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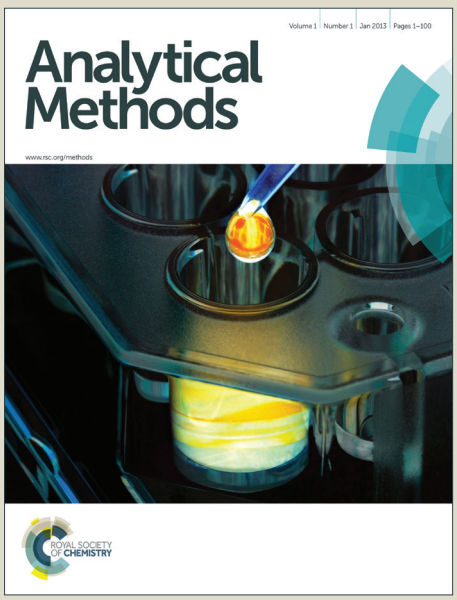

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ARTICLE

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

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A dispersive liquid–liquid microextraction based on the solidification of floating organic drop (DLLME–SFO) method followed by graphite furnace atomic absorption spectrometry (GFAAS) was developed for the extraction, preconcentration and determination of ultra-trace amounts of heavy metals in farmed trout fish samples. The influences of analytical parameters, including pH, extraction solvent type and its volume, disperser solvent type and its volume, concentration of chelating agent, salt effect and extraction time on the quantitative recoveries of cadmium, lead and mercury ions were investigated. Under the best experimental conditions (extraction solvent: 40 µL of 1-undecanol; disperser solvent: 1000 µL of methanol; ligand concentration: 0.15% (v/v); pH: ~2.4 and without salt added), the enhancement factor ranged from 68 to 93. The calibration graphs were linear in the range of 0.5–50 µg kg^{-1} for Hg, 0.1–100 µg kg^{-1} for Cd and Pb with correlation coefficient (r^2) better than 0.990. The detection limits were between 0.04 and 0.1 µg kg^{-1}. Application of the proposed method to the analysis of fish certified reference material produced results that were in good agreement with the certified values. The results obtained for heavy metal ions in analyzed trout fishes were below the established values by various authorities. The results showed that DLLME–SFO is a very simple, rapid, environmental friendly, sensitive and efficient analytical method for the determination of metal ions in fish samples and suitable results were obtained.

1. Introduction

The Fish is widely consumed in many parts of the world by humans because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health.[1] Fish consumption is recommended due to all its nutritional and therapeutic benefits. However, the one potential risk of dietary fish eating is its content of heavy metals in some fish, which affects the health of people consuming large quantities.[2] Heavy metals are emitted to the environment from different sources such as transportation, industrial activities, fossil fuels, agriculture, urbanization and other human activities.[3] Metals, such as iron, copper, zinc and manganese, are essential metals since they play important roles in biological systems, whereas mercury, lead and cadmium are toxic, even in trace amounts. The essential metals can also produce toxic effects at high concentrations. Toxic metals can be very harmful, even at low concentration, when ingested over a long time period.[4] Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms, such as fish.[5] For this reason, determination of chemical quality of aquatic organisms, particularly the contents of heavy metals in fish is extremely important for human health.

Currently, the most commonly used analytical methods that afford high selectivity for the determination of trace amounts of heavy metals are flame atomic absorption spectrometry (FAAS),[6,7] graphite furnace atomic absorption spectrometry (GFAAS),[8] inductively coupled plasma–optical emission spectrometry (ICP–OES),[9,10] and inductively coupled plasma–mass spectrometry (ICP–MS).[11,12] GFAAS is one of the suitable methods for the determination of trace metals in food and biological samples because of its speed, minimum need for sample preparation, possibility of automation, good sensitivity and low detection limit.[13] Some analytical methods have been developed for the determination of mercury at low concentrations, but the most commonly used ones are cold vapor atomic absorption spectrometry (CVAAS), cold vapor atomic fluorescence spectrometry (CVAFS).[14] CVAAS has been widely used technique for mercury in food and environmental samples owing to its simplicity, high sensitivity and speed.[15] But, the main disadvantage of this method is the high consumption of sample solution. In the microextraction techniques that volume of extraction solvent is very small, detection with graphite furnace atomic absorption

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spectroscopy is more appropriate. However, the determination of heavy metals at very low concentrations is often difficult because of insufficient sensitivity of method as well as the matrix interferences occurring during the real sample analysis. For this reason, a preliminary preparation, separation and preconcentration step is often required to enhance the sensitivity of the method.

Various techniques such as co-precipitation,[16] liquid–liquid microextraction (LLE),[17] cloud point extraction (CPE),[18] solid phase extraction (SPE)[19,20] and dispersive liquid–liquid microextraction (DLLME)[21,22] have been applied for separation and preconcentration of trace amounts of heavy metals from environmental samples. Advantages and disadvantages of these techniques have already been discussed in Ahmadi-Jouibari et al. 2013.[23] Recently, a new microextraction method was developed, which is DLLME integrated with the solidification of a floating organic drop (DLLME–SFO).[24] In DLLME-SFO, the extraction solvent after DLLME, was collected in the top of the test tube and was then cooled by inserting it into an ice bath for few minutes. The solidified extraction solvent was transferred into a suitable vial and immediately melted at room temperature; then it was finally injected into a suitable instrument. However, conventional DLLME as well as DLLME–SFO are usually applied to analysis of aqueous sample and only in less extent to analysis of solid biological and food samples. In the analysis of these samples a preparation step is necessary before DLLME-SFO. The combination of microwave-assisted extraction (MAE) and DLLME-SFO as a novel sample pretreatment method can be used successfully in solid and semisolid matrices. Therefore, DLLME and DLLME–SFO are widely applied to the preparation of environmental samples[25–27] and rarely applied to the analysis of heavy metals in complex food samples.[28–30] Also, several reviews have been written on this issue.[31–34]

The aim of the present work was to combine the advantages of MAE and DLLME–SFO to develop a new sample-preparation method for the determination of Cd, Pb and Hg in the farmed trout fish taken from five different farms in Kermanshah, Iran. For this purpose, MAE has been used to extract heavy metals from solid matrices and DLLME–SFO has been applied to simultaneous preconcentration and determination of these metal ions in fish samples. The factors affecting the efficiency of microextraction were thoroughly studied. In addition, the accuracy of the proposed methodology was evaluated by analyzing a standard reference material. To the best of our knowledge, this study is the first report on the application of DLLME–SFO to determine heavy metals in trout fish.

2. Experimental

2.1. Apparatus

Analysis of Pb and Cd were performed using a Model nov AA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany), equipped with deuterium background correction, a transversely heated graphite tube atomizer and a MPE 60 auto-sampler. Pyrolytic graphite coated graphite tubes with integrated PIN platform (Analytik Jena Part No. 407-A81.026) were used for all measurements. The optimum operating parameters for GFAAS are given in Table 1. Argon 99.999 % (Air Products, UK) was used as a purge and protective gas at a flow rate of 500 mL min–1 during all stages, except during atomization, when the flow was stopped. All measurements were based on the peak height. Microwave closed system Multiwave 3000 (Anton Paar, Germany) was used for digestion of samples. The Hettich Zentrifugen (EBA20, Tuttlingen, Germany) was used for centrifugations. The pH values were measured with a Metrohm pHmeter (Model: 692, Herisau, Switzerland) supplied with a glass-combined electrode.

2.2. Standard solution and reagents

All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 18.2 MΩ cm resistivity) was used for all dilutions. HNO_3, H_2O_2 and HCl were of suprapur quality (E. Merck, Darmstadt, Germany). The chelating agent, diethyldithiophosphoric acid (DDTP) with the density of 1.17 kg L^{-1} was supplied from Merck. 1-undecanol, 1-dodecanol and 1-decanol as extraction solvent, methanol (for spectroscopy), acetone (HPLC grade) and acetonitrile (HPLC grade) as disperser solvent and NaCl (analytical grade) were obtained from Merck. All the plastic and glassware were cleaned by soaking in dilute HNO3 (1:9, v/v) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L^{-1} of the given element supplied by Sigma Chem. Co. St. Louis, USA. A mixture of 1000 mg L^{-1} $Pd(NO_3)_2$ and 300 mg L^{-1} $Mg(NO_3)_2$ solutions, both from Merck (Darmstadt, Germany), were used as chemical modifiers.

2.3. Sampling

Farmed trout fish samples were collected from five different farms (Kermanshah, Iran). Three samples of each farm with a different weights of 200 ± 20, 300 ± 30 and 400 ± 40 g were selected. Fish species were labeled, stored in ice and at the same day transported to the laboratory and were washed with distilled water. Fish samples were dried in filter paper and packed in polyethylene bags and stored below –20 °C until further treatment and analysis. The muscle and skin of each sample were analyzed for heavy metals.

2.4. Sample preparation

The samples were thawed to room temperature. Boneless tissue samples (i.e., skin and muscle) were taken for the heavy metal analysis. The body parts were removed with stainless steel knives, homogenized and weighed. Individual samples were then oven-dried to constant weight at 80 °C for 48 h in acid-washed Petri dishes. Samples were allowed to cool in the desiccators and were ground to a fine powder using a porcelain mortar and pestle. The samples were 0.5 g dry weight for powdered skin and muscle.

Samples were processed in triplicate and then digested using closed-vessel microwave digestion (Anton Paar, Germany) with 8 mL nitric acid (65%) and hydrogen peroxide (30%) mixture at a 3:1 ratio at a temperature of 150 °C for 25 min, followed by a 30 min cooling period at room temperature in the microwave.[35,36] The digested samples were diluted with deionized water to a total volume of 50 mL and then filtered through 0.45 µm Whatman filter paper (Germany). Finally, 10.0 mL of this sample solution was subjected to DLLME–SFO procedure.

2.5. DLLME–SFO procedure

A 10.0 mL of ultra-pure water (or sample solution) was placed in a 15-mL screw cap glass test tube and spiked at levels of 2.0 µg L^{-1} of Hg and 0.5 µg L^{-1} of Cd and Pb. One milliliter of methanol (disperser solvent) contains 40.0 µL of 1-undecanol (extraction solvent) and 15.0 µL DDTP (chelating agent) was injected rapidly into a sample solution by using 2.0 mL syringe (gastight, Hamilton, Reno, Nevada, USA). A cloudy solution (water, methanol and 1-undecanol) was formed in a test tube. In this step, metal ions react with DDTP and extract into the fine droplets of 1-undecanol.[22,37] The overall chemical reaction between metal ions and DDTP is shown in Fig. 1. The mixtures were centrifuged for 3 min at 6000 rpm. Accordingly, the dispersed fine droplets of the extraction solvent were collected at the top of test tube. The sample solution was transferred into a beaker containing ice pieces and the organic solvent was solidified after 5 min and then, the solidified solvent was transferred into a conical vial where it was melted immediately. Finally, for quantitation of metal ions, 20.0 µL of the extract using auto-sampler was injected into the GFAAS and was subjected to the temperature program of Table 1.

3. Results and discussion

In the present work, DLLME–SFO combined with GFAAS was developed for the determination of Cd, Pb and Hg in farmed trout fish samples. In order to obtain a high enrichment factor and extraction recovery, the effect of different parameters affecting the complex formation and extraction conditions, such as kind of extraction and disperser solvent and their volume, pH, concentration of the chelating agent, extraction time and salt addition were optimized.

3.1. Selection of extraction solvent and its volume

The selection of appropriate extraction solvent was essential for the development of an efficient DLLME-SFO procedure. Several extracting solvents including 1-undecanol, 1-dodecanol and 1-decanol were investigated. The experiments were performed by using 40.0 µL of each extracting solvent and 1.0 mL of methanol (as the disperser solvent), and all experiments were performed in triplicates. The results revealed that 1-undecanol has the highest recoveries in comparison with the other tested solvents. Therefore, 1-undecanol was chosen for further experiments.

To study the effect of extraction solvent volume on the extraction recovery in DLLME–SFO method, different volumes of 1-undecanol (20, 30, 40, 50 and 60 µL) containing 1.0 mL of methanol were tested to select the optimum volume of extraction solvent to be applied in subsequent experiments. The results are shown in Fig. 2. As can be seen, when the volume of 1-undecanol is increased, the analytical signal of the ions increases until 40.0 µL, by further increasing the volume of 1-undecanol, it decreases, because of dilution effect. Therefore, 40.0 µL of 1-undecanol was chosen as the optimum extracting solvent volume.

3.2. Selection of disperser solvent and its volume

The main criterion for disperser solvent in DLLME–SFO is its miscibility with both water and the extraction solvent. In this study acetone, methanol and acetonitrile were evaluated as disperser solvents and the effect of these solvents on the performance of DLLME–SFO was investigated. For this purpose, various experiments were performed by using 1.0 mL of each disperser solvent containing 40.0 µL of 1-undecanol as extraction solvent and 15.0 µL DDTP as chelating agent. The volume of floated phase for all dispersers was the same (30 ± 2 µL). The result of this study shows that the analyte signal with methanol as the dispersive solvent was higher than that with acetone and acetonitrile. In this study methanol was selected as the most suitable disperser solvent due to high analyte signal.

For obtaining optimized volume of methanol, various experiments were used by using different volumes of methanol (0.25, 0.5, 1.0, 1.5 and 2.0 mL) containing different volumes of 1-undecanol and 15.0 µL DDTP as chelating agent. Variation of the volume of methanol causes changes in the volume of the floated phase; hence, it is impossible to consider the influence of methanol volume on the extraction efficiency. In order to avoid this and also to achieve a constant volume of the floated phase, the volume of methanol and 1-undecanol were changed simultaneously. According to the results in Fig. 3, with low volumes of disperser solvent, the cloudy state would not form properly. On the other hand, at high volumes of disperser solvent, the polarity of the sample is reduced and partition coefficients of analytes are decreased accordingly, leading to a marked decrease of extraction efficiency. Thus, 1.0 mL of methanol was selected as the volume of dispersive solvent in subsequent experiments.

3.3. Effect of pH

The pH of the solution plays a unique role on metal-chelate formation and subsequent extraction. The extraction yield depends on the pH at which the complex formation occurs. In the present work, diethyldithiophosphoric acid was totally transformed to the DDTP ammonium salt with the NH_3 and the effect of pH upon the

complex formation of target ions was studied within the pH range of 1–7, using hydrochloric acid and sodium acetate. Fig. 4 shows the influence of the sample pH on the analytical signal intensity. As it is demonstrated, the recovery of complexed ions is nearly constant and maximum in the pH range of 1–4 and it reduced on the higher pH value. On the other hand, because the aqueous solution of DDTP was almost acidic (pH 2.4 in 10.0 mL aqueous solution), in this study, the use of an acidic solution for the pH adjustment, being the contamination sources, was not necessary.

3.4. Effect of the DDTP concentration

The influence of DDTP concentration on the DLLME−SFO extraction of Cd(II), Pb(II) and Hg(II) was evaluated in the concentration range of 0.05–0.25% (v/v). In this case, the absorbance signal increased with the increase of the DDTP amount up to 0.15% (v/v), reaching a plateau. Therefore, the concentration of 0.15% (v/v) was chosen as the optimum amount for the determination of the heavy metal ions to prevent any interference.

3.5. Effect of salt addition and extraction time

To study the effect of salt addition on the analytical signal of the metal ions, the concentration of NaCl was changed in the range of 0–10% (w/v). The results demonstrated that salt addition has no effect on extraction recovery. However, the experimental results showed that enrichment factors decreased slightly with the increasing of salt amount. The reason is that the decreased solubility of floating solvent in the aqueous phase resulted in the increasing of the volume of floating phase. As a result, the peak signal of analytes and the EFs decreased slightly.[22,25,26,38,39] Consequently, further extractions were performed in the absence of any salt.

In DLLME–SFO, extraction time is defined as the time interval between injecting the mixture of disperser and extraction solvents and starting to centrifuge. The effect of extraction time on the extraction efficiency was examined in the range of 0–50 min under constant experimental conditions. The obtained results showed that the extraction time did not have significant influence on the absorbance of the metal ions. Because of the infinitely large surface area between extraction solvent and aqueous phase after the formation of cloudy solution, the complexes were formed instantly and diffused into extraction solvent quickly. This is the great advantage of DLLME–SFO technique, which is independent of time.

3.6. Interferences

It is known that DDTP as chelating agent can form complexes with several transition metals and semi-metals in acidic media, but it does not react with alkali and alkaline earth metals and other elements.[22] In order to demonstrate the selectivity of the proposed method, the recovery of 2.0 μg L^{-1} of Hg and 0.5 μg L^{-1} of Cd and Pb solution in the presence various amounts of interfering ions were treated according to the recommended procedure. investigated. The tolerance level was defined as the maximum concentration of the foreign ion causing a change in the analytical signal no higher than 5%, when compared with the signal of 2.0 μg L^{-1} of Hg and 0.5 μg L^{-1} of Cd and Pb. The results obtained are given in Table 2.

3.7. Figures of merit of the proposed method

The analytical characteristics of the method, i.e., precision, detection limits and linearity, were investigated under the chosen experimental conditions. The results are listed in Table 3. The percent relative standard deviations (RSDs %) were between 3.5 and 6.2. The limit of detection, defined as $C_L = 3S_B/m$ (where C_L, S_B, and m are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively), were obtained between 0.04 and 0.1 μg kg^{-1} for different metal ions. Linear ranges (LRs) of 0.5−50 μg kg^{-1} for Hg and, 0.1–100 μg kg^{-1} for Cd and Pb were obtained. The correlation coefficient of the calibration curves were in the range of 0.991–0.997. The enhancement factor, obtained from the slope ratio of calibration graph after and before extraction, were in the range of 68−93.

3.8. Analysis of fish certified reference material

The accuracy of the developed procedure for the determination of Cd, Pb and Hg ions was assessed by determining the concentration of these ions in two fish certified reference material (DORM-2-dogfish muscle, NRC, Canada; Muscle tissue-NIST SRM 2976). The CRM samples were subjected to the DLLME−SFO procedure. No significant difference was found between the results obtained by employing the proposed method and the certified values (Table 4).

3.9. Analysis of real samples

The proposed method was applied to the determination of Cd, Pb and Hg ions in fish samples, which were taken from the five different farms. Each of the dried fish sample was initially digested using the sample pre-treatment procedure, after which each sample was subjected to the proposed procedure. The accuracy of the proposed method was also tested by calculating the recovery of the heavy metal ions from spiked fish samples. As shown in Table 5, the recoveries of the spiked fish samples were in the range of 86.6–112%, which indicated that good recoveries can be obtained using this method. The quantitative results show that the method is accurate and reliable and could be applied for the determination of heavy metals in other food samples.

3.10. Comparison of the proposed method with other methods

Determination of heavy metals in fish samples by DLLME–SFO procedure and GFAAS detection was compared with other methods to extract metal ions from different fishes in Table 6. As can be seen, the relative standard deviations (RSDs) of the proposed method are similar to other methods. The LODs values in DLLME–SFO were low and the required sample and solvent volume are smaller. The extraction of analytes from solid or semisolid samples is usually time-consuming, because it needs one step sample preparation before extraction procedure. In DLLME–SFO method the extraction time is shorter than other extraction methods. All these results indicate that DLLME–SFO is a fast, reproducible and simple technique that can be used for the extraction and determination of heavy metals from food samples.

4. Conclusions

In the present study, a novel, simple, and environmental friendly method based on DLLME–SFO coupled with GFAAS was developed to determine three metal ions in fish samples. This technique provides good precision, simplicity, multi-element enrichment capability, ease of operation, good recovery and low detection limits within a short time compared to other techniques. The proposed method avoided the use of chlorinated solvents, which are commonly used as extraction solvents in conventional DLLME. The performance of this procedure in the extraction of heavy metal ions from fish samples is excellent. Moreover, these results can also be used to understand the chemical quality of farmed trout fish and to evaluate the possible risk associated with their consumption. In this study, the results obtained for heavy metal ions in analyzed trout fishes were below the established values by various authorities.

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Figure of merits

Fig. 1 Schematic representation of the overall reaction between metal ions and DDTP.

Fig. 2 Effect of the volume of extraction solvent on the absorbance of heavy metals obtained from DLLME–SFO. Extraction conditions: type of extraction solvent, 1-undecanol; sample amount, 0.5 g; volume of disperser solvent (methanol), 1.0 mL; floated phase volume, 30 ± 2 µL; DDTP concentration, 0.15% (v/v); pH, 2.4; concentration of heavy metals, 2.0 µg L^{-1} for Hg and 0.5 µg L^{-1} for Cd and Pb; room temperature.

Fig. 3 Effect of volume of disperser solvent on the absorbance of heavy metals obtained from DLLME–SFO. Extraction conditions: similar to those in Fig. 1, except for volume of extraction solvent (1-undecanol), 40.0 µL.

Fig. 4 Effect of pH on the absorbance of heavy metals obtained from DLLME–SFO. Extraction conditions are similar to those of Fig. 2.

Table 1 Instrumental parameters for the heavy metal ions determination using GFAAS

Spectrometer parameters	Cd	Pb	Hg
Wavelength (nm)	228.8	283.3	253.7
Slit width (nm)	0.7	0.7	0.2
Lamp current (mA)	8	10	5

Step	Temperature (°C)			Time (s)		Gas flow (mL min^{-1})
	Cd	Pb	Hg	Ramp	Hold	
Pre-warming	50	50	50	1	2	500
Inject modifier	80	80	80	5	20	500
Inject sample						
Drying I	90	120	90	10	15	500
Drying II	110	250	110	5	20	500
Ashing	300	600	200	10	10	500
Gas stop step	300	600	200	0	1	0
Atomization	2100	1800	1200	0	3	0
Cleaning	2400	2200	1600	0	2	1000

Table 2 Effect of interferences ions on preconcentration and determination of metal ions

Interference	Interference to metal ions ratio[a]	(%) Recovery		
		Cd	Pb	Hg
Na^{+}	20000	98	101	94
K^{+}	3000	101	98	97
Ca^{2+}	1000	101	96	99
Zn^{2+}	1000	89	105	78
Zn^{2+}	500	98	102	93
Mn^{2+}	1000	87	97	75
Mn^{2+}	100	99	102	94
Cu^{2+}	1000	105	102	94
Al^{3+}	500	96	99	98
Co^{2+}	500	102	97	101
As^{5+}	500	86	88	77
As^{5+}	100	98	100	93
Fe^{3+}	500	99	89	96
Cr^{3+}	200	101	96	93
As^{3+}	200	95	102	108
Sn^{2+}	100	97	99	98
Ni^{2+}	100	78	83	90
Ni^{2+}	20	96	99	103
Cl^{-}	200000	98	96	103
NO^{3-}	10000	104	94	101
SO_4^{2-}	10000	97	99	86
SCN^{-}	10000	99	107	96

[a] Concentration of Cd and Pb ions are 0.5 µg L^{-1} and Hg ion is 2.0 µg L^{-1}

Table 3 Figures of merit of the proposed method

Analyte	Enhancement factor	Detection limit (μg kg^{-1})[a]	RSD%[b] (n = 7)	Linear range (μg kg^{-1})	r^2
Cd	85	0.04	4.3	0.1-100	0.995
Pb	93	0.04	3.5	0.1-100	0.997
Hg	68	0.1	6.2	0.5-50	0.991

[a] Detection limits were calculated based on $3S_B/m$

[b] Percent relative standard deviation for seven replicate measurements of the elements with the concentration of 0.5 μg L^{-1} for Hg and 2.0 μg L^{-1} for Cd and Pb

Table 4 Concentrations of heavy metals found in certified references material

Certified reference material	Element	Certified values ± SD ($\mu g\ kg^{-1}$)	Measured values ± SD (n=3, $\mu g\ kg^{-1}$)	Recovery (%)
DORM-2-dogfish muscle, NRC, Canada	Cd	43.0 ± 8.0	41.4 ± 5.4	95.5
	Pb	65.0 ± 7.0	66.5 ± 4.2	102.3
	Hg	4640 ± 260	4480 ± 438	96.5
Muscle tissue-NIST SRM 2976	Cd	820 ± 160	780 ± 93	95.1
	Pb	1190 ± 180	997 ± 105	83.8
	Hg	61.0 ± 3.6	56.0 ± 3.2	91.8

Table 5 Heavy metal concentrations in the muscle and skin of farmed trout fishes

Farm No.	Tissue	Analyte	Added ($\mu g\ kg^{-1}$)	Found($\mu g\ kg^{-1}$) ± SD (n=3)	Relative recovery (%)
1	Muscle	Cd	-	18.5 ± 1.6	-
			10	27.4 ± 2.0	89.0
			50	70.2 ± 4.2	103.4
		Pb	-	155 ± 8.6	-
			30	181 ± 7.5	86.6
			50	201 ± 11.3	92.0
		Hg	-	46.4 ± 3.7	-
			20	64.5 ± 4.1	90.5
			40	88.4 ± 7.5	105.0
	Skin	Cd	-	65.3 ± 5.8	-
			10	74.7 ± 6.0	94.0
			30	96.2 ± 8.4	103.0
		Pb	-	220 ± 15.6	-
			50	266 ± 18.3	92.0
			100	311 ± 21.6	91.0
		Hg	-	55.3 ± 6.2	-
			20	76.4 ± 8.3	105.5
			40	91.5 ± 7.6	90.5
2	Muscle	Cd	-	33.6 ± 4.4	-
			10	42.9 ± 3.6	93.0
			50	85.2 ± 6.7	103.2
		Pb	-	95.4 ± 5.5	-
			30	121.6 ± 9.6	87.3
			50	148.5 ± 11.7	106.2
		Hg	-	33.7 ± 4.5	-
			20	52.4 ± 6.3	93.5
			40	73.2 ± 7.5	98.7
	Skin	Cd	-	44.6 ± 5.1	-
			10	54.2 ± 6.4	96.0
			30	78.2 ± 8.3	112.0
		Pb	-	115 ± 7.4	-
			30	143.2 ± 12.6	94.0
			50	160.8 ± 10.1	91.6
		Hg	-	48.5 ± 3.3	-
			50	102.5 ± 6.4	108.0
			100	142 ± 13.5	93.5
3	Muscle	Cd	-	12.6 ± 0.8	-
		Pb	-	105 .4 ± 4.8	-
		Hg	-	28.5 ± 1.7	-
	Skin	Cd	-	26.2 ± 3.0	-
		Pb	-	166.3 ± 12.3	-
		Hg	-	33.2 ± 2.6	-
4	Muscle	Cd	-	8.6 ± 0.7	-
		Pb	-	98.5 ± 6.8	-
		Hg	-	43.3 ± 3.5	-
	Skin	Cd	-	18.4 ± 1.5	-
		Pb	-	146.7 ± 11.0	-
		Hg	-	37.5 ± 4.0	-
5	Muscle	Cd	-	27.6 ± 3.2	-
		Pb	-	96.7 ± 6.8	-
		Hg	-	16.5 ± 1.2	-
	Skin	Cd	-	39.6 ± 3.1	-
		Pb	-	118.3 ± 9.5	-
		Hg	-	44.1 ± 5.2	-

Table 6 Comparison of DLLME-SFO with other extraction methods for determination of heavy metals in different fishes

Methods	LOD[a] (μg kg^{-1})	LR[b] (μg kg^{-1})	RSD[c] %	Extractant volume (mL)	Sample amount (gr)	Metal ions	Samples	Reference
SPE–ETAAS[d]	0.16–0.65	–	2–9	2	3	Pb, Cd	Fresh fish	[40]
MAE–ICP–OES[e]	5–10	–	–	–	0.5	Cr, Mn, Zn	Silverside fish	[41]
SLSDE-ILDLLME–FAAS[f]	90–400	1000–5000	2.3–2.5	400 μL	1	Pb, Ni	Local fish	[42]
SPE–FAAS	0.21–0.63	0.5–60	1.5–4.8	3	2	Pb, Cd, Zn, Cu	Seawater, fish and oysters	[43]
CPE–FAAS[g]	0.67-3.42	250-10000	1.7–4.8	2	1	Pb, Cu, Co	Canned fish	[44]
DLLME–SFO–GFAAS	0.04-0.1	0.1-100	3.5–6.2	40 μL	0.5	Pb, Cd, Hg	Trout fish	This work

[a] LOD, limit of detection.
[b] LR, linear range.
[c] RSD, relative standard deviation.
[d] Soli phase extraction and electrothermal atomic absorption spectroscopy.
[e] Microwave-assisted extraction and inductively coupled plasma-atomic emission spectrometry.
[f] Solid–liquid–solid dispersive extraction-ionic liquid-based dispersive liquid–liquid microextraction and flame atomic absorption spectroscopy.
[g] Cloud point extraction and flame atomic absorption spectroscopy.
[h] Dispersive liquid–liquid microextraction based on the solidification of floating organic drop and graphite furnace atomic absorption spectrometry.

Fig. 1

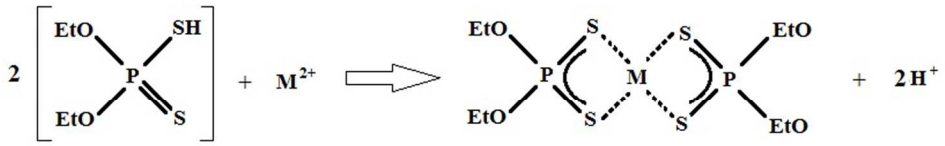

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Fig. 2

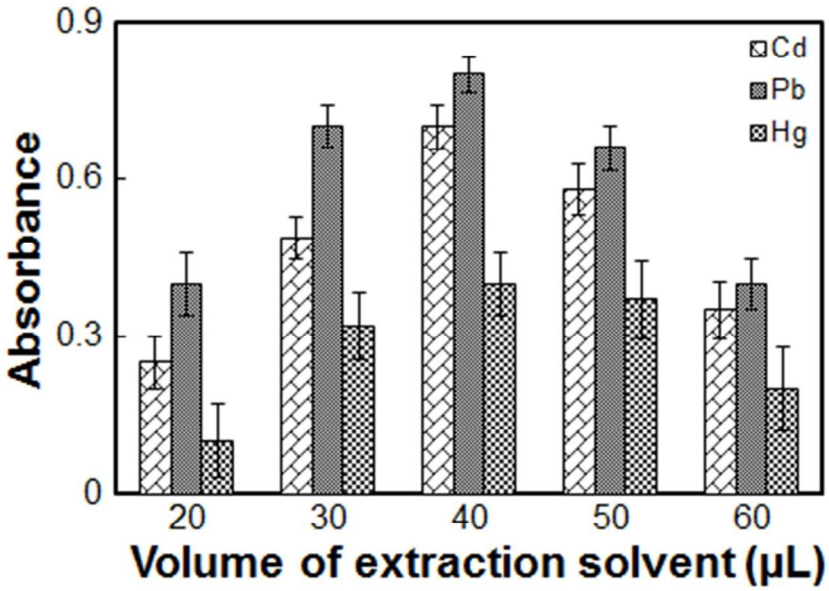

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Fig. 3

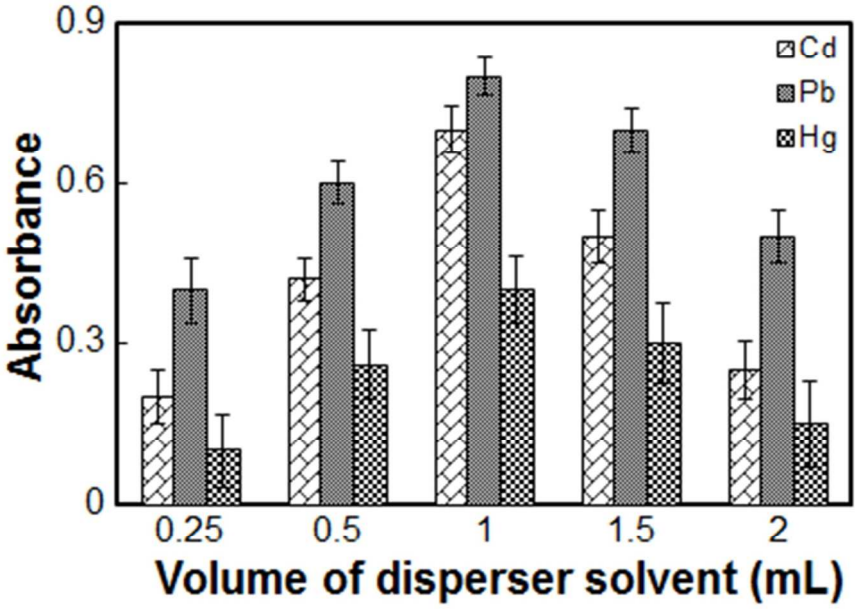

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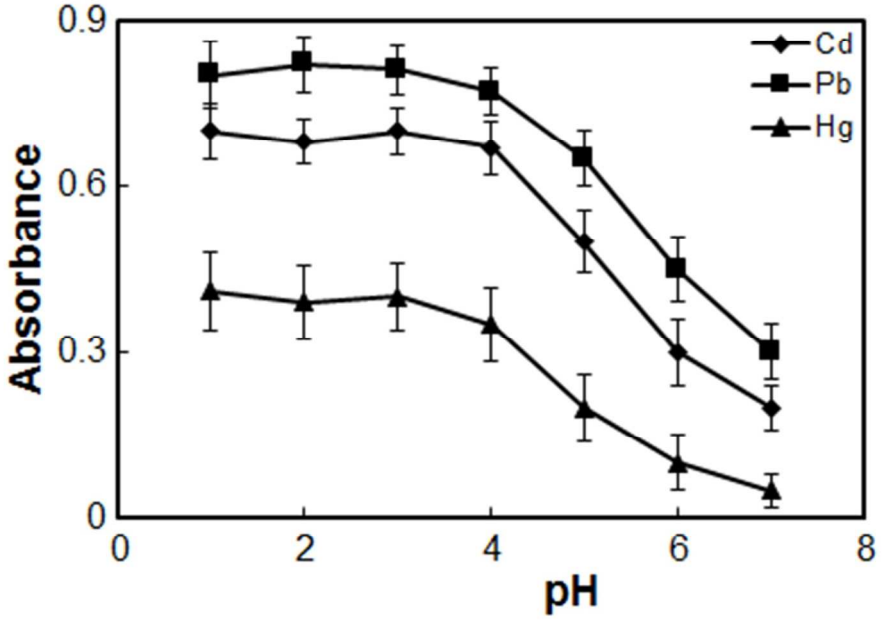
Fig. 4



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