



Vortex Assisted Dispersive Liquid-Liquid Microextraction: Determination of Cobalt by HPLC

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

A simple and effective method was developed for preconcentration of cobalt in water samples. In the proposed approach, sodium diethyl dithiocarbamate (DDTC) was used as chelating agent, and vortex-assisted dispersive liquid-liquid microextraction combined with high performance liquid chromatography (HPLC) with UV detection was developed. In this method a small amount of chloroform as extracting solvent is rapidly injected by syringe into the water sample containing cobalt ions complexed by DDTC. This complex is extracted into the fine chloroform droplets and vortex agitation takes place during five minutes. The influence of variables affecting to the microextraction process were studied. Under the selected conditions, the preconcentration factor of 148 was achieved and the relative standard deviations (RSD) were 1.86% and 2.68% for 50 and 20 $\mu\text{g L}^{-1}$ of cobalt, respectively. The proposed method was applied to analysis of water standard reference materials and the recovery range from 90.0 to 110.0%.

Keywords: Cobalt; vortex assisted dispersive liquid-liquid microextraction; HPLC; waters.

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1. INTRODUCTION

In the recent years, pollution of the environment by heavy metals has received considerable attention. Cobalt is an essential trace element present in most body tissues and is a component of vitamin B₁₂, which is involved in the production of the blood red cells and the prevention of pernicious anemia [1], nevertheless excess amounts of cobalt lead to toxic effects. For this reason, it is clear that the determination of cobalt at trace levels is very important in the field of environmental analysis.

Although there are diverse analytical techniques for the determination of trace metal with sufficient sensitivity however, the determination of trace cobalt in natural waters and environmental samples is difficult due to its low concentrations and matrix effects.

On the other hand, despite the advances in analytical instrumentation, sometimes a pretreatment of samples is necessary [2,3].

From the introduction of the first works on liquid-phase microextraction in 1996 [4,5], different approaches of this methodology such as single drop microextraction [6,7], hollow fiber liquid-phase microextraction [8], dispersive liquid-liquid microextraction [9] and solidified floating organic drop microextraction [10], among others, were developed.

In particular, dispersive liquid-liquid microextraction is a very simple and rapid extraction method, based on the use of a ternary component solvent system, which has been applied to the extraction and preconcentration of both organic and inorganic compounds from aqueous samples. This procedure has attracted more and more attention, due to its superior advantages of high enrichment factor, high recovery, and high extraction speed, low cost and easy operation [11,12]. The main drawbacks associated with this approach have been the difficulty to automate and the necessity of using a third component (disperser solvent), which commonly decreases the partition coefficient of analytes into the extractant solvent.

In the year 2010, Yiantzi et al. [13] introduced a new microextraction method termed vortex-assisted liquid-liquid microextraction (VALLME), whereby dispersion of low density extraction solvent into water is obtained through using vortex mixing, a mild emulsification procedure.

The fine droplets can rapidly extract target analytes from water because of the shorter diffusion distance and larger interfacial area. After centrifugation, the floating extractant phase restores its initial single-drop shape for the following instrumental analysis. Recently, applications of VALLME are presented in two interesting review, where the authors principally discuss the application of ultrasonic irradiation, and in minor extension, the application of vortex agitation in solvent microextraction procedures [14,15].

In previous years the authors have published several papers about the use of DLLME for determination of different metal ions by flame atomic absorption spectrometry [16-19].

In this work, we present a method for preconcentration of cobalt from natural waters based on VALLME after complexing this metal ion with DDTc and its final determination by liquid chromatography. Under optimum conditions high preconcentration factor was obtained and for this reason cobalt determination can be carried out at ppb levels by HPLC.

2. EXPERIMENTAL

2.1 Standard Solutions and Reagents

Standard solution for Co(II) (1000 mg L⁻¹) was supplied by Merck, Darmstadt, Germany. Daily, standard solutions were obtained by appropriate dilution.

High purity water (resistivity 18.2 MΩ cm) obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used in all study.

Sodium diethyl dithiocarbamate (DDTC) was supplied by Merck, Darmstadt, Germany. DDTC 0.8% (w/v) solution in water was prepared daily.

All the other reagents were analytical-grade reagents, as well as the reagents mentioned above.

2.2 Instrumentation

The instrumental analysis was carried out with an Agilent HPLC model LC 1220 Infinity equipped with VWD. A Zorbax Eclipse XDB-C18 column (250 mm x 4.6 mm, 5 μm particle size) was employed at room temperature. All injections were made manually with 20 μL sample loop. The signal from the detector was recorder and

integrated with a PC HP Pro 3010 Desktop VN934EA. The methanol/water in 80/20 v/v was used at a flow rate of 1.5 mL/min as a mobile phase. Analyte was monitored at $\lambda = 284$ nm. A VX-2500 Multi-Tube Vortexer was used to mix the samples. Phase separation was achieved with a centrifuge PACISA Orto Tornax in 15 mL calibrated conical tubes.

2.3 Sample Analysis

The method's accuracy was proven by analyzing the CRMs: Fortified lake water (TMDA 54.4), Estuarine water (SLEW-3), Eau de mer (CASS-5), Riverine water (SLRS-5), all supplied by National Research Council of Canada. Standard addition method was used for the analysis of these samples and in the case the content of cobalt was under detection limit of the method, recovery studies were performed. Tap water was collected from our laboratory just before the determination of Co.

2.4 VALLME Procedure

10 mL analyte solution with variable amounts of cobalt, 3 mL acetate buffer solution (0.2 M, pH 5), 400 μ L of 0.8% (w/v) DDTC solution in water as chelating agent was placed in a 15 mL screw cap glass test tube. Then, 3 mL of ethanol (as disperser solvent) were added and 200 μ L of

chloroform (as extraction solvent) was rapidly injected into a sample solution by using a microsyringe. A cloudy solution was formed in the test tube and vortex agitation take place during five minutes. Separation of the phases was achieved by centrifugation at 3800 rpm for 5 min. Schematic diagram is shown in Fig. 1. HPLC method was used as detection technique and chromatogram was registered during 8 minutes and the retention time was 6.5 min as can be seen in Fig. 2. All experiments were performed in triplicate and the mean of results was used in plotting of curves or preparation of tables.

3. RESULTS AND DISCUSSION

Univariate method was employed to study the effect of the parameters affecting to the VALLME procedure.

3.1 pH Study

The separation of metal ions by VALLME requires previous formation of a hydrophobic complex to be extracted into the small volume of the organic phase. pH is an important variable on metal chelate formation and its posterior extraction. The effect of pH was investigated in the range of 3.0–8.0 by using acetate or phosphate buffer [20]. The results reveal that the

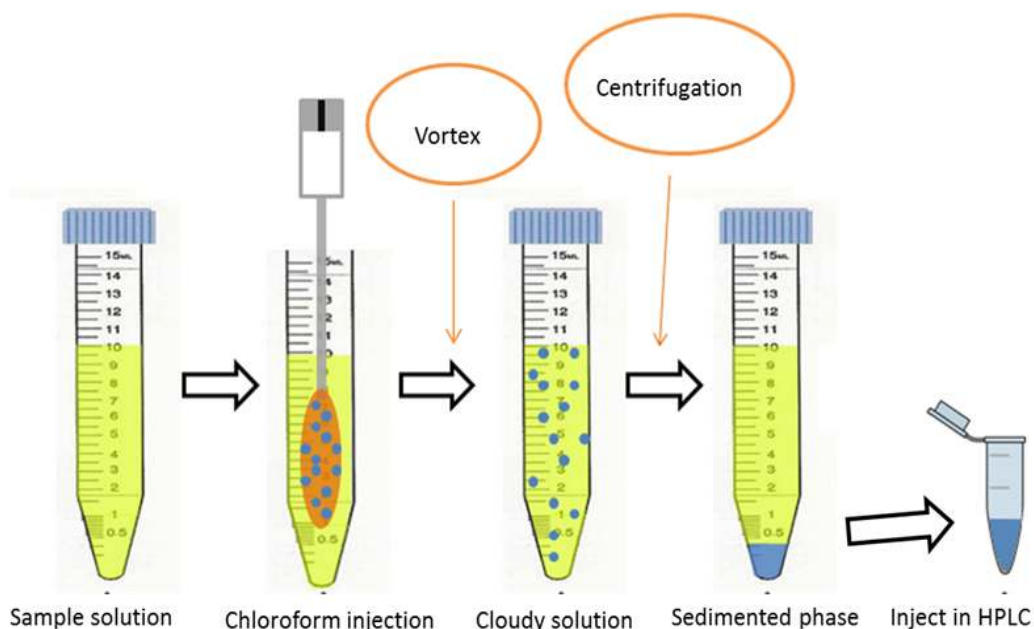


Fig. 1. Schematic diagram

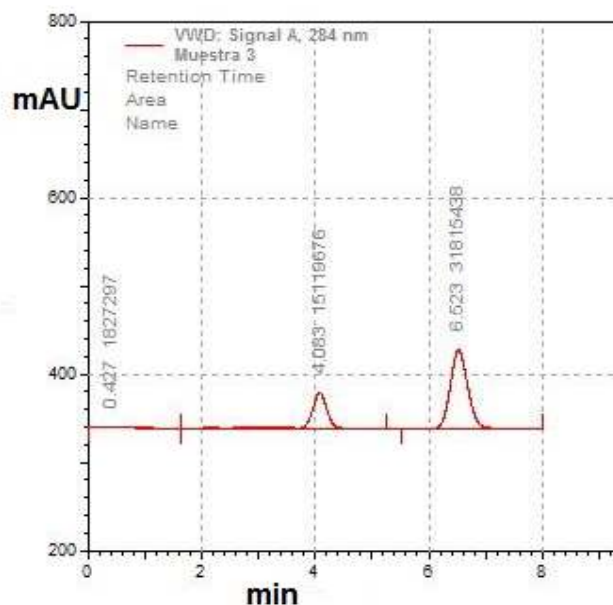


Fig. 2. Chromatogram for Co-DDTC: [Co(II)]=50 $\mu\text{g L}^{-1}$; 0.4 mL DDTC 0.8% (w/v); 3 mL buffer pH 5; 3 mL ethanol; 200 $\mu\text{L HCCl}_3$; five min vortex

absorbance is nearly constant in the pH range of 4.5–6. Thus, the value of pH 5 was selected for the following experiments. Besides, the influence of 0.2 M acetate buffer solution amount was investigated (1-4 mL). The extraction efficiency was constant starting 3 mL. For this reason 3 mL was selected as the optimum value for posterior work.

3.2 Effect of Chelating Reagent (DDTC) Concentration

The effect of DDTC concentration on the signal was examined using increasing volumes of 0.8% (w/v) DDTC from 100 μL to 1000 μL . The effect on analytical signals is shown in Fig. 3, thus the volume of 0.4 mL, was used in other experiments.

3.3 Effect of Ionic Strength

Ionic strength had not effect upon percent recovery and sensitivity for Co extraction when different amounts of NaCl between 0-1% (w/v) were added. Then no NaCl solution was used in subsequent experiment.

3.4 Effect of Type and Volume of Extractant

In this study, chloroform and carbon tetrachloride were compared in the extraction of cobalt.

Results showed that the maximum extraction recovery was obtained by using chloroform. Then the effect of chloroform volume was examined (100-500 μL). When the volume of chloroform was increased, the volume available for the measurement also increased, but the enrichment factors decreased. Thereby, in the following studies, the optimum volume of 200 μL was selected for the chloroform.

3.5 Effect of Type and Volume of Disperser Solvent

In this section the ability of ethanol and methanol was investigated. The results (Fig. 4) show differences between disperser solvents containing 200 μL chloroform or CCl_4 (extraction solvent). As can be seen from this figure better results were obtained by using chloroform and ethanol as disperser solvent. Then, extraction of analytes was carried out by using 1 to 3 mL of ethanol. A 3 mL ethanol was selected for subsequent studies.

3.6 Effect of Vortex Agitation

For this purpose five samples prepared in the same manner were treated at different time between one and six minutes by vortex. Peak area increased gradually when the sample is agitated up five minutes and then remained nearly constant.

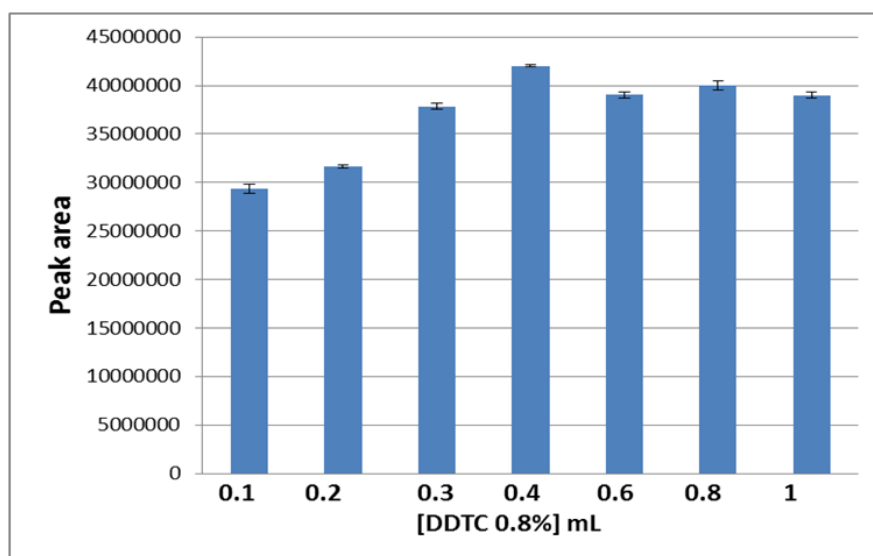


Fig. 3. Influence of reagent volume 0.8% (w/v); [Co(II)]=50 µg L⁻¹

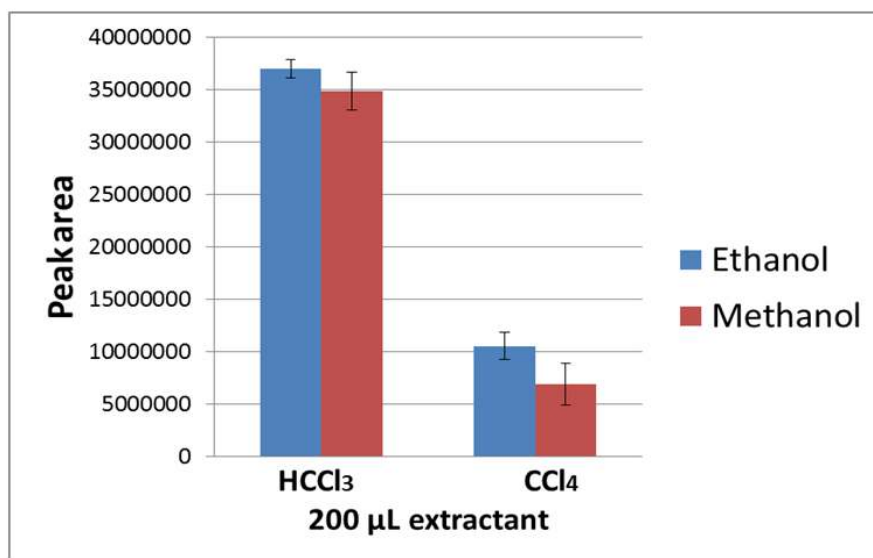


Fig. 4. Influence of disperser and extraction solvents; [Co(II)]=50 µg L⁻¹

3.7 Interferences

Because DDTC is a versatile chelating agent, interferences may occur due to the competition of other heavy metal ions and their subsequent co-extraction with Co(II). For this purpose, the effect of typical potential interfering ions was investigated. The tolerance limit was defined as the concentration of added ion that caused less than ± 5% relative error in the determination of Co. About 50-fold excess of Ni²⁺, Ca²⁺, Ba²⁺, Al³⁺, K⁺, I⁻, F⁻, SO₄⁼ do not affect Co signal. Cr³⁺ does not interfere at 20-fold

excess. Cd²⁺ and Cu²⁺ does not interfere at 5-fold excess and Fe³⁺ can be tolerated at 1:1 ratio.

3.8 Analytical Figures of Merit

Table 1 summarize the analytical figures of merit of the proposed VALLME-HPLC method. The preconcentration factor was determined as the ratio of the slopes of the linear section of the calibration graphs before and after preconcentration, and also by the ratio of the volumes.

Table 1. Analytical figures of merit

	Co
Dynamic range ($\mu\text{g L}^{-1}$)	10-100
Regression equation	Slope: $626754 \pm 12555^{(a)}$ Intercept: $7 \times 10^6 \pm 761981^{(a)}$ $R^2=0.998$
Detection limit ($\mu\text{g L}^{-1}$) [22]	2
Determination limit ($\mu\text{g L}^{-1}$) [22]	10
Regression equation without extraction	$A = 4234[\text{Co}] + 35673$; $R^2=0.988$
Precision (% RSD) n=6	1.86 ($50 \mu\text{g L}^{-1}$ Co) 2.68 ($20 \mu\text{g L}^{-1}$ Co)
Preconcentration factor	
Slope ratio	148
Volume ratio	50

^(a) Standard error for regression equation obtained by Statgraphic program [21]

Table 2. Analysis of four certified reference materials for the determination of Co with VALLME-HPLC method

Sample	Certified ($\mu\text{g L}^{-1}$)	Found* ($\mu\text{g L}^{-1}$)	% Recovery
Fortified lake water. TMDA 54.4	309 ± 27	300 ± 50	97
Sample	Added ($\mu\text{g L}^{-1}$)	Found* ($\mu\text{g L}^{-1}$)	% Recovery
Tap water	50.0	52.0 ± 4.0	104
Eau de mer. CASS-5	10.0	11.1 ± 1.2	111.0
	20.0	18.0 ± 1.0	90.0
	30.0	31.1 ± 2.4	103.6
	50.0	50.3 ± 1.4	100.6
Riverine water. SLRS-5	10.0	11.0 ± 2.0	110.0
	20.0	20.0 ± 3.0	100.0
	30.0	33.0 ± 3.0	110.0
	50.0	50.0 ± 3.0	100.0
Estuarine water. SLEW-3	10.0	10.6 ± 0.5	106.0
	20.0	19.6 ± 0.3	98.0
	30.0	29.6 ± 0.3	98.7
	50.0	49.4 ± 0.2	98.8

*Mean \pm standard deviation; n=3

3.9 Analysis of Real Sample

For this purpose, standard solution containing Co was added to tap water and the resulting material was prepared as described under Experimental. Standard additions method was used to avoid matrix effects in all instances and the results were obtained by extrapolation. The result of this analysis is summarised in Table 2.

3.10 Analysis of Standard Reference Materials

In order to assess the accuracy and validity of the presented procedure, the method was applied to the determination of cobalt in certified reference materials, Fortified lake water (TMDA 54.4), Estuarine water (SLEW-3), Eau de mer (CASS-5), Riverine water (SLRS-5), which were

analyzed according to the proposed method. Standard additions method was used to avoid matrix effects. It was found that analytical results were in good agreement with the certified values (Table 2). Contents of Co in CASS-5, SLRS-5 and SLEW-3 were under detection limits of the method, so different amounts of Co ion were added to the samples for to verify if it is possible to determine Co under the proposed procedure. Good recoveries were obtained in all cases.

4. CONCLUSIONS

A fast, simple and environmentally friendly sample preparation method termed vortex-assisted dispersive liquid-liquid microextraction combined with HPLC-UV has been investigated. In this procedure, a vortex-assisted process was applied to accelerate the formation of the fine

cloud, which significantly increased extraction efficiency and reduced extraction time. Under optimum conditions, the preconcentration factor was 148 and so, cobalt determination can be carried out by HPLC at ppb levels. Notwithstanding atomic spectrometric techniques are frequently utilized in the determination of metal ions principally flame atomic absorption spectrometry, required samples at milliliter volumes. Besides spectrometric techniques such as inductively coupled plasma-mass spectrometry are costly and required complicated instruments. On the other hand, VALLME is a microextraction technique where the analyte is extracted in a small volume of the order of microlitres, compatible with HPLC [23]. All variables that influence in the formation of the complex Co-DDTC and then application of VALLME procedure have been optimized. To study the accuracy of the proposed method, certified reference materials have been analysed with good agreements.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nordberg GF, Fowler BA, Nordberg M, Friberg LT. Handbook on the toxicology of metals. Elsevier: Amsterdam; 2007.
2. Cruz-Vera M, Lucena R, Cardenas S, Valcarcel M. Sample treatments based on dispersive (micro) extraction. *Anal. Methods*. 2011;3:1719–1728.
3. Yan H, Wang H. Recent development and applications of dispersive liquid-liquid microextraction. *J Chromatogr A*. 2013;1295:1–15.
4. Liu H, Dasgupta PK. Analytical chemistry in a drop, solvent extraction in a microdrop. *Anal. Chem*. 1996;68:1817–1821.
5. Jeannot MA, Cantwell FF. Solvent microextraction into a single drop. *Anal. Chem*. 1996;68:2236–2240.
6. He Y, Lee HK. Liquid-phase microextraction in a single drop of organic solvent by using a conventional microsyringe. *Anal. Chem*. 1997;69:4634–4640.
7. de Jager LS, Andrews ARJ. Solvent microextraction of chlorinated pesticides. *Chromatographia*. 1999;50:733–738.
8. Pedersen-Bjergaard S, Rasmussen KE. Liquid-liquid-liquid microextraction for sample preparation of biological fluid prior to capillary electrophoresis. *Anal. Chem*. 1999;71:2650–2656.
9. Rezaee M, Assadi Y, Hosseini MRM, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid-liquid microextraction. *J. Chromatogr. A*. 2006;1116:1–9.
10. Zanjani MRK, Yamini Y, Shariati S, Jonsson JA. A new liquid-phase microextraction method based on solidification of floating organic drop. *Anal. Chim. Acta*. 2007;585:286–293.
11. Ojeda CB, Rojas FS. Separation and preconcentration by dispersive liquid-liquid microextraction procedure: A review. *Chromatographia*. 2009;69:1149–1159.
12. Ojeda CB, Rojas FS. Separation and preconcentration by dispersive liquid-liquid microextraction procedure: Recent applications. *Chromatographia*. 2011;74:651–679.
13. Yiantzi E, Psillakis E, Tyrovola K, Kalogerakis N. Vortex-assisted liquid-liquid microextraction of octylphenol, nonylphenol and bisphenol-A. *Talanta* 80. 2010;2057–2062.
14. Ojeda CB, Rojas FS. Vortex-assisted liquid-liquid microextraction (VALLME): Applications. *Chromatographia*. 2014;77:745–754.
15. Andruch V, Burdel M, Kocúrová L, Sandrejová J, Balog IS. Application of ultrasonic irradiation and vortex agitation in solvent microextraction. *Trends Anal. Chem*. 2013;49:1–19.
16. Rojas FS, Ojeda CB. Dispersive liquid-liquid microextraction combined with flame atomic absorption spectrometry for determination of cadmium in environmental, water and food samples. *Anal. Methods*. 2011;3:1652–1655.
17. Ojeda CB, Rojas FS, Pavón JMC. Determination of cobalt in food, environmental and water samples with preconcentration by dispersive liquid-liquid

- microextraction. American J. Anal. Chem. 2012;3:125–130.
18. Rojas FS, Ojeda CB, Pavón JMC. Simultaneous separation/preconcentration of nickel and cobalt by dispersive liquid-liquid microextraction prior to determination by FAAS. European Scientific J. 2013;9:20–31.
19. Ojeda CB, Rojas FS, Pavón JMC. Preconcentration of copper from different samples by dispersive liquid-liquid microextraction. European Scientific J. 2013;9:1857–7431.
20. Lemos VA, Vieira EVS. Method for the determination of cadmium, lead, nickel, cobalt and copper in seafood after dispersive liquid-liquid microextraction. Food Additives & Contaminants: Part A. 2014;31:1872–1878.
21. Statgraphics® Centurion XVI, © 2010 StatPoint Technologies, Inc. Available:www.STATGRAPHICS.com
22. Miller JN, Miller JC. Statistics and chemometrics for analytical chemistry. Sixth Edition, Pearson Education Limited; 2010. ISBN: 978-0-273-73042-2
23. Farajzadeh MA, Bahram M, Vardast MR. Optimization of dispersive liquid–liquid microextraction of Co(II) and Fe(III) as their oxinate chelates and analysis by HPLC: Application for the simultaneous determination of Co(II) and Fe(III) in water samples. J. Sep. Sci. 2009;32:4200–4212.

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