Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-022-23942-5

RESEARCH ARTICLE

Evaluation of blood lead levels in opium addicts and healthy control group using novel deep eutectic solvent based dispersive liquid–liquid microextraction followed by GFAAS

Toraj Ahmadi Jouybari[1] · **Hadi Ahmadi Jouybari**[2] · **Fatemeh Hosseini**[1] · **Mohsen Nesari**[1] · **Nazir Fattahi**[3]

Received: 29 June 2022 / Accepted: 28 October 2022

Abstract

Today, drug dealers and sellers add lead compounds to these substances to get more profit. As a result, drug users are heavily exposed to lead, and lead poisoning is clearly seen in most of them. Therefore, it is especially important to check the blood lead levels in these people. In this research, an efficient and eco-friendly pretreatment method was established by deep eutectic solvent for dispersive liquid–liquid microextraction (DES – DLLME) followed by graphite furnace atomic absorption spectrometry (GFAAS) analysis. The selected hydrophilic deep eutectic solvent consists of *l*-menthol and (1S)-(+)-camphor-10-sulfonic acid (CSA) at a 5:1 molar ratio as a green solvent instead of traditional toxic organic solvents. Under the optimal extraction conditions, the introduced method exhibited good linearity with coefficient of determination (r^2) 0.9975 and an acceptable linear range of 0.3–80 μg L^{-1}. Accordingly, the detection limit was 0.1 μg L^{-1} (S/N = 3) for lead ions, and the high enrichment factor (240) was obtained. The proposed method was successfully applied to analysis lead ions in real blood samples, which is a promising technique for biological samples. The case samples were classified and analyzed based on age, duration of consumption, and type of substance. The results showed that there was no significant difference between blood lead levels in different age groups and different duration of use, while blood lead levels were higher in opium residue (shireh) users than in opium users.

Keywords Liquid–liquid microextraction · Eutectic solvent · Lead · Blood analysis · Drug addicts

Introduction

One of the most important heavy metals that exists both naturally and due to industrial and chemical activities in the environment is lead, which causes acute and chronic poisoning (Mostafazadeh et al. 2017; Farnia et al. 2021). Industrial wastewater, lead pigment paints, fossil fuels, lead in gasoline, contaminated food, and soil are the most important sources of lead, which is readily absorbed through organs such as the skin, respiratory tract, and gastrointestinal tract. Of course, it is necessary to mention that lead exists in two organic and inorganic forms. The inorganic form of lead is not absorbed through the skin (Kushwaha et al. 2018; Nakhaee et al. 2021; Champion et al. 2022). Muscle aches, headaches, constipation, irritability, myalgia, anorexia, decreased libido, and decreased concentration are the effects of lead poisoning (Farnia et al. 2022; Malik et al. 2022; Khoshnamvand et al. 2021; Gautam et al. 2015). Lead contamination occurs during contact with contaminated sources and food consumption, but in recent years, occupational health strategies have drastically reduced the rate of poisoning. However, new types of non-occupational poisoning have become prevalent in various societies and have created many problems (Soltaninejad et al. 2011).

Responsible Editor: Ester Heath

✉ Nazir Fattahi
nazirfattahi@gmail.com

[1] Clinical Research Development Center, Imam Khomeini and Dr. Mohammad Kermanshahi and Farabi Hospitals, Kermanshah University of Medical Sciences, Kermanshah, Iran

[2] Infectious Disease and Tropical Medicine Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

[3] Research Center for Environmental Determinants of Health (RCEDH), Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

Lead-contaminated drug use is one of the most important non-occupational resources that has recently been introduced in some countries, including Iran (Khatibi-Moghadam et al. 2016). Lead poisoning from contaminated heroin or intravenous injections has been reported since 1989 (Daglioglu et al. 2021). Traditional Indian herbal medicines, opium, processed cannabis, and methamphetamine are reported to be important sources of lead contamination (Karrari et al. 2013). Illegal opium may be combined with various substances such as strychnine, paracetamol, and heavy metals such as lead and thallium (Ghaderi et al. 2015). Sellers and traffickers may add heavy metals to opium to increase its weight and subsequently make more profit (Karrari et al. 2012; Azadi et al. 2022). Addition of lead into opium can cause an important health problem in Iran, especially in Kermanshah, and growing reports of lead poisoning due to drug abuse are alarming (Ghaemi et al. 2017). Due to the high prevalence of drug users in Kermanshah (Moradi et al. 2015) and the confirmation of more lead than expected in 65–85% of opium samples detected in this province (Ahmadi-Jouibari et al. 2020) as well as non-specific clinical manifestations due to poisoning with lead, which in some cases is confused with other health consequences and leads to unnecessary clinical and diagnostic processes that are sometimes invasive and can have their own side effects, checking the blood lead levels in drug addicts seems necessary.

In previous researches, several methods of analysis, including flame atomic absorption spectrometry (FAAS) (Zaman et al. 2020), inductively coupled plasma–optical emission spectrometry (ICP–OES) (Rehan et al. 2018), laser-induced breakdown spectroscopy (LIBS) (Varotsis et al. 2021), graphite furnace atomic absorption spectrometry (GFAAS) (Ahmadi-Jouibari et al. 2020), and inductively coupled plasma mass spectrometry (ICP–MS) (Terán-Baamonde et al. 2018), have been applied for the detection of lead ions in various matrices.

Sample preparation plays a significant role in purifying matrix interference and enrichment of target compounds prior to instrumental analysis. Therefore, an effective extraction step is indispensable to obtain the highest accuracy in determining of trace-level lead ions in biological samples. Liquid–liquid extraction (LLE) (Comitre and Reis 2005), solid-phase extraction (SPE) (Mehdinia et al. 2017), cloud point extraction (CPE) (Wang et al. 2016), co-precipitation [20], solid-phase microextraction (SPME) (Liu et al. 2020), liquid–phase microextraction (LPME) (Borahan et al. 2019), and dispersive liquid–liquid microextraction (DLLME) (Shamsipur et al. 2014; Martínez et al. 2018) are widely used in the extraction of lead ions in various samples. Hence, it is indispensable to find a less toxic and environmentally friendly green solvent. In recent years, new green solvents such as deep eutectic solvents (DESs) have received great attention in the field of green analytical chemistry in recent years. DES includes hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), which are bound by hydrogen bonding at suitable temperatures and specific proportions (Shakirova et al. 2022; Akramipour et al. 2018; Golpayegani et al. 2022). Low cost, simple synthesis process, and good biocompatibility are important advantages of DES compared to ionic liquids and toxic organic solvents. These advantages have led to DESs being widely used as an alternative to conventional organic solvents in the extraction and preconcentration of various organic and inorganic compounds (Ahmadi-Jouibari et al. 2022; Fattahi et al. 2022).

The main purpose of the current study was to develop a novel deep eutectic solvent based dispersive liquid–liquid microextraction followed by GFAAS for the extraction and determination of lead in blood of drug addicts and healthy control group. A new DES was successfully synthesized, and the optimal conditions were determined combine with DLLME method. As far as we know, it is the first report that for extraction and detection of lead in blood based on DES assisted DLLME combined with GFAAS. Another goal of this research was to investigate the blood lead level in the studied cases based on age, duration of consumption, and type of substance consumed. The results of the present study can be a basis for further research in this field and a warning to organizations related to the fight against drugs. Also, for clinical and basic science groups that are somehow related to lead poisoning and related diseases, it can be used in making better decisions in the direction of diagnosis and treatment of related diseases.

Experimental

Reagents and standards

All chemicals and reagents including *l*-menthol, sodium chloride (NaCl, analytical grade), acetonitrile (AN, HPLC grade), acetone (AC, suprasolv), methanol (MeOH, for spectroscopy), HNO_3 (65%, suprapure), $Pb(NO_3)_2$ (analytical grade), the chelating agent, diethyl dithiophosphoric acid (DDTP) with the density of 1.17 kg L^{-1}, and mixture of $Mg(NO_3)_2$ (300 mg L^{-1}) and Pd $(NO_3)_2$ (1000 mg L^{-1}) as chemical modifiers were obtained from Merck company (Darmstadt, Germany). The (1S)-(+)-camphor-10-sulfonic acid (CSA) was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Stock standard solution of Pb (1000 mg L^{-1}) was produced by dissolving of $Pb(NO_3)_2$ in ultra-pure water.

Apparatus

The Pb concentration was detected by a Model nov AA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany), combined with auto-sampler MPE-60, deuterium lamp for background correction, and transversely heated graphite tube atomizer. Analytik Jena pyrolytic graphite-coated tubes and argon (99.999%, Air Products, UK) were used. The instrument working conditions and temperature program are shown in Table S1. The lead hollow cathode lamp (Analytik Jena, Jena, Germany) was run under the conditions which recommended by the manufacturer (Table 1).

Sampling and sample preparation

Among the people referring to the centers of addiction treatment clinics in Kermanshah who are opium users, 45 people were included in the study by convenience sampling method, and among the companions or family members of the case, one person with no history of substance abuse was included in the study as a control. Finally, at least 45 people in each group (case and control) and a total of 90 samples were included in the study. The inclusion criteria of the case group included people who had a history of using opium and opium residue. Exclusion criteria also included groups with chronic illnesses, people with a history of other drug use, and people undergoing treatment.

Sample preparation is done as follows: in a 15-mL test tube containing 500 µL HNO_3 (65%) and H_2O_2 (30%) mixture at a 3:1 volume ratio, 200 µL of plasma sample, 100 µL of 15%(w/v) zinc sulfate solution, and 70 µL of acetonitrile are added and vortexed for 15 min. Then, the test tube maintained at 5 °C for 5 min followed by centrifugation at 4500 rpm for 3 min. The supernatant is transferred to another clean test tube and increased to 10 mL with distilled water to reduce the effects of the matrix (Akramipour et al. 2020). The obtained solution is subjected to the presented method.

Table 1 Blood lead level of opium addicts based on group, age, type of substance, and time of consumption

Variable	Variable type	*N*	BLL (µg L^{-1})	*P* value
Group	Case	45	214.6 – 341.2	0.011
	Control	45	85.4 – 128.5	
Age (year)	20 – 40	16	214.6 – 307.1	0.058
	41 – 60	22	296.5 – 330.7	
	˃ 60	7	244.2 – 341.2	
Type of substance	Opium	21	214.6 – 266.3	0.030
	Opium residue (shireh)	26	278.1 – 341.2	
Duration of consumption (year)	3 – 10	9	214.6 – 297.5	0.165
	11 – 20	20	264.8 – 316.4	
	˃ 20	16	274.3 – 341.2	

DES preparation

DES was prepared by simply mixing of *l*-menthol and (1S)-(+)-camphor-10-sulfonic acid (CSA) at a 5:1 molar ratio on a thermostatic magnetic at 40 °C for 30 min until a clear and transparent was formed without any precipitate. After the reaction, the synthesized DES was cooled to room temperature and stored for further use. The resulting DES was employed as extractant in DES – DLLME. In the previous report (Fattahi et al. 2022), the formation of *l*-menthol:CSA at molar ratio of 5:1 was studied, and the DES is characterized using Fourier transform-infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) (Fattahi et al. 2022).

Statistical evaluation

Results were presented as mean ± SD or median and quartile range for numerical variables and number (percentage) for categorical variables. To assess the differences in subject characteristics, a *t*-test or Chi-squared test was used as appropriate. Normality assumption was assessed using the D'Agostino test. To guard against normality violation of data and also extreme outliers, robust one-way ANOVA was used to make comparisons between groups (Wilcox 2011).

DES – DLLME procedure

Firstly, a volume of 10 mL of ultra-pure water (spiked at the level of 5.0 µg L^{-1} of lead) or sample solution was placed in a test tube, and 0.50 mL of acetone (disperser solvent) containing 50.0 µL of DES (extraction solvent) and 80.0 µL of DDTP (chelating agent), were injected rapidly into a test tube. Instantly, a cloudy solution was formed in the test tube resulting from the dispersion of the fine droplets of DES in the sample solution. Subsequently, the lead ions formed complexes with the DDTP and were simultaneously extracted into droplets of DES. After centrifugation at 4500 rpm for 2 min, the dispersed fine droplets of DES were floated at the top of the test tube. The density of DES is lower than water, and it collects on the surface of the aqueous solution during centrifugation. The test tube was transferred into a freezer for DES solidification. The resulting DES was transferred into a sample cup, and 20 µL of this phase using an auto-sampler was injected into the GFAAS (Akramipour et al. 2018; Ahmadi-Jouibari et al. 2022; Borahan et al. 2019). The temperature program and other instrumental parameters are illustrated in Table S1.

Results and discussion

Selection of HBA:HBD molar ratio

The molar ratio of the components of the DES is the most important parameter in the synthesis of the DES. To achieve this, different *l*-menthol:CSA molar ratios were used (1:1, 2:1, 3:1, 4:1, 5:1, 6:1, and 7:1). As illustrated in Fig. 1A, the extraction efficiency of the lead ions increases with increasing the ratio of *l*-menthol:CSA from 1:1 to 4:1. The extraction recovery in the molar ratio of 4:1 to 6:1 is not statistically different. The extraction efficiency decreases when the molar ratio of *l*-menthol increases again. Therefore, a molar ratio of 5:1 was selected as the best ratio for the synthesis of DES, because the extraction efficiency is slightly higher and it has a better standard deviation.

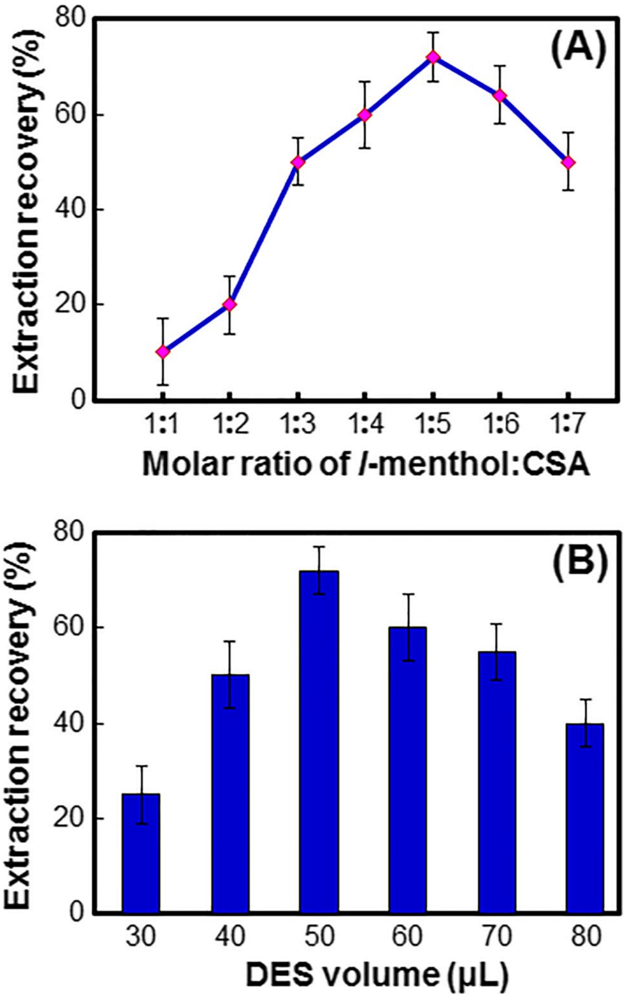

Fig. 1 Effect of the molar ratio of HBA to HBD (**A**) and volume of DES (**B**) on the extraction recovery of the lead ions

Selection of DES volume

The volume of DES also plays a significant role in the extraction efficiency of the target analytes. Less volume of DES will cause high enrichment factor of the target analytes with small impact on the environment. However, insufficient DES volume may also result in incomplete extraction of target analytes or incomplete collect of DES. In this work, the volumes of 30, 40, 50, 60, 70, and 80 μL of DES as extractant were selected. The results are shown in Fig. 1B. The maximum extraction efficiency was obtained for the lead ions detected after extraction with 50 μL of DES. Therefore, 50 μL of DES was selected for subsequent tests.

Selection of type and volume of disperser solvent

The main criterion for selecting the disperser solvent is its miscibility in the aqueous sample and dissolves the DES. For this purpose, various solvents with this property such as acetone, acetonitrile, methanol, and THF were used. A series of experiments were studied by using 0.50 mL of each disperser solvent containing 50.0 μL of DES (extraction solvent) and 80.0 μL of DDTP (chelating agent). The enrichment factors obtained for acetone, acetonitrile, methanol, and THF were 240.0 ± 18.5, 235.2 ± 19.8, 227.4 ± 21.3, and 244.6 ± 22.5, respectively. The results show no statistical significant differences between disperser solvents; however, less toxicity and cheaper acetone make it a better choice.

The effect of disperser solvent volume on extraction efficiency was also investigated. Changing the volume of the disperser solvent causes a change in the volume of the extraction phase. Therefore, to keep the volume of the extraction phase constant, the volume of acetone and DES was changed simultaneously. For this purpose, different volumes of acetone 0.25, 0.50, 0.75, 1.0, 1.25, and 1.50 mL containing 30.0 μL of DDTP and different volumes of DES were used. According to the results, when volumes of 0.25 and 0.50 mL of disperser solvent are used, there is no significant change in the extraction efficiency. As the disperser solvent volume increases by more than 0.5 mL, the extraction efficiency decreases slowly. Increasing the volume of acetone increases the solubility of the complex in water and decreases the extraction efficiency. Thus, 0.50 mL of acetone was selected as the optimum.

Selection of sample solution pH

The formation of the complex and its extraction depends on the pH of the sample solution. Because the ligand used in this study has its own acidic properties, in order to study the effect of pH, this ligand must first be converted to its salt. Therefore, DDTP was converted to the salt of DDTP ammonium using NH_3, and the pH effect upon the formation of lead complex was investigated in the pH range of 1–7, using HCl and CH_3COONa. According to Fig. 2A, the absorbance of target analytes in the pH range of 1–4 is almost constant and shows the highest value, and with further increase of pH, the absorbance decreases. Because the DDTP ligand (chelating agent) itself has a strong acidic property, it forms a complex in acidic pHs, and the molecular form of the complex is preserved. As the pH of the environment increases, the formed complex is ionized, and its solubility in the aqueous solution increases, and the extraction efficiency decreases. The pH of the ligand itself in solution is close to 2; therefore, no acid or secondary buffer was used to adjust the pH.

Effect of the DDTP concentration

Sufficient ligands must be used to ensure high extraction of lead ions. DDTP is a suitable and somewhat specific ligand for lead ions, and its chelating ratio is 2:1. To study the effect of the concentration of DDTP, experiments with various concentration of DDTP were done over the range of 0.02 to 0.20% (v/v). Figure 2B exhibits that when the concentration of DDTP was up to 0.08% (v/v), lead ions will be extracted with the highest efficiency, and with the further increase of the concentration of DDTP, the extraction efficiency had no significant increase. It seems that slight reduction of absorbance in high concentration of DDTP is due to the extraction of DDTP itself, which can easily saturate the small volume of extraction solvent. Therefore, 0.08% (v/v) concentration of DDTP was chosen as optimum amount.

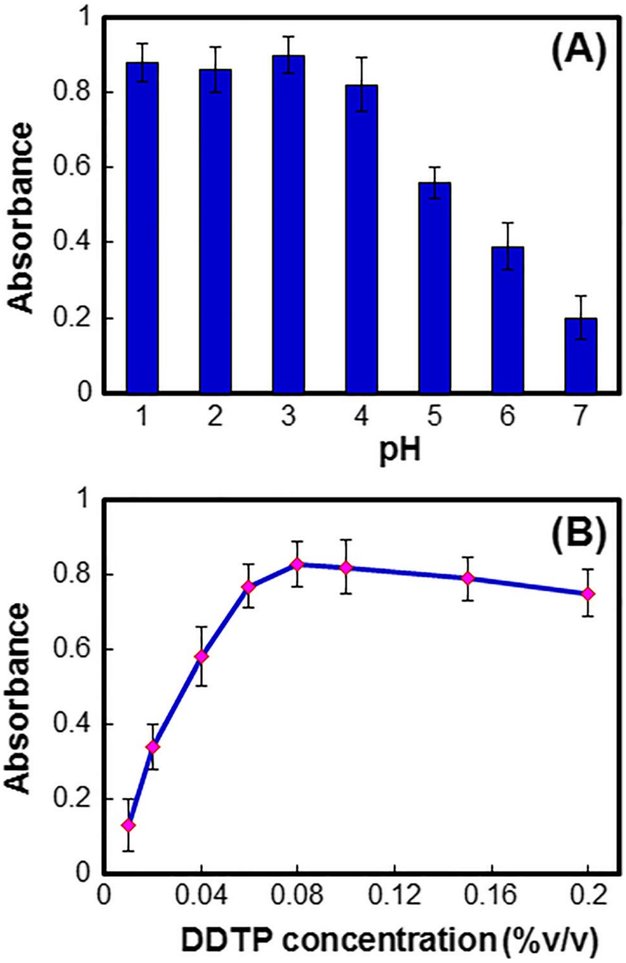

Fig. 2 Effect of the sample solution pH (**A**) and concentration of DDTP (**B**) on the absorbance of the lead ions

Effect of the ionic strength

To study the effect of ionic strength on the performance of DES–DLLME, various tests were done by adding different concentration of NaCl in the range of 0–10% w/v. Based on the results shown in Fig. 3, with increasing concentration of NaCl from 0 to 5%, the extraction efficiency remains almost constant. Because the ionic strength of the solution reduces the solubility of the analytes in the aqueous solution and increases the transfer efficiency of the analytes to the organic phase (salting-out), conversely, increasing the concentration of NaCl reduces the solubility of DES and increases the volume of the obtained organic phase and the dilution effect occurs. It is quite clear that “salting-out” effect happens with the increase of salt. This means that the increase of salt decreases the solubility of organic compounds in water. Although the DES has a low solubility in water, but with the increase of salt, this partial solubility also decreases, and the volume of the collected DES increases slightly. These two inverse effects keep the extraction efficiency constant. By increasing the concentration of NaCl more than 5%, the dilution effect prevails on salting-out, and the extraction efficiency decreases. Therefore, no salt was added in subsequent experiments.

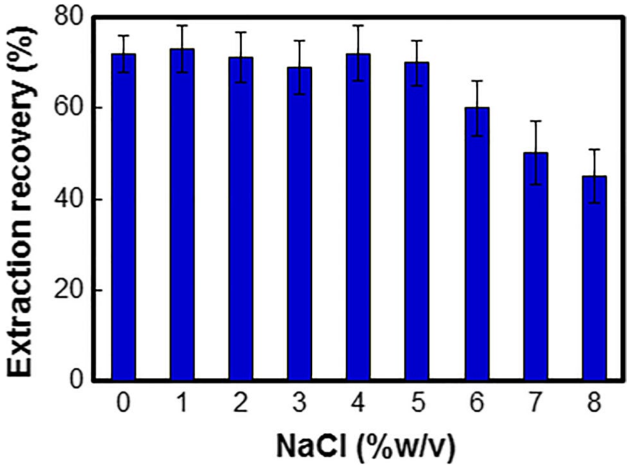

Fig. 3 Effect of the salt addition on the extraction recovery of the lead ions

Effect of the coexisting ions

The proposed working method was used to extract lead ions in the presence of common coexisting ions. To do this, 10 mL of 5.0 μg L^{-1} lead solution in the presence of various concentrations of coexisting ions was extracted by DES–DLLME procedure and analyzed by GFAAS. Any ion that changes the extraction recovery of lead ions by more than ± 5% is known as an interfering ion. Table S2 shows tolerance limits of the interfering ions.

Quantitative analysis

The specificity, limit of detection (LOD), linearity (LDR), precision, accuracy, extraction recovery (ER), and enrichment factor (EF) were evaluated to demonstrate the practical application of the method in the optimal conditions. The results are listed in Table S3. A good linear relationship was obtained in the range of $0.3 - 80$ μg L^{-1} with a correlation coefficient (r^2) of 0.9975, and the linear regression equation was $y = 34.03x + 18.65$. To calculate the LOD, the blank test is repeated 7 times, and its standard deviation is obtained (SD). Then, the calibration curve of the method is drawn in the concentration range of 0.3 to 80 μg L^{-1}, and the slope of the curve is obtained (m). Three times the standard deviation of the blank divided by the slope of the calibration curve is equal to the LOD. In simpler words, the LOD, defined as $C_L = 3S_b/m$ (where C_L, S_b, and m are the LOD, SD of the blank, and slope of the calibration graph, respectively), was 0.1 μg L^{-1}. Standard solutions with different concentrations (low, medium, high) were added to the sample solution to calculate the recovery of lead ions and evaluate the accuracy of the method. Moreover, intra-day and inter-day precisions were completed within 1 day and 3 days respectively, expressed by relative standard deviation (RSD%). The recoveries of the method were in the range of $90.8 - 108.5\%$, and the RSD% of intra-day and inter-day precisions were 3.2 and 5.6%, respectively, which proved that the proposed method has a good accuracy and precision. The EF was 240, and the enhancement factor was obtained from the slope ratio of calibration graph after and before extraction, which was about 500.

Determination of lead in real blood samples

The efficiency of the DES–DLLME method in the determination of lead ions was investigated by analyzing blood samples of opium addicts and healthy control group. In this study, 45 people were selected as the case group and 45 as the control group. Considering that more than 90% of addicts and opium users in Kermanshah province are men, all samples were selected from the male population. The samples were classified and analyzed based on age, duration of consumption, and type of substance. The results are collected in Table 1. According to the results, the concentration of lead in case and control group are in the range of 214.6–341.2 and 85.4–128.5 μg L^{-1}, respectively. There is a significant difference in blood lead levels between the case group and the control group ($P < 0.05$). It is clear that the high blood lead levels in the case group are related to the use of lead-contaminated drugs. The case group was classified by age, and blood lead levels are shown in Table 1. The results showed that there was no significant difference between blood lead levels in different age groups ($P > 0.05$). Considering that the consumption source of all participants in the study is approximately the same, it is expected that blood lead levels in different age groups do not have significant differences. Blood lead levels were also measured based on the type of opium used. Blood lead levels in opium and opium residue (shireh) users were 214.6–266.3 and 278.1–341.2 μg L^{-1}, respectively. The case group also is classified by duration of consumption, and the ranges of blood lead levels are shown in Table 1. The results showed that there was no significant difference between blood lead levels in different durations of consumption ($P > 0.05$). It is probably because the biological half-life of lead in the blood is short, and it is excreted over time and does not accumulate in the blood. The results showed that there was a significant difference between blood lead levels in opium and opium residue (shireh) users ($P < 0.05$). It seems that drug retailers add lead compounds to opium and opium residue (shireh) to increase weight and profit more, but they contaminate the opium residue (shireh) more than opium. To investigate the

Table 2 Relative recoveries of lead ions from spiked blood samples

Sample No	Added (μg L^{-1})	Found mean ± SD (μg L^{-1}) ($n = 3$)	Relative recovery (%)
1	0	157.3 ± 6.5	-
	20	176.1 ± 8.7	94.0
	50	211.2 ± 12.5	107.8
	100	249.5 ± 19.4	92.2
2	0	213.5 ± 15.1	-
	20	231.9 ± 18.6	92.0
	50	266.3 ± 16.7	91.3
	100	317.8 ± 23.1	105.6
3	0	98.7 ± 5.6	-
	30	130.1 ± 8.7	104.7
	60	162.5 ± 11.3	106.3
	120	215.2 ± 10.9	98.0
4	0	158.5 ± 13.4	-
	30	186.5 ± 14.8	93.3
	60	221.9 ± 17.5	105.7
	120	267.4 ± 20.7	90.7

accuracy and matrix effect, two opium and two opium residue (shireh) samples were spiked with the lead at different concentration levels, and the DES–DLLME was applied to them ($n = 3$). The results showed (Table 2) that the relative recoveries of lead were in the range of 90.7−107.8%. The results indicate that the DES – DLLME procedure can be reliably used for the extraction and preconcentration of lead in biological samples.

Table 3 Comparison of DES – DLLME with other extraction methods for determination of lead in different samples

Methods	LOD[a] (μg L^{-1})	LR[b] (μg L^{-1})	RSD[c] %	Extractant volume (μL)	Extraction time (min)	Samples	Reference
MADLLME-SFO-GFAAS	0.1	0.3 – 50	3.2	530	< 10	Lipsticks and hair dyes	Sharafi et al. 2015
DLLME-SFO-GFAAS	0.05	0.1-50	4.7	1 mL + 40	< 10	Rice, wheat and cereal	Ataee et al. 2016
SPE-DLLME-GFAAS	0.001	0.003–0.06	5.2	1.5 mL + 18	< 15	Water	Shamsipur et al. 2014
DLLME-SDES-GFAAS	0.01	0.02–200	2.3	50	< 15	Soil and vegetables	Habibollahi et al. 2018
DLLME-SFO-GFAAS	0.04	0.1–100	3.5	1 mL + 40	< 10	Trout fish	Pirsaheb and Fattahi 2015
HLLME-LNPS-GFAAS	0.2	0.6–100	2.8	400	< 1	Opium and refined opium	Ahmadi-Jouibari et al. 2020
CLPME – GFAAS	2.0	5.0–50.0	2.1	30	< 10	Blood samples	Akramipour et al. 2020
DES – DLLME–GFAAS	0.1	0.3 – 80	3.2	0.5 mL + 50	< 5	Blood samples	This work

[a] *LOD*, limit of detection

[b] *LR*, linear range

[c] *RSD*, relative standard deviation

Comparison with other methods

The presented method was compared with other methods reported in the literatures for the extraction and determination of lead ions based on some analytical properties such as limit of detection, linear range, RSD%, extractant volume, extraction time, and sample type. The specific data comparison is shown in Table 3. By comparing the types of extraction solvents, it can be seen that the developed method in this study is more in line with the concept of green chemistry than other methods and showed a low detection limit within a suitable linear range, and the enrichment factor also fulfills the analysis requirements. These merits emphasize the fact that DES – DLLME method is a simple, efficient, economical, reliable, and environmentally friendly pretreatment method for the analysis and determination of lead ions in blood samples.

Conclusions

A relatively efficient and green method named DES−DLLME was introduced to enrich lead ions in blood samples for the first time. The selected hydrophilic deep eutectic solvent consists of *l*-menthol and CSA at a 5:1 molar ratio as a green solvent instead of traditional toxic organic solvents. In order to obtain the optimal extraction conditions, the extraction process was systematically optimized by a single factor experiment. The developed method was validated including linear range, precision, and accuracy, and satisfactory results were obtained. The presented procedure was successfully used for the preconcentration and analysis of lead in blood samples of men addicted to opium and opium residue. Blood lead levels were studied based on age, duration of consumption, and type of substance consumed. In addition, the proposed procedure in this study is a relatively simple, efficient, green, and economical extraction method for the extraction of lead ions in blood samples and has potential application in the analysis of contaminants in biological samples.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-23942-5.

Acknowledgements The authors appreciate the Clinical Research Development Center experts of Imam Khomeini and Dr. Mohammad Kermanshahi and Farabi hospitals for their cooperation in the preparation of plasma samples. We thank Mr. Nesari for his help in collecting samples and completing the checklist of patients. We are also very grateful to all of the patients who participated in this project.

Author contribution T. AJ., H. AJ., F. H., M. N., and N. F. contributed to the design of the study, the interpretation of the results, and the drafting of the manuscript. T. AJ. and F. H. conducted the collection of the data. H. AJ. and M. N. conducted the statistical analyses. N. F. conducted the sample preparation and analysis. All authors have read and approved the final version of the manuscript.

Funding The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 4000583) for financial support.

Data availability The datasets used and analyzed during the current research are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate This study was approved by the Research and Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1400.304).

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Ahmadi-Jouibari T, Aghaei A, Sharafi K, Fattahi N (2020) Homogeneous liquid–liquid microextraction based on liquid nitrogen-induced phase separation followed by GFAAS for sensitive extraction and determination of lead in lead-adulterated opium and refined opium. RSC Adv 10:29460–29468

Ahmadi-Jouibari T, Shaahmadi Z, Moradi M, Fattahi N (2022) Extraction and determination of strobilurin fungicides residues in apple samples using ultrasound-assisted dispersive liquid-liquid microextraction based on a novel hydrophobic deep eutectic solvent followed by HPLC-UV. Food Addit Contam Part A 39:105–115

Akramipour R, Golpayegani MR, Gheini S, Fattahi N (2018) Speciation of organic/inorganic mercury and total mercury in blood samples using vortex assisted dispersive liquid-liquid microextraction based on the freezing of deep eutectic solvent followed by GFAAS. Talanta 186:17–23

Akramipour R, Golpayegani MR, Noori N, Fattahi N (2020) Monitoring of blood lead level in young children using new mode of liquid phase microextraction and graphite furnace atomic absorption spectrometry. Toxin Rev 39:180–187

Ataee M, Ahmadi-Jouibari T, Fattahi N (2016) Application of microwave-assisted dispersive liquid–liquid microextraction and graphite furnace atomic absorption spectrometry for ultra-trace determination of lead and cadmium in cereals and agricultural products. Int J Environ Anal Chem 96:271–283

Azadi N, Nakhaee S, Farnia V, Pirsaheb M, Mansouri B, Ahmadi-Jouybari T, Khanegi M (2022) Multivariate statistical evaluation of heavy metals in the urine of opium individuals in comparison with healthy people in Western Iran. Environ Sci Pollut Res 29:8232–8241

Borahan T, Unutkan T, Turan NB, Turak F, Bakırdere S (2019) Determination of lead in milk samples using vortex assisted deep eutectic solvent based liquid phase microextraction-slotted quartz tube-flame atomic absorption spectrometry system. Food Chem 299:125065

Champion WM, Buerck AM, Nenninger C, Yusuf K, Barrett LJP, Rakotoarisoa L, Rakotondrazaka R, Alfredo K, Cunningham J, Khaliq M, Mihelcic JR (2022) Childhood blood lead levels and environmental risk factors in Madagascar. Environ Sci Pollut Res 29:68652–68665

Comitre ALD, Reis BF (2005) Automatic flow procedure based on multicommutation exploiting liquid–liquid extraction for spectrophotometric lead determination in plant material. Talanta 65:846–852

Daglioglu N, Guzel EY, Atasoy A, Gören IE (2021) Comparison of community illicit drug use in 11 cities of Turkey through wastewater-based epidemiology. Environ Sci Pollut Res 28:15076–15089

Farnia V, Pirsaheb M, Mansouri B, Azadi NA, Radmehr F (2021) Blood lead concentration among oral/inhaled opium users: systematic review and meta-analysis. Crit Rev Toxicol 51:24–35

Farnia V, Nakhaee S, Azadi, N, Khanegi M, Ahmadi-Jouybari T, Mansouri B (2022) Comparison of urine trace element levels in tramadol addiction alone and its co-abuse with cigarette and opium in Western Iran. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-022-21271-1

Fattahi N, Shamsipur M, Nematifar Z, Babajani N, Moradi M, Soltani S, Akbari S (2022) Novel deep eutectic solvent-based liquid phase microextraction for the extraction of estrogenic compounds from environmental samples. RSC Adv 12:14467–14476

Gautam RK, Soni S, Chattopadhyaya MC (2015) Functionalized magnetic nanoparticles for environmental remediation. In: Handbook of research on diverse applications of nanotechnology in biomedicine, chemistry, and engineering. IGI Global, p 518–551. https://doi.org/10.4018/978-1-4666-6363-3.ch024

Ghaderi A, Vahdati-Mashhadian N, Oghabian Z, Moradi V, Afshari R, Mehrpour O (2015) Thallium exists in opioid poisoned patients. DARU J Pharm Sci 23(1):39

Ghaemi K, Ghoreishi A, Rabiee N, Alinejad S, Farzaneh E, Amirabadi Zadeh A, Abdollahi M, Mehrpour O (2017) Blood lead levels in asymptomatic opium addict patients; a case control study. Emergency 5(1):e69

Golpayegani MR, Akramipour R, Gheini S, Amini MV, Fattahi F, Mohebbi A, Fattahi N (2022) Sensitive determination of vincristine in plasma of children with leukaemia using vortex-assisted dispersive liquid–liquid microextraction based on hydrophobic deep eutectic solvent. RSC Adv 12:3611–3617

Habibollahi MH, Karimyan K, Arfaeinia H, Mirzaei N, Safari Y, Akramipour R, Sharafi H, Fattahi N (2018) Extraction and determination of heavy metals in soil and vegetables irrigated with treated municipal wastewater using new mode of dispersive liquid-liquid microextraction based on the solidified deep eutectic solvent followed by GFAAS. J Sci Food Agric 99:656–665

Karrari P, Mehrpour O, Abdollahi M (2012) A systematic review on status of lead pollution and toxicity in Iran; guidance for preventive measures. DARU J Pharm Sci 20(1):2

Karrari P, Mehrpour O, Afshari R, Keyler D (2013) Pattern of illicit drug use in patients referred to addiction treatment centres in Birjand, Eastern Iran. J Pak Med Assoc 63(6):711–716

Khatibi-Moghadam H, Khadem-Rezaiyan M, Afshari R (2016) Comparison of serum and urine lead levels in opium addicts with healthy control group. Hum Exp Toxicol 35(8):861–865

Khoshnamvand N, Azizi N, Hassanvand MS, Shamsipour M, Naddafi K, Oskoei V (2021) Blood lead level monitoring related to environmental exposure in the general Iranian population: a systematic review and meta-analysis. Environ Sci Pollut Res 28:32210–32223

Kushwaha A, Nidhi H, Sanjay K, Radha R (2018) A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. Ecotoxicol Environ Saf 147:1035–1045

Liu S, Yang H, Yang S, Huang Y, Xiang Z, Ouyang G (2020) Carbon dots based solid phase microextraction of 2-nitroaniline followed by fluorescence sensing for selective early screening and sensitive gas chromatography-mass spectrometry determination. Anal Chim Acta 1111:147–154

Malik VS, Singh M, Pradhan P, Singal K, Agarwal A, Chauhan A, Sra H, Singh M (2022) Role of environmental lead in the occurrence of anemia in Indian children: a systematic review and meta-analysis. Environ Sci Pollut Res 29:37556–37564

Martínez D, Grindlay G, Gras L, Mora J (2018) Determination of cadmium and lead in wine samples by means of dispersive liquid–liquid microextraction coupled to electrothermal atomic absorption spectrometry. J Food Compost Anal 67:178–183

Mehdinia A, Ramezani M, Jabbari A (2017) Preconcentration and determination of lead ions in fish and mollusk tissues by nanocomposite of Fe3O4@graphene oxide@polyimide as a solid phase extraction sorbent. Food Chem 237:1112–1117

Moradi M, Aghaei A, Najafi F, Mahboubi M, Ataee M, Lotfi B, Farnia V, Ahmadi-Jouybari T (2015) Epidemiology of drug abuse and drug dependence in individuals visiting drug abuse treatment centers in Kermanshah province in 2013. J Biol Today's World 4(7):156–164

Mostafazadeh B, Shadnia S, Tavakkoli MA, Khoddami Vishteh HR (2017) Evaluation of blood lead level in methamphetamine users in Tehran. Subst Abuse Treat Prev Policy 12(2). https://doi.org/10.1186/s13011-017-0088-3

Nakhaee S, Rezayee M, Mansouri B, Hadianfar A, Amirabadizadeh A, Zardast M, ParsaSefat M, Mehrpour O, (2021) Comparison of thyroid function in lead-poisoned patients and healthy individuals in eastern Iran. Biol Trace Elem Res 200:3097–3102

Pirsaheb M, Fattahi N (2015) Trace determination of heavy metals in farmed trout fish using dispersive liquid–liquid microextraction based on solidification of floating organic drop and graphite furnace atomic absorption spectrometry. Anal Methods 7:6266–6273

Rehan I, Gondal MA, Rehan K (2018) Determination of lead content in drilling fueled soil using laser induced spectral analysis and its cross validation using ICP/OES method. Talanta 182:443–449

Shakirova F, Shishov A, Bulatov A (2022) Hydrolysis of triglycerides in milk to provide fatty acids as precursors in the formation of deep eutectic solvent for extraction of polycyclic aromatic hydrocarbons. Talanta 237:122968

Shamsipur M, Fattahi N, Sadeghi M, Pirsaheb M (2014) Determination of ultra-traces of lead in water samples after combined solid-phase extraction–dispersive liquid–liquid microextraction by graphite furnace atomic absorption spectrometry. J Iran Chem Soc 11:249–256

Sharafi K, Fattahi N, Pirsaheb M, Yarmohamadi H, FazlzadehDavil M (2015) Trace determination of lead in lipsticks and hair dyes using microwave-assisted dispersive liquid–liquid microextraction and graphite furnace atomic absorption spectrometry. Int J Cosm Sci 37:489–495

Soltaninejad K, Flückiger A, Shadnia S (2011) Opium addiction and lead poisoning. J Subst Use 16(3):208–212

Terán-Baamonde J, Bouchet S, Tessier E, Amouroux D (2018) Development of a large volume injection method using a programmed temperature vaporization injector–gas chromatography hyphenated to ICP-MS for the simultaneous determination of mercury, tin and lead species at ultra-trace levels in natural waters. J Chromatogr A 1547:77–85

Varotsis C, Tselios C, Yiannakkos KA, Andreou C, Papageorgiou M, Nicolaides A (2021) Application of double-pulse laser-induced breakdown spectroscopy (DP-LIBS), Fourier transform infrared micro-spectroscopy and Raman microscopy for the characterization of copper-sulfides. RSC Adv 12(2):631–639

Wang Y, Han J, Liu Y, Wang L, Ni L, Tang X (2016) Recyclable non-ligand dual cloud point extraction methodfor determination of lead in food samples. Food Chem 190:1130–1136

Wilcox RR (2011) Introduction to robust estimation and hypothesis testing, 3rd edn. Elsevier, Academic Press

Zaman BT, Erulaş AF, Chormey DS, Bakirdere S (2020) Combination of stearic acid coated magnetic nanoparticle based sonication assisted dispersive solid phase extraction and slotted quartz tube-flame atomic absorption spectrophotometry for the accurate and sensitive determination of lead in red pepper samples and assessment of green profile. Food Chem 303:125396