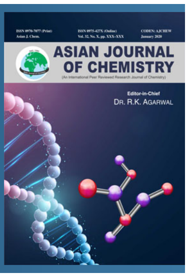
Asian Journal of Chemistry; Vol. 32, No. 12 (2020), 3191-3196

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22863



Spectrophotometric Determination of Zirconium by Dispersive Liquid-Liquid Microextraction based on Solidification of Floating Organic Droplets

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Received: 18 June 2020; *Accepted*: 30 October 2020; *Published online*: 7 December 2020; AJC-20160

In this study, a new method was developed for the spectrophotometric determination of zirconium by the procedure of dispersive liquid-liquid microextraction based on the solidification of floating organic droplets (DLLME-SFO). *o*-Nitrobenzene-azopyrocatechol (*o*-NBAP) and non-ionic surfactant (OP-10) were used in the complex formation. At the first stage, a separation and solidification processes were carried out, after which the solidified samples were examined by spectrophotometric method. The effect of various parameters *viz*. pH effect, ion resistance, ligand effect, volume and type of extraction and dispersion solution, extraction time and temperature effect were studied. The results of the experiment were optimized with design programs. The calibration curve was linear ranging from 0.5 to70 μg L^{-1}, with a correlation coefficient of 0.998. The limit of detection (LOD) is 0.12 μg L^{-1}, the limit of quantification (LOQ) is 0.40 μg L^{-1} and the relative standard deviation (RSD) at 60 μg L^{-1} is 1.6% (n=6). This method was also applied to determine zirconium in various water samples. The obtained reextraction amount was 98-110%.

Keywords: Dispersive liquid-liquid microextraction, Placked Burman, Box Behnken.

INTRODUCTION

Dispersive liquid-liquid microextraction method (DLLME) was first used for the determination of phosphorus-organic pesticides, chlorobenzenes and later for the determination of organic and inorganic compounds in water, food, plant and soil samples [1]. The method enables to perform both the process of solidification (enrichment) and separation at the same time. Therefore, it is an easy, fast, reliable and accurate method to analyze very small samples of heavy metal ions. High volumes of solvents are used in the classical liquid-liquid extraction method and the solvents are mostly toxic. In order to eradicate this problem, drop-in-drop system [2], single-drop microextraction (SDME) [3,4], homogeneous liquid-liquid microextraction (HLLME) [5,6], solid phase microextraction (SPME) [7,8] and dispersive liquid-liquid microextraction (DLLME) [9-11] methods have been developed. The solid floating organic droplet microextraction (SFODME) method has a number of advantages [12-14]. The SFODME method is a easy, simple, inexpensive method, comsume small volume and non-toxic solvents, moreover, does not have a complex mechanism and gives accurate results in quick time.

Dispersive liquid-liquid microextraction method (DLLME) method, combined with several other methods have also been applied. for example, DLLME and gas chromatography were combined for the determination and extraction of polycyclic aromatic hydrocarbons [9]. HPLC has been combined with DLLME for the determination of antioxidants in various plants. Later, 100% recovery and 2-3 times enrichment factor have been obtained by this method [15]. Apart from organic compounds, DLLME has been used in combination with other methods to determine inorganic compunds too. Element atoms [16-22] have been analyzed in water samples by combining DLLME with electrothermal atomic absorption spectroscopy (ETAAS), flame atomic absorption spectrophotometry (FAAS) [23-26], solid phase extraction (SPE) [27-29], solidified floating organic drop (SFOD) [30,31] and supercritical fluid extraction (SFE) [32].

EXPERIMENTAL

Optical density of coloured solutions was measured on JENWAY 6300 spectrophotometer using quartz microcell. A Denley bench centrifuge model BS400 (Denley Instruments Ltd., Billingshurst, UK) was used to accelerate the phase separation. A Hamilton syringe was used for rapid injection (Hamilton Company, USA). The pH of all solutions was monitored using Cond./TDS/Temp universal pH-meter.

Reagents and solutions: A 5 × 10^{-3} mol L^{-1} solution of *o*-nitrobenzene-azopyrocatechol (Fig. 1) was prepared in acetone. A standard zirconium solution with a concentration of 135 µg mL^{-1} has been prepared by dissolving high purity metallic zirconium in a mixture of HF + $HClO_4$. Then hydrofluoride acid was allowed to evaporate, separated and stirred with 4 M $HClO_4$ and 5 M H_2SO_4 [33]. A working solution of Zr(IV) (27 µg mL^{-1}) was obtained by distilling primary solution with distilled water. Non-ionic surfactant (OP-10) solution (0.1%) were prepared by dissolving 10 mg substances in 10 mL distilled water. Ethanol, methanol, acetone and acetonitrile solutions were used to obtain the dispersed system. Extraction solvent solutions *viz.* 1-undecanol (0.83 g L^{-1}), 1-dodecanol (0.83 g L^{-1}), 2-dodecanol (0.80 g L^{-1}), *n*-hexadecane (0.77 g L^{-1}) were used. For pH solution regulation, 0.5 mol L^{-1} HNO_3 and NH_3 were used.

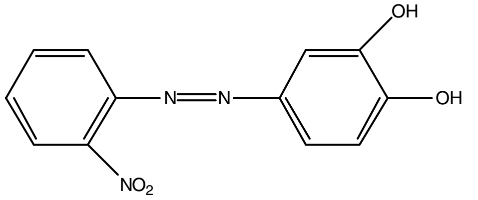

Fig. 1. Structure of *ortho*-nitrobenzene azopyrocatechol

Method: A mixture consisting of 1-undecanol 200 µL^{-1} (extractor), ethanol 300 µL^{-1} (dispersant), 200 µL^{-1} OP-10 (non-ionic surfactant) and *o*-nitrobenzene-azopyrocatechol (0.7 mL) (complexing agent) was added to a buffered zirconium solution (5 µg mL^{-1}, pH = 2.5). The total volume was made up to the level of 10 mL by adding distilled water. An obtained turbid solution was shaken for 2 min, then rotated with a centrifuge at 3000 rpm for 5 min. Since the density of the organic phase is less than the density of the aqueous phase, it accumulates on the surface of the solution. The organic phase was placed in the refrigerator and after 15 min the frozen organic phase is transferred to a separate test tube. The frozen organic phase after thawing was diluted with ethanol up to 250 µL. The optical density was then measured on a spectrophotometer at λ = 510 nm.

RESULTS AND DISCUSSION

Effect of extraction solvent: When extracting metal complexes by dispersive liquid-liquid microextraction and floating organic droplet solidification (DLLME-SFO), the extraction solvent must be poorly soluble in water to achieve high recovery and enrichment, however it must be well soluble in the dispersant solvent. It should also be denser than water with a melting point near room temperature. For this purpose, the extraction capacity of of 1-undecanol (density: 0.83 g mL^{-1}, melting point: 15 ºC), 1-dodecanol (density: 0.83 g mL^{-1}, melting point: 24 ºC), 2-dodecanol (density: 0.80 g mL^{-1}, melting point: 19 ºC) and *n*-hexadecane (density: 0.756 g mL^{-1}, melting point: 6 ºC) solvents was examined. *n*-Hexadecane was poorly soluble in the dispersant solvent and formed an emulsion, while the extraction capacity of 2-dodecanol and 1-dodecanol was weaker than that of 1-undecanol. Therefore, 1-undecanol was chosen as the extraction solvent. 1-Undecanol is less volatile and has less toxic properties.

When the volume of the organic phase is reduced relative to the volume of the water phase, the reextraction factor will increase, but the efficiency of the extraction process will increase. Therefore, effect of 1-undecanol volume on extraction was studied. For this purpose, the effect of 1-undecanol samples on extraction in different volumes (100, 150, 200, 250, 300 µL) was examined. The absorption value was the highest when the volume of 1-undecanol was 200 µL and the absorption value did not increase with subsequent volume increase. Thus, maximum condensation and extraction efficiency was obtained when 1-undecanol of 200 µL was used and the optimum volume of extraction solvent was selected. The effect of the type of extraction solvents and their volume on the recovery is shown in Fig. 2.

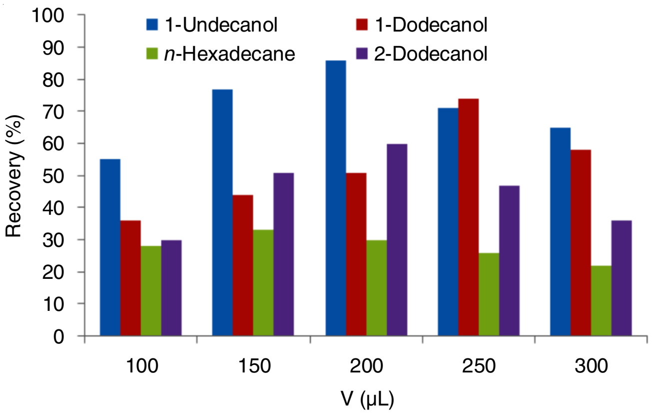

Fig. 2. Effect of type and volume of extraction solvents on recovery. Extraction conditions: sample volume of 10 mL, concentration of zirconium 5 µg mL^{-1}, dispersive solvent 300 µL^{-1} ethanol, complexing agent 129.5 µg L^{-1}, OP-10 200 µL^{-1}, pH = 2.5

Effect of dispersive solvent: The dispersive solvent must be well soluble in both water and the extraction solvent, as the dispersive solvent must dissolve 1-undecanol droplets in the aqueous phase. Because the dipole moment of ethanol is close to the dipole moment of water (water: 1.87D, ethanol: 1.66D, methanol: 2.87D, acetone: 2.69D, acetonitrile: 3.44D) and because it is an active surfactant in the system, the surface activity between the two phases decreases and facilitates the formation of organic phase droplets in the aqueous phase. 1-Undecanol did not disperse well in the aqueous phase when the volume of ethanol was less than 300.0 µL and no cloudy form was shaped when it was more than 350 µL. Therefore, the optimal volume of the dispersive solvent was selected to be 300 µL. The effect of the type and volume of dispersive solvents on the recovery is shown in Fig. 3.

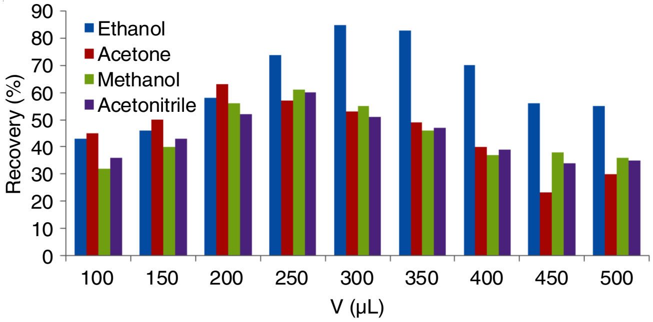

Fig. 3. Effect of type and volume of dispersive solvent on recovery. sample volume of 10 mL, concentration of zirconium 5 µg mL^{-1}, complexing agent 129.5 µg L^{-1}, 1-undecanol 200 µL^{-1},OP-10 200 µL^{-1}, pH = 2.5

Effect of pH: The pH of the sample solution has a great impact on the formation of the metal complex and its subsequent extraction into the organic phase. For this purpose, samples were prepared in the range of pH 1-5 and the effect of pH on the extraction of zirconium complex was studied (Fig. 4). In the range of pH = 1.5-3.0, the extraction and absorption of the complex were maximal, at pH = 1.5 the protonization of *o*-NBAP occurs and the its complex formed by zirconium dissolves, at pH > 3.5 the extraction weakens and at pH > 5 it hydrolyzes zirconium ions and as a result the solution becomes turbid and sediment is formed. Therefore, the optimal value of pH was chosen pH = 2.5. If the metal complex is well soluble in the extraction solvent, it will be better extracted.

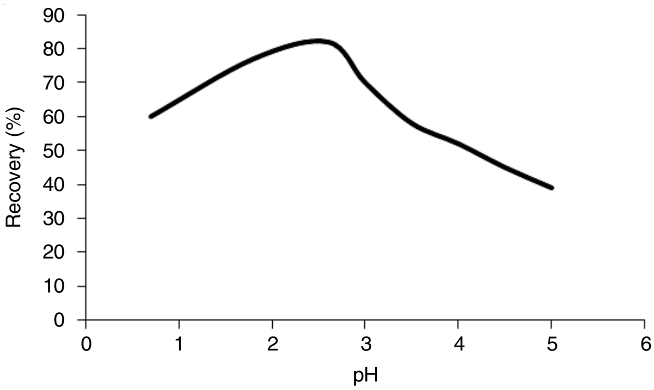

Fig. 4. Effect of pH on recovery. Sample volume of 10 mL, concentration of zirconium 5 µg mL^{-1},complexing agent 129.5 µg L^{-1}, 1-undecanol 200 µL^{-1}, OP-10 200 µL^{-1}, dispersive solvent 300 µL^{-1} ethanol

Effect of ligand and active surfactant concentrations: If the metal complex is stable, less complex forming ligand is used during its extraction. To determine the amount of ligand in which the reagent forms the best complex with zirconium ions, different amounts of ligand samples at the appropriate pH were added to the sample containing metal ions. It was found out that,the recovery of Zr(IV) complex was higher when the concentration of *o*-NBAP was 3.5×10^{-5} mol L^{-1} and the extraction recovery was stable in the subsequent high concentrations of the ligand. Therefore, the concentration of the ligand taken in the experiment with the highest recovery was chosen as the optimal concentration. Volumes of 100, 150, 200, 250 and 300 µL of 0.1 % OP-10 solution were used to study the effect of OP-10 on the recovery. Better results were obtained when the volume of OP-10 was 200 µL and therefore, 200 µL was chosen for further studies.

Effect of other experimental conditions: The addition of salt facilitates the extraction of metal complexes into the organic phase in the liquid-liquid extraction method. For this purpose, NaCl concentration was changed between 0.0-3.0 mol L^{-1}. The results showed that the efficiency of extraction was not affected by the concentration of NaCl. Therefore, the method of salt addition was not used.

In the temperature range of 25-40 ºC and the volume of 10-50 mL of aqueous phase, the extraction recovery was high and in parallel, the colour intensity of the complex does not decrease.

The mixed solution is rotated with the centrifuge to separate the organic phase from the aqueous phase. The speed of the centrifuge was changed in the range of 2000-5000 rpm and it was observed that the organic phase was better separated when the speed was 3000 rpm. The effect of centrifugation time on extraction recovery was also studied and the results of experiments performed in the interval of 2-10 min were compared, the best results were obtained during 5 min of centrifugation.

Analytical performance: Analytical parameters of the method were measured with standard Zr(IV) solutions. The calibration curve was prepared by selecting the optimal conditions and conducting three parallel experiments. The equation of the calibration curve is A = 0.1608 + 0.3804 (where A = absorbance and C = concentration of zirconium (µg mL^{-1}) in the aqueous phase), the correlation coefficient is 0.998. In the range of 0.5-70 µg L^{-1}of zirconium concentration is subject to Beer's law. The relative standard deviation was 1.6 % for Zr(IV) at a concentration of 60 µg L^{-1} in six repeated experiments. According to the calculations, the limit of detection (LOD) is 0.12 µg L^{-1}, the limit of quantification (LOQ) is 0.40 µg L^{-1}.

Influence of foreign ions: A systematic study of the effect of foreign ions on the selectivity of determination of Zr(IV) was carried out to determine the maximum concentration that causes interference. Natural water is a complex system that contains several cations and anions which have potential sources of interference. In this study, a tolerance limit of the interfering species was fixed at concentrations that caused no more than 5% change in the recovery of a 2 µg L^{-1} Zr(IV) solution (Table-1).

TABLE-1
TOLERANCE LIMITS OF SEVERAL IONS FOR THE RECOVERY OF 2 µg Zr(IV) (RESULTS GIVING ≤ 5.0 % ERROR) USING 1000 mL SAMPLE OPTIMUM CONDITIONS

Ions	Tolerance limit
Na^+, K^+, Mn^{2+}, Mg^{2+}, Ni^{2+}	– 1340
Be^{2+}, Cu^{2+}, NO_3^-, IO_3^-,	+ 1500
Pb^{2+}, Fe^{3+}, Ag^+,	+ 2500
Ti^{4+}, Al^{3+}, Hg^{2+}, Br^-,	– 750
PO_4^{3+}, Co^{2+},	+ 800
Sr^+, Rb^+	– 450

Analysis of zirconium in different water samples: The amount of zirconium was determined in various water samples to examine the accuracy of the method. Samples of zirconium of known concentration were added to 1000 mL of water and

TABLE-2
ANALYSIS OF ZIRCONIUM IN VARIOUS WATER SAMPLES.
A certain amount of zirconium was added to 1000 mL of water

Sample	Amount added (μg L^{-1})	Amount found (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})	Recovery (%)
Tap water	4.0	3.98	0.11	0.42	98
River water	3.0	3.01	0.14	0.44	97
Industrial waste water	6.0	5.97	0.09	0.35	93
Polluted water	5.0	4.95	0.12	0.40	92

TABLE-3
COMPARISON OF THIS TECHNIQUE WITH REPORTED METHODS

Reagent	Analized obyect	Linear range	λ_{max} (nm)	Molar absorptivity × 10^4 (L mol^{-1} cm^{-1})	pH	Ref.
2, 2', 3, 4-tetrahydroxy-3'-sulpho-5'-carboxyazobenzene (tetrahydroxyazon SC)	Alloys	0.10-2.80 mg mL^{-1}	495	4.9	6.0	[34]
Dibromo-*p*-chloroarsenazo7 (DBC-ASA)	Water samples	0-1.20 μg mL^{-1}	560	4.56		[35]
1,4-Dichloro-2,5-dihydroxyquinone	Alloys	0-5 μg mL^{-1}	331	1.6	3.0	[36]
2-(5-Bromo-2-pyridylazo)-5-(diethylamino) phenol.	Water, artificial saliva and dental materials	1.4-714 μg mL^{-1}	578		4.5	[37]
2-Hydroxy naphthaldehyde-*p*-hydroxybenzoic hydrozone	Steel samples	0.456-4.56 μg mL^{-1}	415	0.986 ± 0.002	1.0	[38]
5,7-Dibromo-8-hydroxyquinoline	Wide variety of samples	0.2-9.0 μg mL^{-1}	416	1.05		[39]
ortho-Nitrobenzene-azopyrocatechol	Water samples	0.1-70 μg mL^{-1}	510	1.8	2.5	This study

analyzed by the proposed method. The limit of detection (LOD) and the limit of quantification (LOQ) of the zirconium, as well as the absorption recovery were determined in each water sample. The best analytical results were obtained with tap water. The results are shown in Table-2.

Compared to different methods of analysis of zirconium (according to Beer-Lambert's law and the limit of detection), this method can be considered a sensitive and selective method (Table-3). The simplicity of the method allows to perform analysis in the laboratory with less time and simple devices.

Statistical calculations and optimization of results: Box Behnken design was used to optimize the extraction process of zirconium(IV) complex by DLLME-SFO method (Minitab 19). First, a normality test was performed for graphs of factors affecting the recovery. Based on histogram, variation coefficient skewness kurtosis, detrended, test of normality (Shapiro Wilk), it was decided to optimize statistical calculations. Plackett Burman statistics were used to determine which of the 8 variables that could influence the results of the analysis were "effective" or "ineffective". For this purpose, a design matrix consisting of 12 experiments with 8 factors was prepared.

The Plackett Burman design is based on Hadamart matrices and the data are primarily polynomial:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + ...\beta_iX_i$$

where, Y = estimated response function, β_o = constant, β_1-β_i = coefficient of the factors, and X_1-X_i = factors. The variables are the volume of extraction solvent, dispersive solvent, aqueous phase, pH, concentration of the ligand, centrifugation time, centrifugation speed and the temperature. The values of the independent variables calculated by the Plackett Burman design are shown in Table-4.

TABLE-4
POINTS OF DEPENDENT VARIABLES
CALCULATED BY PLACKETT BURMAN DESIGN

Non-dependent variables	Symbol	Low point	Upper point
Volume of extraction solvent (μL)	VES	100	300
Volume of dispersion solvent (μL)	VDS	200	450
pH	pH	1	5
Ligand concentration (× 10^{-5} mol L^{-1})	LC	1	4.5
Temperature	T	20	45
Centrifugation speed (rpm)	CS	2000	5000
Centrifugation time (min)	CT	2	10
The volume of the waters phase (mL)	VWP	5	60
The volume of the surfactant (μL)	VS	50	350

During the initial statistical study of the results with the Plackett Burman design, it was determined that the parameters had the greatest impact on the extraction process and the statistically significant were the ligand concentration, pH and the volume of extraction solution (Fig. 5).

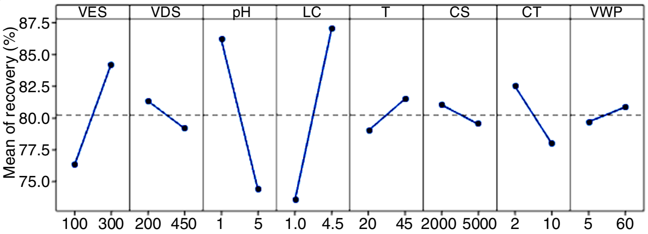

Fig. 5. Factorial plots for recovery for extraction

A three-level, three-factor Box Behnken design was used to optimize the effect of these three parameters on the extraction process. R^2 = 97.41, the p value for pH, VES and LC were 0.009, 0.020 and 0.036, respectively. These values indicate that the mathematical model established for the response variables is meaningful. To evaluate the effects of the factors in more detail, their 3D graphs are shown in Fig. 6.

If the effects of pH and VES were considered at the average value of ligand concentration (LC = 2.75 × 10^{-5} mol L^{-1}), as

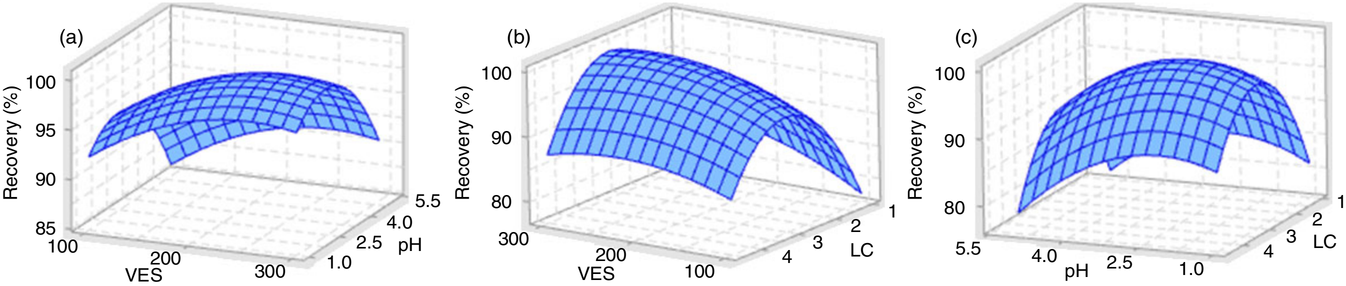

Fig. 6. During extraction (a) VES and pH, (b) VES and LC, (c) effect of pH and LC on recovery

can be seen from the graphs, pH has a greater impact on the recovery (Fig. 6a). In particular, at pH = 2.5, the recovery is up to 95 %. When VES = 200 µL is kept constant, the recovery is still maximal at pH = 2.5 (Fig. 6b). When investigated the effect of SEV and ligand concentration on the recovery at pH = 2.5, it appears that ligand concentration has a greater effect on the recovery (Fig. 6c). According to the results of statistical analysis, the optimal values of independent variables to obtain the highest recovery percentage are pH = 2.5, LC = 2.75×10^{-5} mol L^{-1} and VES = 200 µL. Pareto graph was prepared to show the effect of factors (Fig. 7).

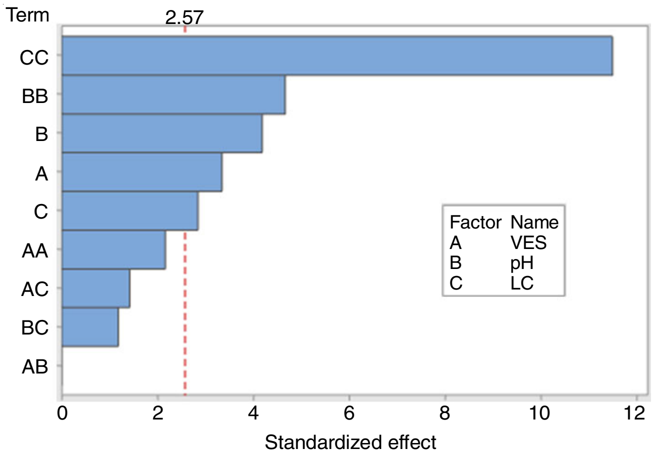

Fig. 7. Pareto analysis of the factors affecting the extraction recovery

Conclusion

By means of DLLME-SFO method, zirconium was determined in the range of 0.5-70 µg mL^{-1}, which obeys Beer's law at the optimal pH = 2.5. Molar extinction coefficient is 1.8×10^4 L mol^{-1} cm^{-1}, λ_{max} = 510 nm. Comparing the analytical performance of this method with other methods of determination of zirconium, it can be said that this method is selective, optional and efficient. Placket Burman design was applied to determine the more important factors as the recovery is affected by a large number of parameters. The most influential factors were the ligand concentration, volume of extraction solution and pH. Applying the Box Behnken design, it was determined that the concentration of the ligand affects the recovery more than other factors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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