Ultra-Trace Determination of Co (II) in Real Samples Using Ion Pair-Based Dispersive Liquid-Liquid Microextraction Followed by Electrothermal Atomic Absorption Spectrometry

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Abstract Ion pair-based dispersive liquid-liquid microextraction technique was used for preconcentration and determination of ultra-trace levels of Co (II) followed by electrothermal atomic absorption spectrometry (ETAAS). Thiocyanate (SCN^-) forms an anionic complex with Co (II) followed by addition of cetylpyridinium chloride (CPC) as a positive counterion to produce hydrophobic cobalt-thiocyanate-CPC complex. The resulting hydrophobic complex was extracted into the fine droplets of carbon tetrachloride by dispersive liquid-liquid microextraction (DLLME). In DLLME, a mixture of 1.5 mL of acetone (as disperser solvent) containing 40 µL of carbon tetrachloride (as extraction solvent) was rapidly injected into the sample solution to extract the hydrophobic cobalt-thiocyanate-CPC complex. Under the optimum conditions, the calibration curve was linear in the range of 0.08–1.5 µg L^{-1} of Co (II) with a correlation coefficient of 0.9997. The relative standard deviation (RSD, %) based on six replicate analyses of 0.5 µg L^{-1} of Co (II) was 3.7 %, and the limit of detection (LOD) was 0.02 µg L^{-1}. The accuracy of the proposed method was evaluated by the analysis of a certified reference material and spike method. The proposed method was successfully applied for determination of ultra-trace levels of Co (II) in different water samples and spinach leaves.

Electrothermal atomic absorption spectrometry · Food and water analysis

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Introduction

Cobalt is essential for human and animals because it is a part of vitamin B_{12}. However, it can be harmful at high levels since its accumulation promotes organ damage and dysfunction due to enhanced oxidative stress (Nordberg et al. 2007). Different techniques including atomic absorption spectrometry (Citak and Tuzen 2010; Jafarvand and Shemirani 2011), UV-vis spectrophotometry (Gharehbaghi et al. 2008; Gharehbaghi et al. 2009), and inductively coupled plasma optical emission spectrometry (Ranjbar et al. 2012: Cerutti et al. 2003) have been used for determination of cobalt in different real samples. Electrothermal atomic absorption spectrometry (ETAAS) is one of the most commonly used analytical techniques for determination of heavy metals. However, to obtain reliable results at trace levels and eliminating the effects of interfering ions, sample preparation techniques are necessary. A variety of procedures for preconcentration of cobalt, such as solid phase extraction (SPE) (Ghaedi et al. 2007; Dadfarnia et al. 2013; Praveen et al. 2005; Pourreza et al. 2010; Azizi et al. 2015), liquid-liquid microextraction (LLME) (Chamsaz et al. 2013; Rajabi et al. 2014; Hosseini et al. 2014; Bahar and Babamiri 2015; Mohammadi et al. 2011), and cloud point extraction (CPE) (Bahram and Khezri 2012; Safavi et al. 2004; Ghaedi et al. 2008; Han et al. 2015), have been developed. Dispersive liquid-liquid microextraction (DLLME) is a kind of liquid phase microextraction technique which has advantages such as ease of operation, use of small amounts of sample and organic solvents, speed of analysis, low cost, and high recoveries (Rezaee et al. 2006; Chamsaz et al. 2014). In DLLME, an appropriate mixture of a water-immiscible

extraction solvent and a water-miscible dispersive solvent is rapidly injected into an aqueous sample solution. This forms a cloudy solution that increases the contact surface between the organic and aqueous phases and therefore reduces the extraction time.

One of the multiple options for the extraction of metallic ions by LLME is based on the formation of ion pairs followed by their extraction in organic solvents. DLLME was used to preconcentrate the ion pair in a microliter volume of organic solvent. In this paper, ion pair-based DLLME has been used as a rapid, very sensitive, and low-cost technique for preconcentration and determination of ultra-trace levels of Co (II) followed by ETAAS. Thiocyanate (SCN^-) was used to form the anionic complex of cobalt-thiocyanate followed by addition of cetylpyridinium chloride (CPC) as a positive counterion. The resulting hydrophobic complex of cobalt-thiocyanate-CPC was extracted into the extraction solvent by DLLME. Based on our knowledge, there is no report on the use of thiocyanate anion as a complexing agent for LLME of cobalt ion. The effects of different parameters affecting the extraction efficiency were investigated, and optimum conditions were selected.

Experimental

Instrumentation

Perkin Elmer HGA 700 model 4100 (Norwalk, CT, USA), a graphite furnace atomic absorption spectrometry equipped with deuterium lamp as a background correction system was used for determination of cobalt in the extraction solvent. A cobalt hollow cathode lamp (Perkin Elmer, USA) operated at a current of 20 mA and a wavelength of 240.7 nm with a spectral bandwidth of 0.2 nm was used. Pyrolytic-coated graphite tubes (Perkin Elmer, HGA 700) with L'vov platform were used. Argon of 99.99 % purity was used as the inert gas with a flow rate of 300 mL min^{-1} during all the stages, except atomization when the gas flow was stopped. The instrumental parameters are given in Table 1. The pH values in the aqueous phase were measured with Metrohm 827 pH lab (Switzerland) glass-electrode pH-meter. Phase separation was assisted using Centurion Scientific Centrifuge (model Andreas Hettich D72, Tuttlingen, Germany).

Reagents and Solutions

All reagents were of analytical reagent grade, and deionized water was used throughout. A stock solution of 1,000 mg L^{-1} cobalt (II) ion was prepared by dissolving appropriate amounts of cobalt nitrate hexahydrate (Merck, Darmstadt, Germany) in 1 % (v/v) HNO$_3$. Working standard solutions were prepared freshly at various concentrations by diluting

the stock standard solution with deionized water. Suprapur HNO$_3$ (65 %, wt) and concentrated H$_2$O$_2$ (30 %) were used for sample digestion and purchased from Merck, Darmstadt, Germany. Acetone, acetonitrile, methanol, and ethanol as disperser solvents and carbon tetrachloride, chloroform, and dichloromethane as extraction solvents were purchased from Merck, Darmstadt, Germany. A solution of 2.0 mol L^{-1} thiocyanate anion was prepared by dissolving appropriate amounts of potassium thiocyanate (Merck, Darmstadt, Germany) in deionized water. A solution of 10^{-2} mol L^{-1} of CPC (Merck, Darmstadt, Germany) was prepared in deionized water.

Microextraction Procedure

Ten milliliters of sample solution containing 1.0 μg L^{-1} Co (II); 0.7 mol L^{-1} SCN$^-$; and 0.8×10^{-4} mol L^{-1} CPC was adjusted at pH 1 and transferred into a conical centrifuge tube. 1.5 mL of acetone containing 40 μL of carbon tetrachloride was rapidly injected into the sample solution. A cloudy solution (water, acetone, and carbon tetrachloride) was formed in the test tube, and the hydrophobic cobalt-thiocyanate-CPC complex was extracted into the fine droplets of carbon tetrachloride. After a 60-s extraction time, the cloudy solution was centrifuged at 3000 rpm for 10 min. The volume of the sedimented phase was determined using a 100-μL micro-syringe which was about 30.0 μL. Finally, 20 μL of sedimented phase was removed by the syringe and diluted to 40 μL with ethanol, and subsequently, 20 μL of the resulting solution was injected into the electrothermal atomizer using an autosampler for determination of Co.

Preparation of Real Samples

Water Samples

Different water samples including tap (Mashhad, Iran) and spring waters (Nowchah, Mashhad, Iran) were collected from their local sources and filtered through No. 42 Whatman® paper to remove any suspended particles and then acidified with dilute nitric acid and stored in glass bottles at 5 °C. After addition of optimum concentrations of thiocyanate and CPC to 6 mL of each sample and diluting to 10 mL, it was analyzed for determination of Co (II) according to the microextraction procedure.

Spinach Leaves

Spinach leaves were purchased from Mashhad, Iran, and washed with deionized water for several times and dried in oven at 50 °C for 6 h. 0.5000 g of powdered spinach leaves was digested in 10 mL of HNO$_3$ (65 %) and heated on heating block at 60–70 °C for 2 h. In order to complete the digestion,

Table 1 Instrumental parameters and temperature program for cobalt analysis

Step	Temp. (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying 1	90	3	15	300
Drying 2	130	10	20	300
Ashing	1000	30	20	300
Atomization	2400	0	5	0
Cleaning	2550	2	3	300

5 mL of H_2O_2 was added to the sample solution and heated for 30 min. The resulting solution was filtered through No. 42 Whatman® paper and diluted to 50 mL with deionized water. One milliliter of sample solution was adjusted at pH 1 and diluted to 10 mL with deionized water and analyzed for its cobalt contents according to the microextraction procedure.

Results and Discussion

Optimization of ETAAS

Drying, pyrolysis, and atomization temperatures for determination of Co in the extraction solvent were optimized, and the optimum conditions are presented in Table 1. The influence of pyrolysis temperature on the absorbance of Co was studied in details within a range of 800–1100 °C. Optimal pyrolysis temperature was observed at 1000 °C, with a hold time of 20 s. The effect of atomization temperature on the absorbance of Co was studied within the interval of 2200–2400 °C. The maximum absorbance was observed at 2400 °C with a hold time of 5 s. Finally, a temperature of 2550 °C with a hold time

of 3 s was chosen for the cleaning step. No matrix modifier was found to be useful for determination of cobalt in the extraction solvent.

Effect of pH

pH has an important effect on the stability of the formed ion pair complex (Uslu et al. 2013). The effect of pH on the absorbance of Co (II) was investigated in the range of 1–9. The results are shown in Fig. 1. Based on the results, the absorbance remains constant at the pH range of 1–6, and it decreases gradually at higher pH values which can be attributed to the formation of cobalt hydroxide in solution or the less stability of cobalt-thiocyanate-CPC complex in weak basic solutions. As at low pHs, metal ions are less likely to be precipitated; pH 1 was selected as the optimum value.

Effect of Thiocyanate Concentration

Thiocyanate anion concentration has an important effect on the formation of negatively charged cobalt-thiocyanate complex and subsequent extraction of cobalt-thiocyanate-CPC
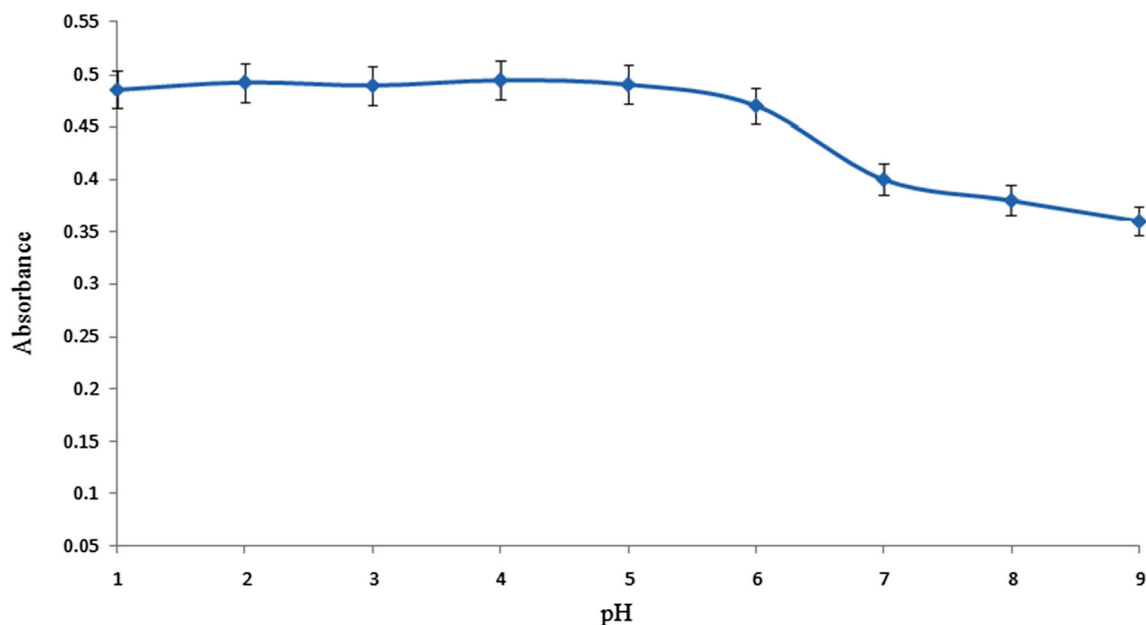

Fig. 1 Effect of pH on the absorbance of Co (II). Conditions: 1 μg L^{-1} Co (II); 0.7 mol L^{-1} SCN$^-$; 0.8 × 10^{-4} mol L^{-1} CPC; and 1.5 mL of acetone containing 40 μL of CCl$_4$. Experiments were performed in triplicates ($n = 3$)

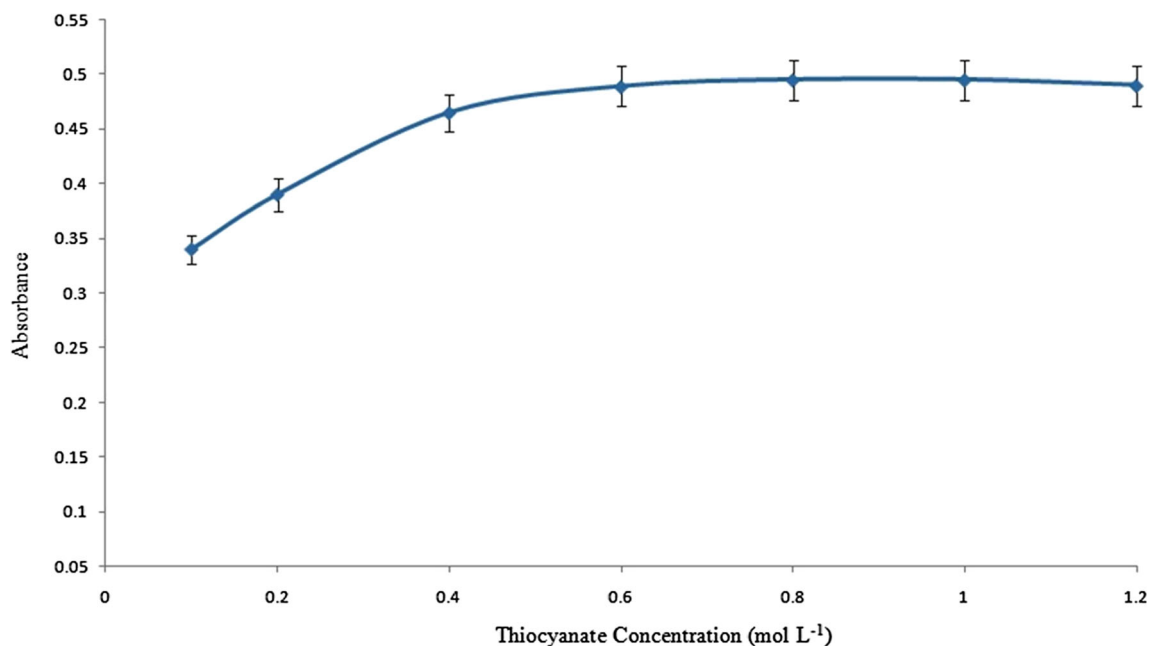**Fig. 2** Effect of thiocyanate concentration on the absorbance of Co (II). Conditions: 1 μg L^{-1} Co (II); pH 1; 0.8×10^{-4} mol L^{-1} CPC; and 1.5 mL of acetone containing 40 μL of CCl$_4$. Experiments were performed in triplicates ($n = 3$)

complex. The effect of thiocyanate concentration on the absorbance of Co (II) was studied in the range of 0.1–1.2 mol L^{-1}. The results are given in Fig. 2, showing that absorbance reaches its maximum value up to 0.6 mol L^{-1} thiocyanate and remains constant afterwards. Therefore, a concentration of 0.7 mol L^{-1} thiocyanate anion was selected as the optimum value.

Effect of CPC Concentration

In order to extract the anionic complex of cobalt-thiocyanate into the hydrophobic extraction solvent, a positive counterion should be added into the sample solution to form hydrophobic cobalt-thiocyanate-CPC complex. In order to study the effect of CPC concentration on the absorbance of Co (II),
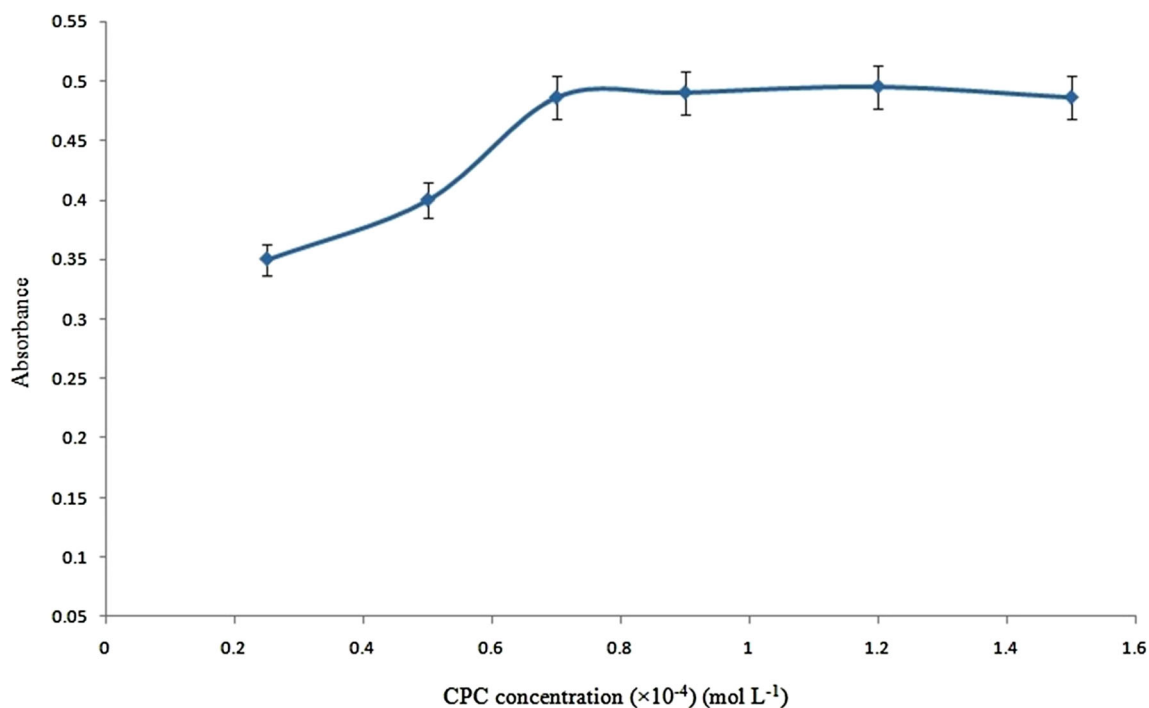

Fig. 3 Effect of CPC concentration on the absorbance of Co (II). Conditions: 1 μg L^{-1} Co (II); pH 1; 0.7 mol L^{-1} SCN$^-$; and 1.5 mL of acetone containing 40 μL of CCl$_4$. Experiments were performed in triplicates ($n = 3$)

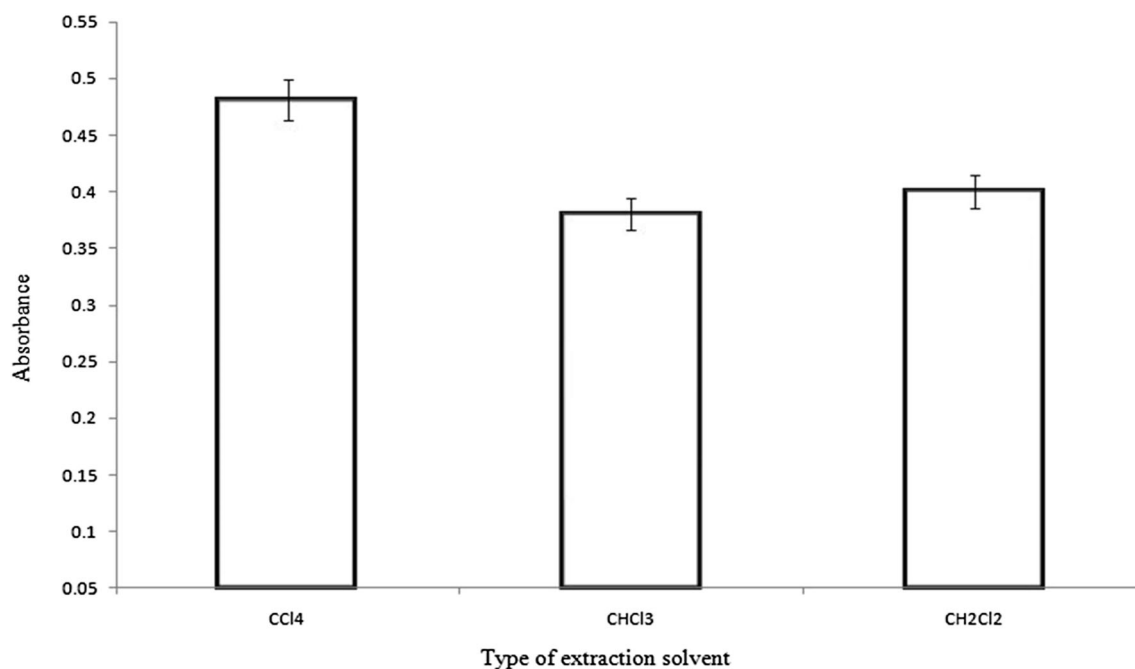**Fig. 4** Effect of type of extraction solvent on the absorbance of Co (II). Conditions: 1 μg L^{-1} Co (II); pH 1; 0.7 mol L^{-1} SCN$^-$; and 0.8×10^{-4} mol L^{-1} CPC. Experiments were performed in triplicates ($n = 3$)

different concentrations of CPC were tested, and the results are shown in Fig. 3. Based on the results, the absorbance reaches its maximum value at a CPC concentration of 0.7×10^{-4} mol L^{-1} and remains constant afterwards. Therefore, to maintain sufficient concentration of CPC in solution, a concentration of 0.8×10^{-4} mol L^{-1} CPC was selected as the optimum value.

Effect of Type and Volume of Extraction Solvent

Selection of an appropriate extraction solvent which has higher density than water plays an important role in DLLME. Different water-immiscible extraction solvents, such as

chloroform, carbon tetrachloride, and dichloromethane, were tested. 1.5 mL of acetone, containing different volumes of chloroform, carbon tetrachloride, and dichloromethane, was injected into the sample solutions to obtain 30 μL of sedimented extraction solvents. The results are shown in Fig. 4, and as can be seen, carbon tetrachloride provides maximum efficiency for extraction of Co (II) and was selected as the optimum extraction solvent. The effect of volume of carbon tetrachloride on the absorbance of Co (II) was studied in the range of 30–80 μL. The results show that the absorbance reaches its maximum value for 40 μL of carbon tetrachloride and decreases gradually at higher volumes. Therefore, 40 μL of carbon tetrachloride was selected as the optimum value.

Table 2 The effect of interfering ions on the absorbance of 1 μg L^{-1} Co (II). Experiments were performed in triplicates for each analysis (mean ± standard deviation, $n = 3$)

Interfering ions	Added as	Concentration (μg L^{-1})	Recovery (%)
Na$^+$	NaNO$_3$	200,000	99.0 ± 3.5
Mg^{2+}	Mg(NO$_3$)$_2$·9H$_2$O	100,000	97.8 ± 2.5
Zn^{2+}	Zn(NO$_3$)$_2$	1000	98.0 ± 3.0
Cu^{2+}	Cu(NO$_3$)$_2$·5H$_2$O	1000	98.9 ± 2.8
Mn^{2+}	Mn(NO$_3$)$_2$·H$_2$O	1000	99.1 ± 4.0
Ni^{2+}	Ni(NO$_3$)$_2$	1000	97.7 ± 2.5
Pb^{2+}	Pb(NO$_3$)$_2$	500	97.5 ± 2.0
Cd^{2+}	Cd(NO$_3$)$_2$·H$_2$O	500	98.7 ± 3.2
Fe^{3+}	Fe(SO$_4$)$_2$·NH$_4$·12H$_2$O	500	98.1 ± 3.0
Al^{3+}	Al(NO$_3$)$_3$·9H$_2$O	500	98.5 ± 2.2
SO$_4^{2-}$	K$_2$SO$_4$	200,000	97.2 ± 4.0
NO$_3^-$	KNO$_3$	200,000	99.0 ± 3.8
Cl$^-$	KCl	100,000	98.9 ± 3.9

Table 3 Determination of Co (II) in different real samples

Sample	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery (%)
Tap water[a]	–	0.31 ± 0.02	–
	0.50	0.79 ± 0.04	97.5
	1.00	1.29 ± 0.07	98.5
Springer water[b]	–	0.52 ± 0.03	–
	0.50	1.05 ± 0.05	102.9
Spinach leaves ($\mu g\ g^{-1}$)	–	0.51 ± 0.03	–
	0.50	1.02 ± 0.06	101.0

Results: mean ± standard deviation based on three replicate analyses of the samples

[a] Obtained from Mashhad, Iran

[b] Obtained from Nowchah, Mashhad, Iran

Effect of Type and Volume of Disperser Solvent

The selection of a disperser solvent is limited to solvents such as methanol, acetonitrile, ethanol, and acetone that are miscible with both water and extraction solvents (Chamsaz et al. 2014). 40 μL and 45 μL of carbon tetrachloride were diluted to 1.5 mL by disperser solvents of acetone and acetonitrile and methanol and ethanol, respectively (to obtain 30 μL of sedimented extraction solvent). Based on the results, acetone provides maximum extraction efficiency for microextraction of Co (II). Therefore, acetone was selected as the optimum disperser solvent. The effect of volume of disperser solvent on the absorbance of Co (II) was also studied by injection of different volumes of acetone in the range of 0.5–2 mL containing 40 μL of carbon tetrachloride. The results show that 1.5 mL of acetone containing 40 μL of carbon tetrachloride provides maximum efficiency for microextraction of Co (II).

Effect of Extraction Time

The extraction time, defined as the interval between injection of the mixture of acetone and carbon tetrachloride and the time of centrifugation process started, was evaluated in the range of 0–180 s. The results (data not shown) show that the absorbance reaches its maximum value at 60 s, and longer extraction time did not significantly change the absorbance of cobalt. Therefore, a 60-s extraction time was considered as the optimum extraction time.

Effect of Centrifugation Time

Centrifugation time is an important parameter that influences the separation of extraction solvent from the aqueous phase. A centrifugation time was investigated in the range of 5–15 min at a rate of 3000 rpm. The results show that a 10-min centrifugation time is adequate to the satisfactory separation of two immiscible phases. Therefore, a 10-min centrifugation time at 3000 rpm was selected as the optimum value.

Effect of Ionic Strength

In order to investigate the effect of ionic strength of sample solution on the absorbance of Co (II), different concentrations of KNO_3 in the range of 0.5–3 % (g mL^{-1}) were tested. The results show that the absorbance remains constant up to 1 % g mL^{-1} KNO_3 and decreases gradually at higher concentrations. Based on the results, the proposed method can be applied for determination of Co (II) in different water samples.

Effect of Interfering Ions

The effect of different cations and anions on the absorbance of Co (II) was studied by addition of different concentrations of interfering ions. An ion was considered to interfere if it resulted in a ±5 % variation of the absorbance. The results are given in Table 2. Also, the effect of several common cations and anions (with following concentrations: 500 μg L^{-1} of Zn^{2+},

Table 4 Characteristic performance data obtained by using the proposed method and other methods reported for Co determination

Method	Extraction time (min)	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	RSD (%)	PF	Ref.
Online IL-DLLME-ETAAS	<5	0.08–7	0.008	5.1	20	Berton and Wuilloud 2011
DLLME-SFO-FAAS	5	1.15–110	0.35	2.6	26	Zhang et al. 2011
IL-based DLLME	>5	0.4–120	0.10	2.9	–	Yousefi and Ahmadi 2011
IL-DLLME-ETAAS	7	0.038–3.5	0.0038	3.4	–	Berton and Wuilloud 2010
Ion pair-based DLLME-ETAAS	1	0.08–1.5	0.02	3.7	167	Present work

Cu^{2+}, Mn^{2+}, and Fe^{3+} and 100,000 $\mu g\ L^{-1}$ of Na^+, NO_3^-, and Cl^-) in the presence of 1 $\mu g\ L^{-1}$ Co (II) was studied to check the selectivity of the proposed method. By the analysis of the solution according to the microextraction method, 96.1 $\pm 3.8\ \%$ ($n=3$) recovery was obtained for determination of Co (II). Therefore, the proposed method shows good selectivity for determination of Co (II) in different real samples.

Analytical Figures of Merit

Under the optimum conditions, the calibration curve was linear in the range of 0.08–1.5 $\mu g\ L^{-1}$ of Co (II) with a correlation coefficient of 0.9997. The equation of calibration graph was $A = 0.4937 C_{Co\ (II)} - 0.0067$, where A is the analytical signal measured as absorbance and C is the concentration of Co (II) in $\mu g\ L^{-1}$. Moreover, the equation of the calibration curve without the preconcentration step was $A = 0.007\ C - 0.018$ within a dynamic range from 25 to 100 $\mu g\ L^{-1}$ Co (II). The relative standard deviation (RSD, %), based on six replicate analyses of 0.5 $\mu g\ L^{-1}$ of Co (II), was 3.7 %, and the limit of detection (LOD) defined as three times of standard deviation of blank ($n=5$) was 0.02 $\mu g\ L^{-1}$. The obtained LOD value for Co was sufficiently low to be valuable for detecting Co in acid-digested real samples. The LOD for solid samples was 0.0015 $\mu g\ g^{-1}$ and was calculated as the analyte concentration corresponding to three times the standard deviation of six independent measurements of the analytical blank divided by the slope of the calibration curve. The preconcentration factor (PF), calculated as the ratio of aqueous phase volume to the final volume of the extraction phase, was 167. The enrichment factor calculated as the ratio of slope of preconcentrated samples to that obtained without preconcentration was 70.

Analysis of Real Samples

In order to assess the applicability of the proposed method for determination of Co (II), water samples and spinach leaves were analyzed for their cobalt contents. The results are shown in Table 3. Spike tests were also conducted to check the reliability of the proposed method. The accuracy of the proposed method was verified by the analysis of CRM-TMDW (drinking water)-certified reference material, http://www.highpuritystandards.com/store/home.php?cat=44, with a certified value of 25.0 $\mu g\ L^{-1}$ Co (II). Since the certified concentration value in the CRM was higher than the upper limit of the linear range of this method, a 20-fold dilution had to be implemented prior to the analysis. By using the proposed method, Co (II) concentration was found to be 24.6 $\pm 1.12\ \mu g\ L^{-1}$ which is in good agreement with the certified value (95 % confidence interval; $n=6$).

Comparison to Other Methods

A comparison of the present method with other reported methods for preconcentration and determination of cobalt is given in Table 4. As can be seen, the LOD (0.02 $\mu g\ L^{-1}$), PF (167), and extraction time of the proposed method are better or comparable to other reported methods.

Conclusions

Ion pair-based DLLME followed by ETAAS was used for preconcentration and determination of ultra-trace levels of Co (II). The method is based on the complexation of cobalt (II) by thiocyanate anion to form negatively charged cobalt-thiocyanate complexes which can be extracted into the fine droplets of hydrophobic carbon tetrachloride by addition of cetylpyridinium chloride (CPC) as a positively charged counterion. The results show that this method is a sensitive, efficient, and very rapid sample preparation technique for different real samples and can be used as a replacement of the traditional extraction method for determination of cobalt.

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