnken design results

3.2.1. ANOVA analysis

The ER % values obtained for Pb(II) and Cd(II) ions as a result of the application of the BBD design were shown in Supplementary Data Table S2. ANOVA results for Pb (II) and Cd (II) ions from the statistical evaluation of these results were shown in Table 1a. The p-value must be<0.05 for the independent variables to be significant for the optimization step. When the related table is examined, the p-values (<0.0001) of the model established for both Pb (II) and Cd (II) were lower than

Table 1b
Results on important validation parameters for the optimization step.

Analytes	R^2	Adjusted R^2	Predicted R^2	Adeq Precision	Std. Dev
Pb(II)	0.9955	0.9910	0.9889	64.4793	1.01
Cd(II)	0.9918	0.9835	0.9726	47.5843	1.38

1070.22) and C^2 (F-value: 644.48), respectively. In addition, Lack of Fit F-value/p-value of 0.1096/0.9977 and 0.2892/0.9492 for Pb(II) and Cd (II), respectively, indicate that the lack of fit is not significant to pure error. The accuracy and precision of the optimized model are decided by looking at the R-values (see Table 1b). As these values approach 1, the predictive power of the model increases. The R^2, adjusted-R^2, and predicted-R^2 values for Pb(II) were 0.9955, 0.9910 and 0.9889, respectively, while the same values were 0.9918, 0.9835 and 0.9726 for Cd(II), respectively. These values indicate that the optimized model for both analyte ions has high accuracy and good precision. In addition, these results confirm that there is a high agreement and correlation between experimental and predicted results. As a result, the quadratic equations obtained for Pb(II) and Cd(II) were given below.

$$ER\% \text{ for } Pb(II) = +64.62 + 5.29A + 6.00B - 1.23C - 1.76D - 7.35AB - 7.82AC - 1.75AD - 1.48BC - 6.07BD - 8.65CD + 4.52A^2 - 0.8392B^2 + 12.96C^2$$
$$+ 7.42D^2 ER\% \text{ for } Cd(II)$$
$$= +60.24 + 5,08A + 5.68B - 1.12C - 1.74D - 7.35AB - 8.22AC - 1.15AD - 1.40BC - 4.85BD - 8.68CD + 5.14A^2 - 0.9450B^2$$
$$+ 13.75C^2 + 7.62D^2$$

0.05, which indicates that the optimization model is significant. Additionally, B^2 (p-value: 0.0525) interaction was not significant for Pb(II), while AD (p-value: 0.1178), BC (p-value: 0.0619) and B^2 (p-value: 0.1030) interactions were not significant for Cd(II). F-values are used to evaluate the contributions to the optimized model. The higher the F value numerically, the greater its contribution to the model. In the light of this explanation, it can be said that the variables that contribute the most to the optimized model for Pb(II) and Cd(II) are C^2 (F-value:

3.2.2. Response surface plots

The 3D response surface plots obtained for Pb(II) and Cd(II) were shown in Figs. 1a and 1b, respectively. These plots were drawn to evaluate the effect of binary interactions on the ER% of the Pb(II) and Cd (II). All binary interactions were significant for ER% of Pb (II) ions. But,

Table 1a
ANOVA results obtained for Pb (II) and Cd (II) as a result of the application of BBD.

Source	For Pb(II)						For Cd(II)					
	Sum of Squares	df[a]	Mean Square	F_{value}[b]	p_{value}[c,d]	prob > F	Sum of Squares	df[a]	Mean Square	F_{value}[b]	p_{value}[c,d]	prob > F
Model	3146.97	14	224.78	220.78	<0.0001	significant	3208.21	14	229.16	120.34	<0.0001	significant
A	336.02	1	336.02	330.04	<0.0001		309.07	1	309.07	162.30	<0.0001	
B	432.00	1	432.00	424.31	<0.0001		387.60	1	387.60	203.55	<0.0001	
C	18.25	1	18.25	17.93	0.0008		14.96	1	14.96	7.86	0.0141	
D	37.10	1	37.10	36.44	<0.0001		36.40	1	36.40	19.12	0.0006	
AB	216.09	1	216.09	212.24	<0.0001		216.09	1	216.09	113.48	<0.0001	
AC	244.92	1	244.92	240.56	<0.0001		270.60	1	270.60	142.10	<0.0001	
AD	12.25	1	12.25	12.03	0.0038		5.29	1	5.29	2.78	0.1178	
BC	8.70	1	8.70	8.55	0.0111		7.84	1	7.84	4.12	0.0619	
BD	147.62	1	147.62	144.99	<0.0001		94.09	1	94.09	49.41	<0.0001	
CD	299.29	1	299.29	293.96	<0.0001		301.02	1	301.02	158.08	<0.0001	
A^2	132.72	1	132.72	130.35	<0.0001		171.54	1	171.54	90.08	<0.0001	
B^2	4.57	1	4.57	4.49	0.0525		5.79	1	5.79	3.04	0.1030	
C^2	1089.62	1	1089.62	1070.22	<0.0001		1227.24	1	1227.24	644.48	<0.0001	
D^2	357.44	1	357.44	351.08	<0.0001		376.39	1	376.39	197.66	<0.0001	
Residual	14.25	14	1.02				26.66	14	1.90			
Lack of Fit	3.07	10	0.3066	0.1096	0.9977	not significant	11.19	10	1.12	0.2892	0.9492	not significant
Pure Error	11.19	4	2.80				15.47	4	3.87			
Cor Total	3161.23	28					3234.87	28				

[a] Degrees of freedom.
[b] Test for comparing model variance with residual (error) variance.
[c] Probability of seeing the observed F value if the null hypothesis is true.
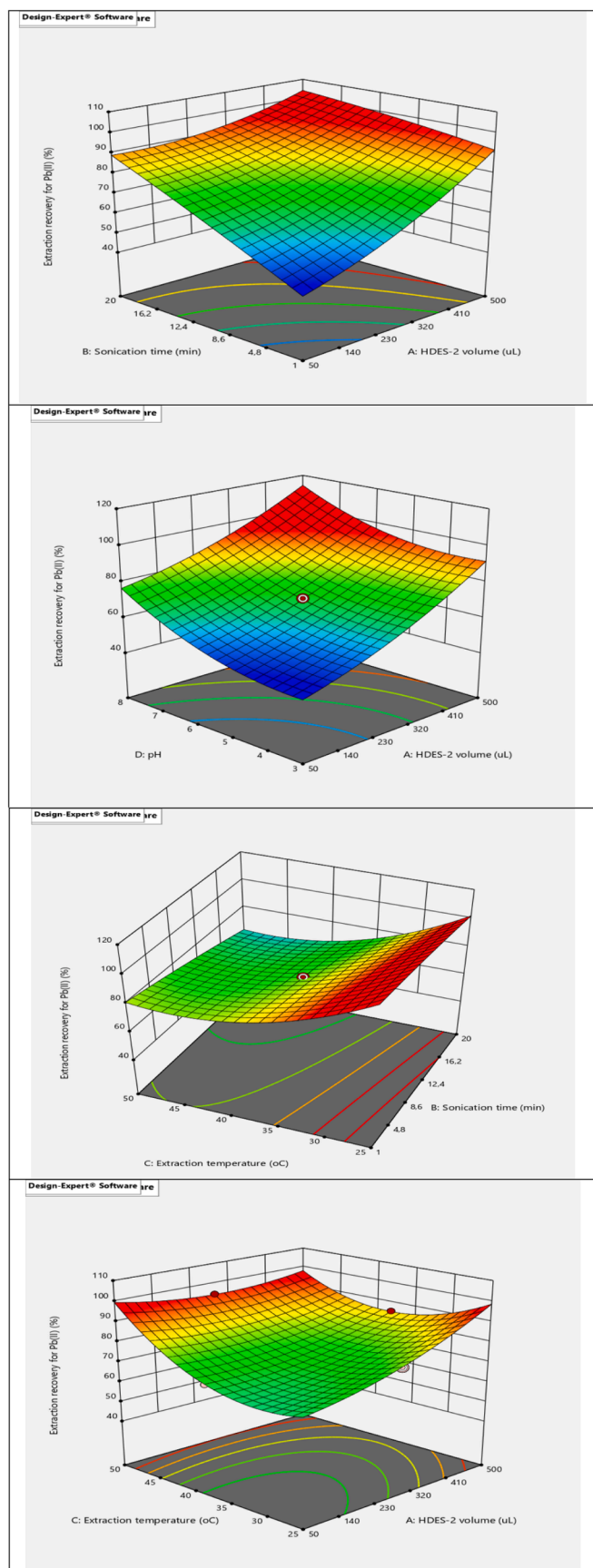
Fig. 1a. 3D graph obtained by BBD design for the optimization of Pb(II).

the HDES-2 vol* sonication time, HDES-2 vol * extraction temperature, sonication time* pH and extraction temperature * pH interactions were significant for ER% of Cd (II) ions. From Figs. 1a and b, it can be seen that the ER% of Pb(II) and Cd(II) ions decreases, especially in the basic region. This may be due to the analyte ions being hydrolyzed. In addition, phase separation could not be achieved in the acidic regions due to possible protonation of the extraction solvent. In addition, the ER% of Pb(II) and Cd(II) ions has decreased due to the increased instability of the extraction solvent at high temperatures. At low temperatures, the microsphere formation required for the extraction solvent to be active could not be achieved. Consequently, low extraction recovery was obtained for Pb(II) and Cd(II) ions. Sonication was applied as a dispersing agent to effectively disperse the extraction solvent in the sample solution. From the relevant Figures, it is seen that approximately 8 min of sonication time is sufficient for quantitative ER% of both analytes.

3.2.3. Optimization

After determining the quadratic equations for Pb (II) and Cd (II) ions, the BBD design suggested optimum values of the variables studied (viz. 375 μL of HDES-2, pH 6.7, 8 min of sonication time and 35 °C of extraction temperature) for the best ER% of Pb (II) and Cd (II) ions at a desirability level of 1.0. As a result of the three-replicate study, the ER% of Pb (II) and Cd (II) ions was 96.5% and 94.8%, respectively. These values were compatible with the values predicted by the BBD design. These results showed that the selected optimum values are suitable for further experimental studies.

3.3. Validation results

Liner dynamic ranges obtained for Pb(II) and Cd(II) were 0.8–350 μg L^{-1} (r^2:09962) and 1.5–500 μg L^{-1} (r^2:09937), respectively. The LOD values were 0.24, and 0.46 μg L^{-1} for Pb(II) and Cd(II), respectively. In addition, LOQ values for Pb(II) and Cd(II) were 0.8 and 1.5 μg L^{-1}, respectively. EFs were 158 and 0163 for Pb(II) and Cd(II), respectively. Other analytical results were presented in Table 2(a).

The repeatability and reproducibility were in the ranges 1.5–2.9% and 1.8–3.3% for Pb(II) ions, respectively. In addition, the repeatability and reproducibility were in the ranges 2.0–3.4% and 2.4–3.8% for Cd(II) ions, respectively. From the results obtained, it can be said that the optimized SA-LPME-HDES method has good precision.

The results of the study for the accuracy of the method are presented in Table 2(b). The findings obtained showed that the experimentally found values for both Pb and Cd are consistent with the certified values. As a result of the analysis of CRMs, RSD and recovery values for Pb(II) were between 1.9 and 2.3% and 98.3–99.1%, respectively, while the same values for Cd(II) were between 2.0 and 4.5% and 96.7–98.8%, respectively. In addition, the t_{exp} (1.09–1.68) obtained from the analysis of both CRMs were found less than t_{crit} (2.13) at the 95% confidence level. These results confirm that Pb(II) and Cd(II) can be accurately determined by the optimized SA-LPME-HDES method.

As a result of the robustness tests, the RSD% of Pb(II) ion for pH, HDES-2 amount, sonication time, and extraction temperature were 1.9%, 1.7%, 2.3%, and 2.0%, respectively, while the RSD% of Cd(II) for the same parameters were 1.6%, 2.4%, 2.6% and 1.9%, respectively. As a result, low RSD% showed the robustness of the optimized SA-LPME-HDES method.

The results of the study for the matrix effect was presented in Supplementary Data Table S3, show good selectivity of the optimized SA-LPME-HDES method toward these interference ions. In addition, quantitative recoveries (91–99%) and high tolerable limits (5000–100) indicate that the SA-LPME-HDES method has a low matric effect.

3.4. Real sample analysis

Following the optimization and validation steps, the applicability of the method was investigated by applying it to different waters and food samples. Preparation of these examples is described in Section 2.3. The
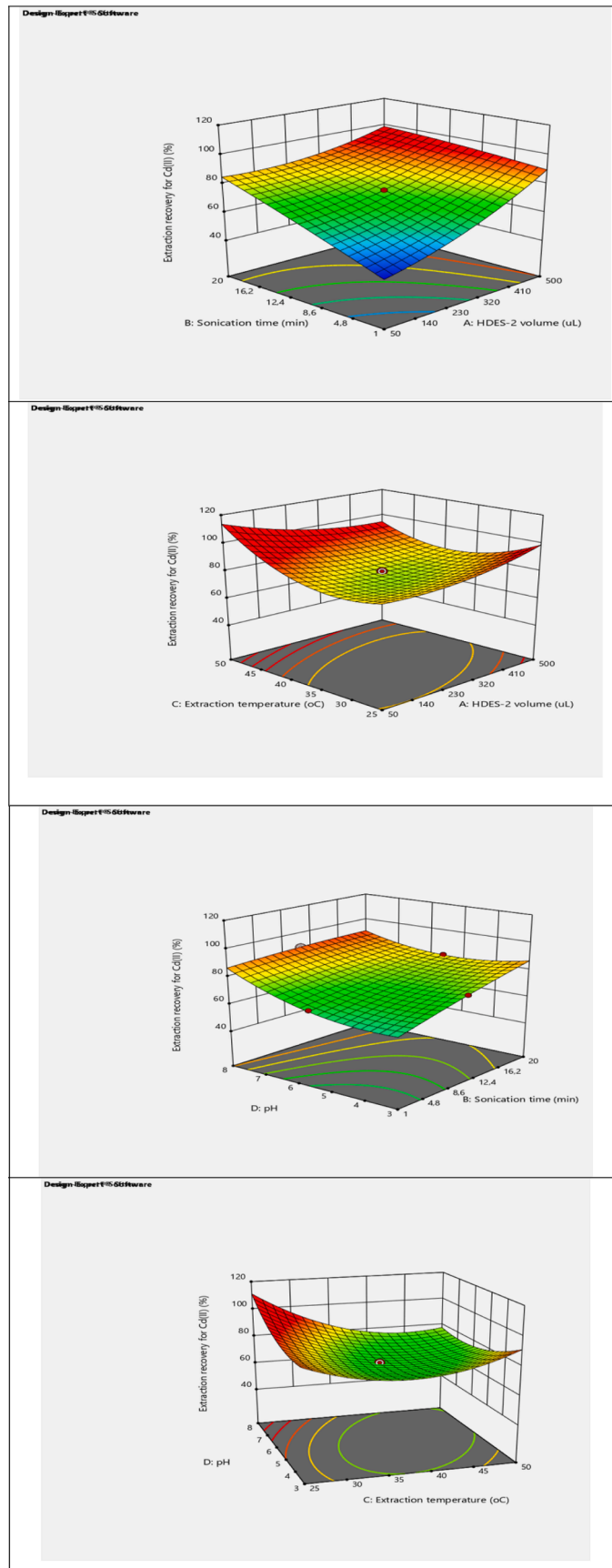
Fig. 1b. 3D graph obtained by BBD design for the optimization of Cd(II).

Table 2a
Analytical characteristics of the optimized SA-LPME-HDES method.

Parameter	For Pb(II)	For Cd(II)
Calibration equation	$A = 0.0022 + 0.0061$[Pb (II) μg L^{-1}]	$A = 0.0038 + 0.0019$ [Cd (II), μg L^{-1}]
Linear dynamic ranges (μg L^{-1})	0.8–350	1.5–500
Correlation coefficient (r^2)	0.9962	0.9937
Limit of detection (μg L^{-1})	0.24	0.46
Limit of quantification (μg L^{-1})	0.8	1.5
Average Recovery (%)	98.1	97.4
RSD% (n = 5, for 10 μg L^{-1})	1.4	1.6
Enhancement factor	158	163

Table 2b
Result of applying the optimized SA-LPME-HDES method to two CRMs (95% confidence interval; t-critical: 2.13. N = 5).

CRMs	[a,b]Certified value	[a,b]Found	Recovery (%)	RSD (%)	t_{-exp}
	For Pb(II)				
INCT-TL-1-tea leaves	1.78 ± 0.24	1.75 ± 0.04	98.3	2.3	1.68
1643e Trace elements in water	19.63 ± 0.21	19.45 ± 0.37	99.1	1.9	1.09
	For Cd(II)				
INCT-TL-1-tea leaves	0.030 ± 0.004	0.029 ± 0.0015	96.7	4.5	1.49
1643e Trace elements in water	6.568 ± 0.073	6.489 ± 0.129	98.8	2.0	1.37

[a]mg kg^{-1}; [b] μg L^{-1}.

analysis results of the water samples are given in Table 2(c). In this study, two-level standard solutions (100 and 200 μg L^{-1}) of Pb(II) and Cd(II) ions were added to the water samples and recovery test was applied. From the results in Table 2(b), the recovery values for Pb(II) and Cd(II) varied between 96.5 and 103.4% and 96.4–102.5%, respectively. In addition, RSD values ranged from 1.7 to 3.3% for Pb(II) and 1.5–3.6% for Cd(II). Both Pb(II) and Cd(II) ions could not be detected in tap water, mineral water and bottled water. Lead was at the level of 1.2 and 15.6 μg L^{-1} in river water and wastewater, respectively, while cadmium was at 1.9 and 25.7 μg L^{-1}, respectively.

The optimized SA-LPME-HDES method was applied after a level standard addition (μg g^{-1}) to food samples prepared by microwave digestion. As a result of the application, Pb(II) could not be detected in wheat, Cd(II) in peanut. Moreover, the highest lead (20.1 ± 1.8 μg g^{-1}) and cadmium (19.5 ± 1.5 μg g^{-1}) contents were detected in soy and eggplant, respectively. Recovery values for Pb(II) and Cd(II) were in the range 95.8–102.7% and 92.8–103.2%, respectively. Comprehensive results were presented in Table 2(d).

3.5. Comparison of the optimized SA-LPME-HDES method with other methods

In order to better evaluate the contribution of the optimized SA-LPME-HDES method to the literature, some analytical parameters of the method

Table 2d
Analysis of real samples using SA-LPME-HDES/FAAS.

Samples	[a]Spiked Pb(II) and Cd(II)	Pb		Cd	
		[a]Found	Recovery (%)	[a]Found	Recovery (%)
Sesame	–	11.5 ± 0.3	–	3.2 ± 0.1	–
	50	60.1 ± 1.2	97.2	49.6 ± 1.7	92.8
Peanut	–	3.6 ± 0.1	–	n.d*	–
	50	52.8 ± 1.8	98.4	47.3 ± 2.2	94.6
Eggplant	–	7.2 ± 0.9	–	19.5 ± 1.5	–
	50	58.6 ± 2.1	102.7	67.9 ± 3.1	96.8
Corn	–	13.8 ± 1.0	–	7.1 ± 0.4	–
	50	61.7 ± 2.5	95.8	58.7 ± 2.6	103.2
Wheat	–	n.d*	–	3.9 ± 0.9	–
	50	48.2 ± 2.0	96.4	52.1 ± 2.2	96.4
Soy	–	20.1 ± 1.8	–	13.4 ± 1.8	–
	50	71.0 ± 3.3	101.8	61.2 ± 3.6	95.6
Cucumber	–	9.5 ± 0.5	–	6.9 ± 0.02	–
	50	58.3 ± 1.9	97.6	58.4 ± 2.9	102.8

[a] μg g^{-1}.

Table 2c
Determination of Pb (II) and Cd(II) in different water samples and recovery test.

Samples	[a]Spiked Pb(II) and Cd(II)	Pb(II)			Cd(II)		
		[a]Found	RSD (%)	Recovery (%)	Found	RSD (%)	Recovery (%)
Tap water	–	*n.d	2.4	–	*n.d	1.9	–
	100	97.3	2.1	97.3	98.1	1.6	98.1
	200	195.2	1.7	97.6	197.4	1.5	98.7
Mineral water	–	*n.d	2.6	–	*n.d	2.7	–
	100	96.5	2.3	96.5	96.4	2.3	96.4
	200	196.2	2.0	98.1	195.8	2.1	97.9
Bottled water	–	*n.d	3.1	–	*n.d	2.8	–
	100	98.3	2.8	98.3	98.4	2.6	98.4
	200	197.6	2.4	98.8	198.0	2.3	99.0
River water	–	1.2	2.6	–	1.9	1.9	–
	100	103.1	2.1	101.9	99.5	1.7	97.6
	200	203.6	1.9	101.2	200.1	1.7	99.1
Wellwater	–	15.6	3.3	–	25.7	3.6	–
	100	119.0	2.9	103.4	128.2	3.0	102.5
	200	220.0	2.5	102.2	228.3	2.6	101.3

[a]μg L^{-1}, * could not be detected.

were compared with previously reported microextraction and detection techniques. It can be seen from Supplementary Data Table S4 that the optimized SA-LPME-HDES method had quantitative recovery, lower LODs and relative low RSDs than the reported analytical approaches. The working range of the method is comparable even to effective techniques, especially ICP-OES and GF-AAS. EF values obtained for analytes in the microextraction step were better than for other microextraction techniques reported. In addition, the use of a small number of chemicals at trace levels in the application of the optimized SA-LPME-HDES method is an important step in both cost and environmental terms. One of the important features that distinguishes the optimized SA-LPME-HDES method from other studies is the simultaneous separation and pre-concentration of Pb(II) and Cd(II) ions without using ligand.

4. Conclusion

In summary, hydrophobic deep eutectic solvents were prepared and used as extraction solvent in SA-LPME for the extraction and pre-concentration of Pb(II) and Cd(II) prior to FAAS determination. The extraction behavior of target ions into extraction solvents has been investigated in detail. The applied sonication accelerates the formation of nano-sized fine droplets that increase the contact surface area between the extraction solvents and Pb(II)/Cd(II) ions. The optimization step was carried out by a Box–Behnken design with minimum number of experiments. The optimized procedure is green, simple and requires a small volume of extraction solvent. Working ranges of 0.8–350 and 1.5–500 $\mu g\ L^{-1}$ were obtained for Pb(II) and Cd(II) ions, respectively. Enhancement factor were in the range of 158–163. R^2, adjusted-R^2 and predicted-R^2 values, which are a measure of reliability in the optimization step, were in the range of 0.9995–0.9898 and 0.9918–0.9726 for Pb(II) and Cd(II) ions, respectively. RSD values for 10 $\mu g\ L^{-1}$ concentration of Pb(II) and Cd(II) ions were 1.4% and 1.6%, respectively. Quantitative recovery (96-4-103.4%) from spiked samples demonstrated the suitability of the optimized method for the quality control of the analyzed samples. Validation of the optimized method was confirmed by analysis of the certified reference materials. As a result, the optimized method is perfectly useful for routine analysis of a variety of samples containing traces of Pb(II) and Cd(II) ions. Also, it is a technique that can be applied with simple equipment in analytical laboratories.

CRediT authorship contribution statement

Adil Elik: Investigation, Conceptualization. **Ahmet Demirbaş:** Investigation, Writing - review & editing. **Nail Altunay:** Supervision, Investigation, Validation, Writing - original draft, Writing - review & editing.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.131138.

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