Development of dispersive liquid–liquid microextraction solidofied floating organic drop (DLLME SFOD) method for determination of cadmium in biological samples

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Abstract

Introduction: Todays, exposure to heavy metals is happened by being produced in various environmental, industrial processes. The production of metals finally results in air pollution as well as contamination in the food chain. There are harmful effects of heavy metals such as cadmium on different organs. Therefore, this study aimed to identify and quantify cadmium in biological samples using DLLME SFOD method.

Materials and Methods: Optimization of the underlying variables played a key role in the process including sample PH, chelator, extractor and disperser solvents, ion concentration, time and rate of centrifugation and extraction time. It was done by employing central composite design (CCD) of the response surface methodology. In the process of optimization, after setting a certain pH, Specific salt concentration and ditizon added to form a complex between the metal and the chelator. A mixture of extraction and dispersant solvents added to the sample. The organic and aqueous phase separations when centrifugation and vortex carried out, the sample vial transferred to a cold ice bath and the organic solvent floated on the aqueous solvent .The organic portion containing the analyte was injected into the analyzer apparatus.

Results. The results showed that variables such as sample PH, complexing solvent, extraction solvent, centrifugation effect and extraction time play an important role in the extraction of cadmium metal ion from biological samples. The optimized method with a minimum detection limit (LOD) of 2 μg / l and a concentration factor (EF) of 50 and a relative recovery (RR) of 1.06.26 used to extract cadmium from urine samples.

Conclusion. According to the pre-test results and the optimization process, they showed that in the three factors of sample PH, salt concentration and extraction solvent volume that play a more effective role in cadmium extraction by DLLME-SFOD method.

Keywords. Dispersive liquid–liquid microextraction solidofied floating organic drop (DLLME SFOD), cadmium, biological samples.

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1. Introduction

Cadmium is used in a variety of industries including painting, plating, bearing, soldering and jewelry. The ways to deal with this metal are through breathing and varying degrees depending on the kind of industry (1). According to the International Organization for Research on Cancer (IARC), cadmium is one of the definitive carcinogens for humans (2). Therefore, cadmium is one of the elements that toxicologists seek to quantify and identify in biological and environmental samples (3). Common methods for metal extraction are two techniques: liquid-liquid extraction and solid-phase extraction (Solid Phase Microextraction) (4-6). The DLLME-SFOD method is controlled by the same variables in the conventional liquid-liquid extraction. The organic solvent is used as the extractor in the DLLME-SFOD. This solvent should not be miscible with water and has low density, high permeability coefficient, melting point and low freezing point. This method is used to extract metals (7). This study investigates occupational samples of workers exposed to cadmium from workers in the metal industry.

2. Experimental

The standard mother cadmium solution was prepared at a concentration of 1000 ppm and standard solutions had made, too. The dissolution of ditizon in ethanol was obtained by complexing those solutions. At first, three variables of sample PH, salt concentration and extraction solvent volume, which play a more effective role in cadmium extraction by DLLME-SFOD method, were selected for investigation in optimization process. Through the Central Composite Design (CCD) method, the 29 stages of testing were determined by the statistical software R Version 1.4.3 as the required sample number. For the sample PH variable, salt concentration and extraction solvent volume, values were presented in the form of upper and lower limits (Table 1). To adjust the PH of the sample, HCl solution and NACL with 1% concentration were used. ditizone 80 ppm ethanol soluble was added to the solution to form a complex. The extraction solution was then prepared by combining the extraction solvent and the dispersing solvent. 1-dodecanol, 2-dodecanol and normal hexadecane were extracted as solvent and methanol, acetone and acetonitrile were used as dispersant solution. The mixture of these two types of solvents was dispersed in the sample solution. Hot plate magnet was also used to investigate the role of temperature and the soluble eddy current flow rate in extraction efficiency was investigated by DLLME-SFOD method. In this process, a hydrophobic metal-ditizon complex was formed and eventually the metal ion transferred into the extraction solvent. The resulting compound was placed under eddy current by vortex and centrifuged for better separation of organic and aqueous phases. After that vial placed in the ice batch to freeze the organic solvent and separated by a syringe containing the sample in a lower liquid phase syringe. After separation, the organic solvent containing the metal ion was melted at room temperature. To reduce the adhesion of the organic drop to the inner wall of the Falcon, it was subjected to heat up to 35 ° C. Adding 200 μl of acid and methanol was diluted 1: 2 with 100 μl of atomic-flame absorption apparatus to measure cadmium metal. The efficiency of extraction was calculated by determining the concentration of cadmium metal extracted by atomic-flame absorption apparatus and having known concentration. In this process, the role of sample PH (2-11), extraction solvent volume (0-100 μL) and extractant solvent type (n-hexanal, dodecanol and n-decanol), dispersant solvent volume (600-300 μL) and disperser solvent type (acetonitrile, acetone and methanol), the effect of centrifuge speed (6000-2000 rpm) and centrifuge time (3–10 min) were investigated on extraction efficiency, ionic strength (0–5 gr) and sample PH on extraction efficiency.

3. Results and Discussion

The relationship between the independent operational variables was investigated with the dependent variable (concentration extracted) using

Table1. Operational range and levels of independent variables for experimental design

Variables	upper levels	lower levels
PH	11	2
Salt concentration(g)	5	0
(μL) Extractor volume	100	10

the linear, reciprocal and quadratic correlation tests. The results of the analysis of variance of three models tested and the results of statistical estimators showed that the quadratic model with F value was 41.88 and the significance level was 0 to 0.001 (P-value = 9.02 *10 -8). It is able to effectively represent the relationship between independent variables and dependent variables. In addition, the value obtained for the Pearson correlation coefficient (Multiple R2) indicates that more than 90% of the experimental data variations in cadmium extraction can be statistically interpreted (Table 2). On the other hand, the small R-Adjusted Factor Distance (percent of variance reduction) and Pearson's correlation coefficient showed the correct selection of the investigated factors for the cadmium extraction process as well as the high correlation between the predicted values and the results . Finally, the proposed Lack-of-fit value ($\geq$ 0.05) confirmed the effective fit and ability of the quadratic model to predict different

conditions. After confirming the tested models, the regression coefficients of each parameter were calculated using multivariate regression and finally the optimal values of effective factors in cadmium extraction from aqueous samples by DLLME-SFOD method, considering the regression equation obtained, was calculated using the Solver extension in Excel software (Table 3).

3.1. Optimizing parameters

Preparation of samples containing cadmium ions by the DLLME-SFOD method involves the formation of hydrophilic complexes with LOD and LOQ of 2 μg / l and 6 μg / l, respectively. The effect of PH was evaluated in the range of 2-11, and optimum PH value was obtained at PH= 6. The effect of the concentration of diisone improved the extraction efficiency of the metal ions. Therefore, different volumes (250 to 750 μL, 1 mg/mL) of ditizon were investigated and 500 μL of diisone was selected as optimium volume. The volume of the acetone as a

Table2. Analysis of variance of three tests to select the appropriate model for optimization of cadmium extraction from aqueous samples by DLLME-SFOD

Cd				Df	Test Type: Response Level Model
P- value	**F-value**	**Mean square**	**Sum of squares**		
0.2843	1.3387	861.00	0.2583	3	First class answer
0.3242	1.1227	57.711	7.2134	3	Mutual interaction
9.10×029^{-8}	41.8863	5.4037	4.12112	3	Quadratic answer
_	_	4.96	4.1831	19	Residuals
0.6726	0.6409	2.68	1.341	5	Lack of fit
Multiple R2 = 0.90 ; Adjusted R2 = 0.8554					

Table 3. Optimal values determined for effective parameters in cadmium extraction from aqueous samples by DLLME-SFOD method

Recovery (%)	Variables	The optimal set of parameters
	PH	6
94	Salt concentration(g)	0.0021
	(μL) Extractor volume	76

organic disperser solvent was studied between 600 and 300 µl and 500 µL was achieved (Table 4). Also, based on the effect of ionic strength, the different salt amount in the range of 0-5 g were investigated and the optimum results were obtained as 0.0021 g. By centrifuging speed of 4000 rpm in 6 min, the extraction time of 5 min with 76 µL of decanol was achieved in optimized conditions. Based on table 5, the interfering ions did not effected on cadmium extraction at 5000 fold concentration.

3.2. Validation

To evaluate the efficacy of the method for extracting biological samples, urine samples were extracted once without increasing standard and twice with the addition of standard optimum conditions. The results of the study of cadmium levels in biological samples are presented in Table 6. This table also shows the results of the accuracy and precision parameters on cadmium metal data.

4. Conclusion

The most important factors were investigated and optimized in the present study. The results, in accordance with Table 6, showed the ability of the method to extract cadmium from aqueous media under optimum conditions and also had successful results in biological samples. The DLLME-SFOD technique is simple, inexpensive, with very low

Table 4. Effect of disperser volume and type on cadmium extraction efficiency in aqueous samples by DLLME-SFOD method (cadmium concentration 30 µg / L)

Recovery (%) (Mean ± SD)	disperser volume acetone (µL)	Recovery (%) (Mean ± SD)	disperser type
75.11 ± 2.14	300	94.31 ± 3.85	Acetonitrile
81.87 ± 3.11	400	96.88 ± 4.72	acetone
96.62 ± 3.73	500	93.12 ± 5.06	Methanol
96.73 ± 3.76	600	95.04 ± 4.33	ethanol

Table 5. The effect of different concentrations of interfering ions on the cadmium extraction efficiency of urine samples by DLLME-SFOD method (cadmium concentration 30 µg / L)

Recovery(٪) (Mean ± SD)	(cadmium) /(Ion)	Ion
98.8 ± 0.24	5000	Na^+
99.87 ± 0.57	5000	NO_3^-
97.09 ± 5.17	5000	SO_4^{2-}
99.62 ± 6.48	5000	Cl^-
99.2 ± 5.13	5000	SO_4^{2-}

Table 6. Check the concentration of cadmium in biological samples.

	cadmium Concentrations added to water and biological samples			Matrix
Relative recovery (%)	Relative standard deviation%	found (µg/l)	Added (µg/l)	
-	0.002	-	-	
99	0.05	0.99	1	Ion-free water
101	0.09	2.02	2	
-	0.07	8.42	-	
98.3	1.12	18.25	10	Urine sample 1
100.7	1.54	28.55	20	
-	4.8	4.64	-	
95.4	2.3	9.41	5	Urine sample 2
97.7	5.4	14.48	10	

solvent consumption and with less environmental and health problems than other conventional methods. It is also in good agreement with the device decomposition methods. Considerable efforts have been made to overcome the time taken to reach equilibrium. The ability to use an atomic absorption device is another advantage of doing this to determine the amount of cadmium in biological samples because it can detect values in ppb using this device, which has a detection power of about ppm. Until now, this method has not been used for toxicology studies and for monitoring exposure levels of cadmium exposed persons and those working in related industries. It is important to introduce a method with the capabilities mentioned to estimate occupational exposure to occupational health and occupational toxicology. Therefore, the occupational applications of the method need to be used to evaluate biological samples.

5. References

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