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Simultaneous Pre-Concentration of Cadmium and Lead in Environmental Water Samples with Dispersive Liquid-Liquid Microextraction and Determination by Inductively Coupled Plasma-Atomic Emission Spectrometry

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Abstract: The dispersive liquid–liquid microextraction (DLLME) method for determination of Pb^{+2} and Cd^{+2} ions in the environmental water samples was combined with inductively coupled plasma-atomic emission spectrometry (ICP-AES). Ammonium pyrrolidine dithiocarbamate (APDC), chloroform and ethanol were used as chelating agent, extraction solvent and disperser solvent, respectively. Some effective parameters on the microextraction and the complex formation were selected and optimized. These parameters included extraction and disperser solvent type as well as their volume, extraction time, salt effect, pH, sample volume and amount of the chelating agent. Under the optimum conditions, the enrichment factor of 75 and 105 for Cd^{+2} and Pb^{+2} ions respectively was obtained from only 5.00mL of water sample. The detection limit (S/N=3) was 12 and 0.8ngmL^{-1} for Pb and Cd respectively. The relative standard deviation (RSDs) for five replicate measurements of 0.50 mgL^{-1} of lead and cadmium was 6.5 and 4.4 % respectively. Mineral, tap, river, sea, dam and spiked water samples were analyzed for Cd and Pb amount.

Keywords: Dispersive liquid-liquid microextraction, ICP-AES, environmental water, Pb ,Cd

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INTRODUCTION

In recent years, the pollution of the environment by heavy metals including lead (Pb) and cadmium (Cd) has received considerable attention. Cadmium enters the environment through fertilizer application, point and non-point source run-off, the burning of fossil fuels, and as a by-product of mining and other industry [1, 2]. Likewise Pb can enter the environment through vehicle and industry exhausts, sewage sledge applications in agriculture, and the use of lead shot [2]. These elements accumulate in living organisms and have high toxic potentials [3]. Exposure to high lead levels can severely damage the brain and kidneys [4]. Chronic exposure can result in decreased neurological performance [5, 6]. Chronic exposure to cadmium may cause kidney damage, bone mineral density loss and hypertension [7, 8]. Cadmium has been classified as carcinogenic by the International Agency for Research on Cancer, with exposure being primarily associated with lung cancer [9]. Heavy metals emitted by industries, traffic, municipal wastes, and hazardous wastes sites have resulted in a steady rise in contamination of natural and ground waters [10].

In natural waters, the concentration of heavy metals, e.g. lead and cadmium, is often trace levels lower than LOD (limit of detection) of highly sensitive instrument such as ICP-MS [11], ICP-AES [12] and GF-AAS [13].

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Therefore, a preliminary pre-concentration in environmental samples is necessary to improve the sensitivity for the determination of trace amounts of these elements. The most frequently employed methods for pre-concentration are liquid–liquid extraction (LLE) (14), sorption and chelating ion exchange [15] and solid phase micro-extraction (SPME) [16].

Recently, dispersive liquid-liquid microextraction (DLLME), developed by Assadi et al. [17], has been successfully applied to the preconcentration of several organic [18, 19] and inorganic species [21, 22] in water samples. DLLME is a miniaturized sample pretreatment technique, which is based on ternary component solvent system. In this method, a cloudy solution is formed after injecting the appropriate mixture of extraction solvent and disperser solvent rapidly into the aqueous sample by syringe. The outstanding advantages of DLLME are its simplicity of operation, rapidity, inexpensive, high recovery and enrichment factor [19].

In previous attempts, we investigated the application of DLLEM in determination of cadmium in different environmental water samples [20]. The present work describes simultaneous pre-concentration of cadmium and lead ions with dispersive liquid-liquid microextraction and determination by inductively coupled plasma-atomic emission spectrometry (ICP-AES) in different water samples. The DLLME technique was used with an appropriate mixture of chloroform, as extraction solvent, ethanol, as disperser solvent, and ammonium pyrrolidine dithiocarbamate (APDC), as chelating reagent, to separate and preconcentrate of lead and cadmium from water samples. The factors influencing the efficiency factor of DLLME such as extraction and disperser solvent type, extraction and disperser solvent volume, pH, chelating reagent concentration, sample size, salt effect and extraction time were systematically studied. The method was successfully applied to determine trace amounts of Pb and Cd in water samples.

MATERIALS AND METHODS

Apparatus

A dual-view ICP-AES instrument, Model Optima 2100DV, Perkin Elmer, equipped with a CCD detector and a Rotofix 32 centrifuge from Zentrifugen Co were used. The pH values were measured with a Mettler Toldo pH/Ion-Analyzer, Model MA235, supplied with a glass-combined electrode. Table 1 showed the instrumental and operating conditions for ICP-AES measurements.

Chemical and Reagents

The stock solution of Pb^{+2} and Cd^{+2} (1000 mgL^{-1} for ICP-AES standard) from Merck (Darmstadt, Germany) were used in this work. All working solutions were prepared by diluting the stock standards as necessary. Deionized distilled water obtained with a Milli-Q system (Millipore, Milford, USA) was used for standard dilutions and other necessary preparations. The chelating agent, 0.1M ammonium pyrrolidine dithiocarbamate (APDC) solution, was prepared daily, on a hot-plate stirrer at approximately 50°C, by dissolving the appropriate amount of APDC (analytical grade, Merck) in ethanol (extra pure, Merck). The rest of the used chemicals were carbon tetrachloride (extra pure), chloroform (extra pure) and dichloromethane (extra pure) as extraction solvent, methanol (for liquid chromatography), acetone (extra pure) and acetonitrile (HPLC grade) as disperser solvent, HNO_3 (65%, supra pure), CH3COONa (supra pure), $NaNO_3$ (analytical grade) and NH3 (25%, supra pure) supplied by Merck.

Table 1 Operation parameters for ICP-AES

Parameters	
RF Power (watt)	1300
Nebulizer gas flow rate ($Lmin^{-1}$)	0.8
Auxiliary gas flow rate ($Lmin^{-1}$)	0.2
Plasma gas flow rate ($Lmin^{-1}$)	15
Sample gas flow rate ($mLmin^{-1}$)	1.2
Wavelength (nm) for Pb^{+2}	220.353
Wavelength (nm) for Cd^{+2}	228.802
Plasma viewing	Axial
Processing mode	Area
Delay time (sec)	20

Dispersive liquid–liquid microextraction procedure

To 5mL of 0.5 mgL^{-1} Cd^{+2} and Pb^{+2} solution in a 12-mL test tube with conical bottom, 1.5mL of ethanol (disperser solvent) contains 150µL of chloroform (extraction solvent) and 0.1M APDC (chelating agent) was injected rapidly into the sample solution by using 2mL syringe. The mixture was immediately centrifuged for 5min at 1000rpm. The volume of the phase (chloroform) was determined using a 200µL HPLC syringe.

The sediment phase was quantitatively transferred to another test tube and allowed to evaporate at room temperature. The residue was dissolved into 0.5mL of 0.1M nitric acid and the Pb and Cd concentration was determined by ICP-AES. Lead and cadmium standard solutions (0.2–10mgL^{-1}) in 0.1M nitric acid were prepared daily. All experiments were performed in duplicate and the mean of results was used in plotting curves or preparation of tables for optimization.

RESULTS AND DISCUSSION

The conditions for the microextraction of the cadmium and lead in environmental water samples with APDC were studied and optimized. The one-variable-at-a-time optimization was used to identify the optimum conditions for the effective parameters such as type and volume of extraction solvent, type and volume of disperser solvent, the chelating agent concentration, pH, salt effect and extraction time on DLLME procedure.

Selection of extraction and disperser solvents

The selection of an appropriate solvent is of high importance for the DLLME process. Extraction solvent for DLLME should be selected on the basis of higher density rather than water, high extraction capability of the interested compounds and low solubility in water. For this purpose, three kinds of solvents: chloroform ($CHCl_3$), carbon tetrachloride (CCl_4) and dichloromethane (CH_2Cl_2) were investigated.

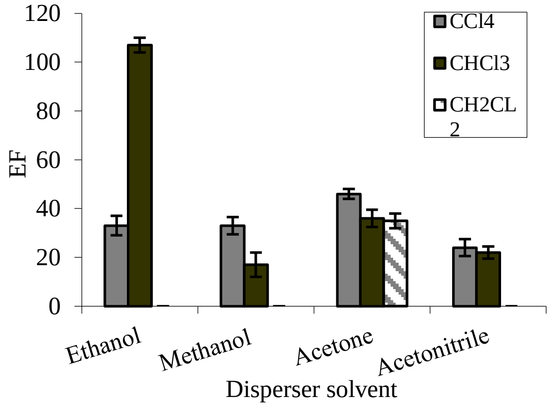
For the DLLME method, miscibility of disperser solvent with extraction solvent and aqueous phase (sample solution) is the main point for the selection of disperser solvent. Therefore, acetonitrile, acetone, ethanol, and methanol were tested as disperser solvent.

In Figure 1 the EF is shown for all combinations of disperser and extraction solvents. For dichloromethane as extraction solvent two-phase system was just observed by acetone as dispersive solvent, on the other hand for methanol, ethanol or acetonitrile as dispersive solvents no sediment phase was obtained with dichloromethane, hence these conditions are useless. Considering the sediment phase volume it was found that with the combination of chloroform and carbon tetrachloride with methanol the sediment phase volume was about 370µL and with acetonitrile the sediment phase volume was about 480µL, therefore these combinations had low efficiency factor. Chloroform with acetone showed attainable recovery but it showed low EF.

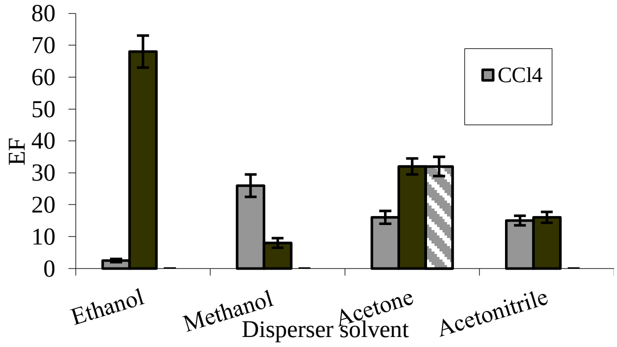
Among these different combinations of extraction and dispersive solvents, chloroform-ethanol showed the highest efficiency and reasonable recovery, therefore, chloroform and ethanol were selected as the extraction and dispersive solvents for Cd^{+2} and Pb^{+2} (Fig. 1a and 1b) respectively.

Effect of volume of the disperser and extraction solvent

The influence of the disperser solvent amount on the extraction efficiency was tested over the ranges of 0–4mL of ethanol as disperser solvent which contain 0.1M APDC and 200µL volume of chloroform as extraction solvent. As shown in Figure 2a, the efficiency factor of cadmium and lead ions increased by increasing of the ethanol volumes but in the case of 1.5mL ethanol the highest recovery and a reasonable EF and sediment phase volume are attainable, therefore we chose 1.5mL ethanol as optimum volume of disperser solvent for Cd^{+2} and Pb^{+2} DLLME procedure.



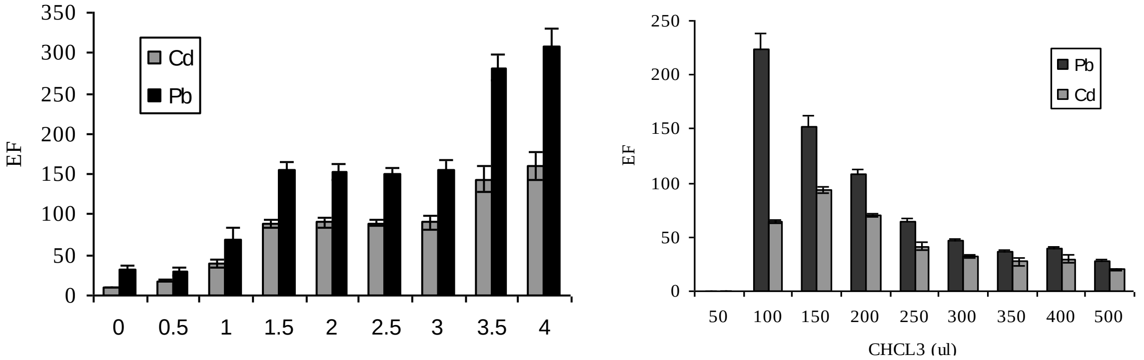
(a)



(b)

Fig.1 Selection of extraction and disperser solvents in DLLME, Conditions: sample, 5mL of (a) Cd^{+2} and (b) Pb^{+2} 0.5µgmL^{-1}; volume of dispersive solvent, 2mL; volume of extracting solvent, 200µL; pH= 4 and APDC 0.1M. The bars show the maximum and minimum levels of determinations.

To evaluate the effect of the extraction solvent volume, a series of sample solutions were tested using 2mL of ethanol (disperser solvent), which contains 0.1M of APDC and different volumes of chloroform (extraction solvent) in the range of 50-500µL. As can be seen from the Figure 2b there was no sediment organic phase in the case of 50µL then the system became useless. By using 100µL chloroform, on the other hand, EF 230 and 75 and recovery 48 and 35% were obtained for lead and cadmium ions respectively. The volume of sediment phase was very low in this condition. In the case of 150µL chloroform high recovery (for both ions more than 90%) and reasonable EF was achieved, therefore 150µL chloroform was selected as optimum value for further studies.



(a)
(b)

Fig.2 Effect of extraction and disperser solvent volume, other conditions are the same as Fig. 1; (a) volume of extracting solvent, 200µL (b) volume of dispersive solvent, 2mL.

Effect of APDC concentration

The influence of the APDC concentration on the DLLME extraction of Cd and Pb was evaluated in the concentration range of 0.002 to 0.20 M. The results (Fig.3) showed that EF of both ions was decreased by decreasing of APDC amount in the range of 0.1 and 0.002M but there was no significant difference (p-value=0.05) in EF between 0.1 and 0.2M of APDC for both ions, therefore 0.1M of chelating agent was employed as optimum value for further studies.

Effect of pH

The separation of metal ions by dispersive liquid–liquid microextraction involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of the sediment phase. PH can play a unique role on metal–chelate formation and the producing extractable species from ionic analyses prior to extraction by DLLME. The effect of pH on the complex formation and extraction of cadmium and lead ions from water samples was studied in the range of 2–12 by using HNO_3, CH_3COONa and NH_3. The results illustrated in Figure 4 reveal that the production of metal-chelate and its extraction is pH-independent between pH 2 and 10 and pH does not influence the sediment phase volume. In this range the highest FE and recovery are obtained. Thus pH 4 was selected for following studies.

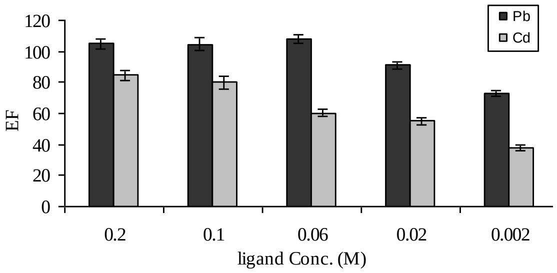


Fig.3 Effect of chelating agent concentration**.** Other conditions are the same as Fig. 1.

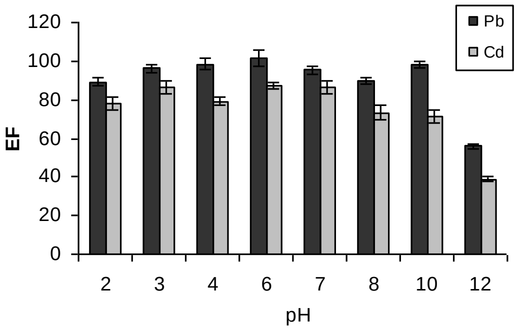


Fig.4 Effect of pH. Other conditions are the same as Fig. 1.

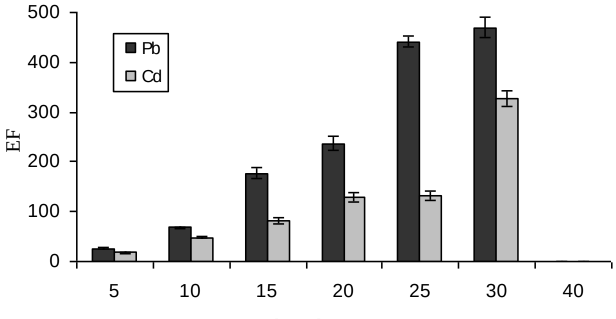


Fig.5 Effect of sample size on the EF in the extraction of Pb^{+2} and Cd^{+2} ions by DLLME. Other conditions are the same as Fig. 1.

Effect of sample volume

Different volumes of sample solution (5–50mL) were used to study the effect of sample size with a constant volume of extracting solvent (200µL) and dispersive solvent (2mL). The results (Fig.5) showed that with a large sample size (40-50 mL) no sediment phase was obtained. By increasing the sample size from 5 to 30mL, the volume of sediment phase and recovery decreased whereas EF increased. With 2mL ethanol and 200µL chloroform, a higher recovery and reasonable EF and sediment phase volume for sample size of 5mL was achieved thus sample size 5mL was chosen in the further studies.

Effect of other parameters

Extraction time, centrifuge speed and salt effect are important factors influencing the EF in DLLME. Extraction time is defined as the time between injection mixture of disperser and extraction solvent, and starting to centrifuge. The dependence of the EF upon the extraction time was studied within the range of 5s–60 min. It was found extraction time has no significant influence on the EF of both analyses. This means that DLLME is a kind of fast equilibrium extraction and in the following experiment 5s was selected as extraction time.

Different centrifuge speed (1000-5000rpm) in a constant time (5min) was studied for illustration of centrifuge speed on EF in DLLME of lead and cadmium, but there was no significant difference (p-value=0.05) between EF of different centrifuge speed. Therefore the 1000rpm in 5min was selected as optimum centrifuge speed.

For investigating the influence of ionic strength on performance of DLLME, various experiments were performed by adding different amount of $NaNO_3$ (0–10% (w/v)) while the rest of the parameters were kept constant. The experiment exhibited the result that the presence of a strong electrolyte had no significant effect on the microextraction efficiency factor. It maybe because of two opposite effects of salt addition in DLLME method. One of them is increasing slightly the volume of sediment phase that decreases the EF and another is salting-out effect that increases the EF. Therefore, the EF is nearly constant by increasing the amount of sodium nitrate. Consequently, all the extraction experiments were performed without salt addition.

Figures of merit

According to IUPAC identification, the limit of detection (LOD, 3δ) of proposed method was 8 and 0.3 ng/mL^{-1} with an enrichment factor of 120 and 70 for Pb and Cd respectively. The relative standard deviation (RSD) was 6.5 and 4.4 %(C=0.5µg mL^{-1}, n=5) for Pb and Cd respectively. The regression equation for calibration curves for Pb and Cd after DLLME were $y=-32.5+2315x$ with correlation coefficient (R^2) of 0.99995 and $y=1111+146300x$ with correlation coefficient (R^2) of 0.99997 respectively where y is signal intensity and x is the concentration of analyses in the extraction solvent.

Interferences effects

The effects of interferences in environmental water samples on the recovery of lead and cadmium were studied. The effect of alkali and alkali earth metals (Li^+, K^+, Na^+, Sr^{+2}, Mg^{+2} and Ca^{+2}) , heavy metal (Mn^{+2}, Fe^{+3}, Cu^{+2},Co^{+2}, Ni^{+2}, Zn^{+2}, Cr^{+3}, Al^{+3}, Si^{+4}) and anions (acetate, sulphate, chloride, nitrate and phosphate) which coexisting in environmental water samples, on the measurement of Cd and Pb was investigated. In these experiments, 5.0mL of solutions contains 0.5µg mL^{-1} of Pb^{+2} and Cd^{+2} and different amounts of foreign ions (analytic: foreign ion ratio for heavy metals 1:1, 1:10 ,1:100 and for alkali, alkaline earth and anions 1:100, 1:1000, 1:2500 and 1:5000) were treated according to the recommended procedure. The tolerable concentration of the coexisting ions defined as the largest amount making 10% variation of the recovery of the analyses in comparison with the case in which interfering ion was absent. The obtained results are given in table 1. Alkali and alkaline earth elements do not react with APDC because of its selectivity. However, large amounts of metal ions which react with APDC appreciably reduce the efficiency of lead and cadmium recovery.

Table 2 Study of interfering ions

Foreign ions	**Tolerance limits(mgL^{-1})**
Si^{+4}, As^{+3}, Co^{+2}, Ni^{+2}, Zn^{+2}, Cr^{+3}, Al^{+3}, Fe^{+3}	1:10
Mn^{+2} , Cu^{+2},	1:100
Li^+, Na^+, K^+	1:5000
Sr^{+2}, Ba^{+2}, Ca^{+2}, Mg^{+2},	1:2500
SO_4^{-2},$H_2PO_4^-$, CH_3COO^-, Cl^-, NO_3^- , PO_4^{-3}	1:5000

Real sample analysis

Tap, mineral, river, dam and sea water samples, used for evaluation of the method, were collected in polypropylene bottles stored at 4°C. Tap water was collected from our laboratory (Tehran, Iran). Dam and river water were collected from Amir Kabir dam and Karaj River (Karaj, Iran). Sea water collocated from Caspian Sea (north of Iran). All of these water samples were firstly filtered through 0.45µm filter and then operated according to DLLME procedure. The real sample analytical results and the recoveries for the spiked samples were shown in Table 3. As can be seen, the recoveries for the spiked real samples ranged from to 93-105% and 92-104% for Pb and Cd respectively. The R.S.D.s for five replicate measurements was from 2.6 to 8.5% for lead and 2.8-9.3% for cadmium.

CONCLUSION

This paper outlined the successful development and application of the DLLME technique, combined with the ICP-AES for the quantitative analysis of lead and cadmium in different natural water samples. High efficiency factor was obtained easily through this method and a detection limit at sub ngmL^{-1} level was achieved with only 5.00mL of sample. In this method sample preparation time as well as consumption of toxic organic solvents was minimized without affecting the sensitivity of the method.

Table 3 Determination of Pb(II) and Cd(II) in five kind of environmental water samples and relative recovery of spiked Pb and Cd in environmental water samples

	Added(μgmL^{-1})		**Found(μgmL^{-1})**		**Recovery (%)**	
	Pb	**Cd**	**Pb**	**Cd**	**Pb**	**Cd**
Tap water	0	0	n.d[a]	n.d		
	1	0.5	1.95	0.49	97	98
Mineral water	0	0	n.d	n.d		
	1	0.5	1.86	0.48	93	96
River water	0	0	n.d	n.d		
	1	0.5	2.045	0.47	102	94
Dam water	0	0	n.d	n.d		
	1	0.5	2.045	0.46	102	92
Sea water	0	0	n.d	n.d		
	1	0.5	2.105	0.52	105	104

[a] n.d: not detected

REFERENCES

1. Fassett D.W., in Metals in the Environment, Academic Press, New York, 1980. pp 61-110.

2. Patriarca M., A. Menditto, B. Rossi, T. D. B. Lyon, G. S. Fell, Environmental exposure to metals of newborns, infants and young children, Microchemical Journal, Volume 67, 2000. Pages 351-361.

3. Hayes R.B., the carcinogenicity of metals in humans, Cancer Causes Control. 1997 May; 8(3):371-85.

4. Bergbäck B., Carlsson M., Heritage of cadmium and lead, A case study of a Swedish accumulator factory. Sci Total Environ 166:35–42,1995.

5. Bellinger D. C., Lead neurotoxicity and socioeconomic status: Conceptual and analytical issues. *Neurotoxicology,* 2008. 29: 828-32.

6. Patocka J., K. Cerny, Inorganic Lead Toxicology, Acta Medica (Hradec Kralove), V. 46, No 2, 65-72, 2003.

7. Waalkes M.P., Cadmium Carcinogenesis, Mutat.Res, 2003. 10:553,107-120

8. Tellez-Plaza M., Navas-Acien A., Crainiceanu C. M., Cadmium exposure and hypertension in the 1999- 2004 National Health and Nutrition Examination Survey (NHANES). *Environmental Health Perspectives,* 2008. 116: 51-6.

9. International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 58: Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry. Summary of Data Reported and Evaluation. 1997. Available at http:// www.monographs.iarc.fr/ENG/Monographs/vol58 /volume58.pdf.Accessed May 1, 2008.

10. Yang R.S.H., Goehl T. J. Brown R. D., Chatham A. T., Arneson D. W., Buchanan R. C., Harris R. K., 1989. Toxicological studies of a chemical mixture of 25 ground water contaminants. I. Chemistry development. Fundam. Appl. Toxicol. 13:366–376.

11**.** Batterham G. J., N.C. Munksgaard , D. L. Parry, Determination of Trace Metals in Sea-water by Inductively Coupled Plasma Mass Spectrometry After Off-line Dithiocarbamate Solvent Extraction, J. Anal. At. Spectrom., 1997, 12, 1277 - 1280

12. Li X., Schramel P., Wang Z., Grill P., Yedile A., Kettrup A., Determination of trace amounts of Sb, Pb, Tl in water samples by inductively coupled plasma atomic emission spectrometry after a diantipyrylmethane-iodide third phase preconcentration, Microchim. Acta, 124:171-178, 1996.

13. Chen J., Xiao S., Wu X., Fang K., Liu W., Determination of lead in water samples by graphite furnace atomic absorption spectrometry after cloud point extraction, Talanta, 67:992-996, 2005.

14. Batterham G. J., Munksgaard N.C., Parry D.L., Determination of Trace Metals in Sea-water by Inductively Coupled Plasma Mass Spectrometry After Off-line Dithiocarbamate Solvent Extraction, J. Anal. At. Spectrom., 1997. 12, 1277 – 1280.

15. Mesquita R. B., Fernandes S. M. V., Rangel A. O. S., A flow system for the spectrophotometric determination of lead in different types of waters using ion-exchange for pre-concentration and elimination of interferences, Talanta 62:395-401, 2004.

16. Dogan C. E., Akcin G., Solid Phase Extraction and Determination of Lead in Water Samples Using Silica Gel Homogeneously Modified by Thiosalicylic Acid, Anal Lett , 40: 2524-2543, 2007.

17. Rezaee M., Assadi Y., Milani Hosseini M. R., Aghaee E., Ahmadi F., Berijani S., Determination of organic compounds in water using dispersive liquid-liquid microextraction ,J chromatogr A 1116:1-9, 2006.

18. Fatahi N., Assadi Y., Milani Hosseini M. R., Zeini Jahromi E., 2007. Determination of chlorophenols in water samples using simultaneous dispersive liquid–liquid microextraction and derivatization followed by gas chromatography-electron-capture detection. J Chromatogr A 1157:23–29

19. Berijani S., Assadi Y., Anbia M., Milani Hosseini M. R., Aghaee E., Dispersive liquid–liquid microextraction combined with gas chromatography-flame photometric detection: Very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water, J Chromatogr A, 1123:1-9, 2006.

20. Salahinejad M., Aflaki F., Optimization and determination of Cd (II) in different environmental water samples with dispersive liquid–liquid microextraction preconcentration combined with inductively coupled plasma optical emission spectrometry, Environ. Monit. Assess. 177, 2011. 115-125

21. Liang P., Sang H., Determination of trace lead in biological and water samples with dispersive liquid–liquid microextraction preconcentration, Anal. Biochem. 380, 2008. 21–25

22. Jiang H., Qin Y., Hu B., Dispersive liquid phase microextraction (DLPME) combined with graphite furnace atomic absorption spectrometry (GFAAS) for determination of trace Co and Ni in environmental water and rice samples, Talanta 74, 2008. 1160–1165.