Optimization of Solid Phase Extraction for Trace Determination of Cobalt (II) Using Chromosorb 102 in Biological Monitoring

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ABSTRACT

Cobalt is widely used in different industrial processes for production of various synthetic materials. For assessment of human exposure to toxic metal of Co (II), environmental and biological monitoring are essential processes, in which, preparation of samples is one of the most time-consuming and error-prone aspects prior to instrumental analysis. The aim of this study was to achieve optimum factors necessary for development of a sample preparation technique for cobalt (II), present in urine, hair, and nail samples followed by atomic absorption spectrometry. Solid phase extraction (SPE) using mini columns filled with Chromosorb 102 resin was optimized regarding sample pH, ligand concentration, loading flow rate, elution solvent, sample volume (up to 500 ml), elution volume, amount of resins, and sample matrix interferences. Cobalt ion was retained on sorbent and was eluted with 2 M HNO3 followed by determination by flame atomic absorption spectrometry. Obtained recoveries of cobalt ion were more than 92%. To evaluate occupational exposure to Co (II), successful applicability of the optimized method for human exposure was used by treatment real samples, including urine, hair, and nail. Suitable results were obtained for relative standard deviation (less than 10%). This optimized method can be considered successful in simplifying sample preparation for trace residue analysis of Co (II) in different matrices when an evaluation of occupational and environmental exposures is required.

Keywords: Cobalt, Atomic absorption spectrophotometry, Chromosorb 102

INTRODUCTION

Heavy metals persist in nature and most of them are advantageous to humans because of their vast usages in different industries, agriculture, and medicine. They pose health hazards to the public because of their presence in air, water, food chains as well as to the workers who are engaged in mining, smelting, alloy, painting, electroplating, pesticides, and the variety of industrial activities. Some heavy metals such as cobalt have a wide range of toxicities, leading to toxic effects on the renal, respiratory, and nervous systems. Occupational exposure to cobalt dust has been associated with cardiomyopathy characterized by functional effects on the ventricles and enlargement of the heart. Cobalt produces an allergic dermatitis of an erythematous popular type. American Conference of Governmental Hygienists (ACGIH) has classified the cobalt and inorganic compounds as animal carcinogen (A3 class) [1-3]. In recent years, some studies have been performed on different environmental samples, including various wastewater samples [4-6], electroplating wastewater [7] and bottled mineral water as well as artificial seawater [8].

Usages of cobalt are unavoidable; therefore, study of this heavy metal is of great interest. In biological and environmental samples, either exposed compounds or their metabolites, metals are mostly present at trace level, causing major problems in their
determination stages [4-6]. Therefore, an essential need for precise, reliable, and sensitive techniques for the analysis of such trace chemicals in biological samples has been clearly recognized [6-9].

Analytical techniques require expensive equipments and may not be available in most laboratories, thus, sample preparation procedures which can be performed in most routine laboratories have been developed to simplify analytical approaches as these approaches reduced expenses too [7, 9-11]. To extract heavy metals, many sample preparation procedures are being used such as Soxhlet extraction [12], liquid liquid extraction (LLE) [13-15], supercritical fluid extraction (SFE) [16], and solid phase extraction (SPE) [17-20], from which, Soxhlet and LLE are time consuming procedures and also the recoveries obtained from such methods are not reproducible and efficient. Therefore, more sensitive and precise methods are required to measure trace heavy metals in biological and environmental samples. In contrast, SPE methods have proven to be useful in simplifying sample preparation prior to analytical technique. This method refers to the adsorption of chemical constituent from a liquid sample (water, urine, etc.) on a solid sorbent and subsequent desorption of retained constituent by elution from the sorbent. Through this procedure, isolation and purification of the compound of interest can be achieved in a short time and only low volumes of solvents are used during the course of desorption process. The use of commercially available low-cost vacuum manifolds allows many samples to be proceeding simultaneously. Furthermore complete automation of procedures based on SPE is now possible using commercially available instrumentation [21-26]. A wide range of phases based on silica are also available from many suppliers, including reversed phase, normal phase, and ion exchange. These phases can be screened and selected, depending on the chemical nature of the analyte, possible interferences, and matrices environment [27]. Therefore, the variety of available phases can improve the selectivity of the sample preparation procedures.

This study was aimed to achieve optimum criteria necessary for development of a sample preparation procedure for cobalt (II), present in urine, hair, and nail samples, leading to a simple protocol of SPE method in environmental and occupational exposures.

Material and Methods

Chemicals and reagents

Cobalt stock solution was prepared from appropriate amount of the nitrate salt of this analyte (Merck, Darmstadt, Germany) as 1000 mg/l solution in 0.01M HNO₃. Working and standard solutions were prepared daily by dilution of the stock solution. Acids and other chemicals used in this study were obtained from Merck, Darmstadt, Germany. Standard buffered solution at various pH values, Ammonium Pirrolidine Dithio Carbamate (APDC) were also purchased from Merck, Germany. Chromosorb 102 resin (80-100 mesh) was purchased from Sigma Chem. Co.

Apparatus

Quantitation of cobalt was made with spectra AAplus 20, a Varian flame atomic absorption spectrometer (FAAS; Varian, Australia), and using air-acetylene flame. The operating parameters were set as recommended by the manufacturer. The pH values of the solutions were measured by a digital pH meter model Metrohm 744. Amount of reagents were measured using a Satorius CP225D balance (Sartorius, Germany).

Mini columns preparation and pre-concentration procedure

Cartridges (100 x 10 mm) were packed with 500 mg resin. After packing, a little amount of glass wool was placed at both ends of the glass tube. Before using the column, Chromosorb 102 resin was washed by methanol, water, 1 M HNO₃, water, 1 M NaOH, and water, respectively. Finally, resin was pre-conditioned with buffer solution. SPE using Chromosorb 102 resin was optimized with regard to sample pH, sample and eluent flow rates, elution solvent, eluent volume, ligand concentration, amount of resin, and sample volume. Fifty milliliters solution containing 20 µg of Co (II), 10 ml buffer solution with desired pH and 6 ml APDC solution was prepared. Samples were then passed through the column packed in our laboratory at a flow rate of 5 ml/min. The column was then washed with 5-10 ml of the same buffer solution. Therefore, the metal ions were eluted from the mini column with 10-15 ml of different solvents. Finally, the cobalt concentration in the solutions including standards, spiked, and real samples were determined by Flame Atomic Absorption Spectrometry (FAAS).

Preparation of urine samples

Urine samples were collected from exposed workers of relevant industries at different job positions. For adjusting the pH, 10 ml buffer solution with pH 9 was used. After adding the 6 ml ligand, the volume of sample solutions were increased to 50 ml, using deionized water for the further process of SPE and FAAS based on optimized method.

Preparation of hair samples

The hair samples were washed in an ultrasonic cleaner and sonicated for 60 min in 100 ml of acetone, water, water, and acetone, respectively. After filtration of samples from the wash liquid, they were dried. The hair samples were then weighed and dissolved in 2.5 ml nitric acid and were kept overnight in an oven at 60 °C. Digested samples were then transferred to volumetric flasks [28]. For adjusting the pH, 2 M NaOH was used. After adding the 6 ml ligand, the volume of sample solutions were increased to 50 ml, using deionized water for the further process of SPE and FAAS based on optimized method.
**Preparation of nail samples**

The nail samples were first cleaned, rinsed by deionized water five times, and dried in an oven. The samples then were digested by 10 ml of 6:1 mixture of concentrated nitric and perchloric acid then kept overnight at room temperature as well as subsequently heated at 160-180 °C until the mixture was water clear and less than 1 ml of the solution remained [29]. 1.5 ml of 2 M NaOH and 6 ml ligand were added. Deionized water was added to each sample to make a 50 ml volume for the further process of SPE and FAAS based on optimized method

**RESULTS**

**Effect of sample pH**

The effect of sample pH on adsorption of Co (II) ion on Chromosorb 102 resin was evaluated, using different pH values of 2, 4, 7, and 9. The pH values were adjusted by buffer solutions. Fifty milliliter of sample containing 20 µg of Co (II) and 6 ml APDC were adjusted by buffer solutions. Fifty milliliter of different pH values of 2, 4, 7, and 9. The pH values were evaluated, using eluent at different flow rates of 2, 5, 7, and 10 ml/min. As the Table 2 shows, metal of interest was quantitatively recovered in eluent flow rate up to 10 ml/min. Flow rate of 5 ml/min was then selected as an optimum value for the next experiments.

**Effect of APDC the amount of**

The concentration of APDC is one of the important parameter could affect on recovery obtained from the optimized method. Through this investigation, the amount of 0.01- 0.07% (w/v) of APDC were used. The obtained results showed that, by increasing APDC concentration up to 0.05%, the recoveries are also increased, afterward, constant values are recovered.

**Effect of eluent type**

Five solvents were screened for their ability to produce optimum elution of the retained Co (II) from the Chromosorb 102 resin. They were 1 M HCl, acetone, 1 M HNO₃ in acetone, 1 M HNO₃, and 2 M HNO₃. The results have been presented in Table 1. A quantitative recovery (>95%) was obtained for Co (II) ion, using 2 M HNO₃ as an efficient eluent and, therefore, it was used as a suitable solvent for further studies.

**Effect of eluent flow rate**

In this evaluation, retained metal ion on sorbent was eluted, using eluent at different flow rates of 2, 5, 7, and 10 ml/min. As the Table 2 shows, metal of interest was quantitatively recovered in eluent flow rate up to 10 ml/min. Flow rate of 5 ml/min was then selected as an optimum value for the next experiments.

**Effect of sample volume**

In this experiment, 20 µg of Co (II) was diluted into different volumes of 50, 150, 250, 500, and 750 ml distilled water. These samples loaded on Chromosorb 102 mini columns. The columns were then washed and the retained analyte was eluted according to the optimized method (Table 2). It can be seen that up to 500 ml of samples could be applied without significant loss of recovery (94%). Therefore, the highest concentration factor was 33.3 when the final volume was 15 ml.

**Table 1.** Effect of sample pH, ligand concentration, eluent type, and eluent volume on recovery of Co (II) from Chromosorb 102 resin (eluent: 2 M HNO₃).

<table>
<thead>
<tr>
<th>Sample pH</th>
<th>Mean(%)±SD (N=5)</th>
<th>Ligand concentration [w/v(%)]</th>
<th>Mean(%)±SD (N=5)</th>
<th>Eluent type</th>
<th>Mean(%)±SD (N=5)</th>
<th>Eluent volume (ml)</th>
<th>Mean(%)±SD (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5±0.00</td>
<td>0.01</td>
<td>50±0.00</td>
<td>1 M HCl</td>
<td>72±4.47</td>
<td>5</td>
<td>46.00±1.36</td>
</tr>
<tr>
<td>4</td>
<td>7±2.73</td>
<td>0.03</td>
<td>72±4.47</td>
<td>Acetone</td>
<td>38±4.47</td>
<td>10</td>
<td>64.00±2.23</td>
</tr>
<tr>
<td>7</td>
<td>72±4.47</td>
<td>0.05</td>
<td>96±5.47</td>
<td>(HNO₃ in Ace-</td>
<td>96±5.47</td>
<td>15</td>
<td>95.00±4.21</td>
</tr>
<tr>
<td>9</td>
<td>98±4.47</td>
<td>0.07</td>
<td>98±4.47</td>
<td>tone)</td>
<td>94±5.47</td>
<td>20</td>
<td>98.00±4.47</td>
</tr>
</tbody>
</table>

**Table 2.** Effect of eluent flow rate, sample volume, sample flow rate, and sorbent mass on recovery of Co (II) from Chromosorb 102 resin (eluent: 2 M HNO₃).

<table>
<thead>
<tr>
<th>Eluent flow rate (ml/min)</th>
<th>Mean(%)±SD (N=5)</th>
<th>Sample volume (ml)</th>
<th>Mean(%)±SD (N=5)</th>
<th>Sample flow rate (ml/min)</th>
<th>Mean(%)±SD (N=5)</th>
<th>Sorbent mass (mg)</th>
<th>Mean(%)±SD (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>98±4.47</td>
<td>50</td>
<td>98±4.47</td>
<td>2</td>
<td>100±0.00</td>
<td>100</td>
<td>62±4.47</td>
</tr>
<tr>
<td>5</td>
<td>98±4.47</td>
<td>150</td>
<td>96±5.47</td>
<td>5</td>
<td>98±4.47</td>
<td>500</td>
<td>98±4.47</td>
</tr>
<tr>
<td>7</td>
<td>96±5.47</td>
<td>250</td>
<td>96±5.47</td>
<td>7</td>
<td>98±5.47</td>
<td>500</td>
<td>98±4.47</td>
</tr>
<tr>
<td>10</td>
<td>92±4.47</td>
<td>500</td>
<td>94±5.47</td>
<td>9</td>
<td>96±5.47</td>
<td>500</td>
<td>98±4.47</td>
</tr>
</tbody>
</table>
Table 3. Effect of matrix ions on the adsorption of Co (II) on Chromosorb 102 resin (eluent: 2 M HNO₃)

<table>
<thead>
<tr>
<th>Ions (added)</th>
<th>Concentration (g/l)</th>
<th>Recovery (%) Mean±SD, N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺(NaCl)</td>
<td>2.5</td>
<td>98±4.47</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>98±4.47</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>94±4.47</td>
</tr>
<tr>
<td>K⁺(KCl)</td>
<td>0.3</td>
<td>100±5.47</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>96±4.47</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>94±2.37</td>
</tr>
<tr>
<td>Mg²⁺(MgCl₂)</td>
<td>0.3</td>
<td>98±0.00</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>96±4.47</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>94±4.47</td>
</tr>
<tr>
<td>Ca²⁺(CaCl₂)</td>
<td>0.3</td>
<td>98±5.47</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>98±4.47</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>94±5.47</td>
</tr>
<tr>
<td>SO₄²⁻ [(NH₄)₂SO₄]</td>
<td>0.5</td>
<td>96±5.57</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>94±5.47</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>94±5.47</td>
</tr>
</tbody>
</table>

**Effect of sample flow rate**

By achievement of the large sample volumes application, the effect of sample flow rate on metal ion adsorption on Chromosorb 102 was studied in different sample flow rate of 2, 5, 7, and 9 ml/min. Fifty ml sample, using optimum pH, containing 20 µg of metal ion and APDC solution were prepared. No significant reduction in recovery was found for sample flow rate up to 9 ml/min. Flow rate of 5 ml/min as an appropriate value was then used to continue further experiments (Table 2).

**Effect of Chromosorb 102 sorbent mass**

The effect of Chromosorb 102 amount was investigated, using 100 and 500 mg sorbent packed in a mini column. The same sequence of preparation procedure was used. The obtained recovery of metal ion was more efficient when 500 mg was utilized (Table 2).

**Effect of matrix**

The influence of ions, mostly present in the environmental and biological samples, including Na⁺, K⁺, Mg²⁺, Ca²⁺, and SO₄²⁻ was another parameter, affecting on efficiencies of analyte recoveries. The procedure was performed, using 50 ml sample containing 20 µg of analyte and different concentration of matrix ions (Table 3).

**Reproducibility**

Validation of the possible use of the optimized method for measuring metal ion of cobalt (II) in urine was carried out, using spiked samples. Samples of 50 ml were used for extraction with subsequent FAAS. Linear standard curves (extracted) over the concentration range of 1, 1.5, and 2 µg/ml were obtained each day (n=6) for six consecutive days with a correlation coefficient of 0.995 or greater. The day-to-day (for six consecutive days) and within-day reproducibility of the method was investigated (Table 4).

**Application of real samples**

Finally, real samples of urine, hair, and nail were obtained from workers employed in relevant industries in Iran. Optimized method was applied for measurement of Co (II) in all different real samples (Table 5).

**DISCUSSION**

High recovery was achieved from Chromosorb 102 resin using sample pH of 9. However, the pH value of the sample should be adjusted according to the chemistry of the compound of interest. It seems that, at sample pH of 9, the analyte of interest is mostly in the ionized form, making it to be easily retained on the ionized ligand already conjugated to the sorbents. From these pH values, sample pH of 9 was selected for further study.

A non polar sorbent was used, in which, there was no affinity between this type of sorbent and the ionized analyte, so, there was a need of conjugating ionized ligand on the sorbents to follow up an ionized extraction mechanism. APDC showed to be an appropriate ligand for capturing cobalt (II) from the

Table 4. Day-to-day (D-day) and within day (W-day) reproducibility of Co (II) spiked in urine, sample volume: 50 ml, N=6

<table>
<thead>
<tr>
<th>Statistical data</th>
<th>Concentration added (µg/ml)</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D-day</td>
<td>W-day</td>
<td>D-day</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.970</td>
<td>0.970</td>
<td>1.440</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>1.095</td>
<td>1.095</td>
<td>1.633</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>1.13</td>
<td>1.13</td>
<td>1.70</td>
</tr>
</tbody>
</table>
sample, however, from the four concentrations of the ligand, the amount of 0.05%, and 0.07% showed to be good enough for efficient retaining of the analyte. However, for preventing saturation of the sorbent with the ligand and also reduce the reagents through extraction process, the lesser percentage of the ligand (0.05%) was used as this amount provides the same recovery needed for the method.

Highly ionic compounds can result in a strongly retained analyte making elution difficult and leading to subsequent poor recovery from ionic conjugated sorbent. From the eluents used in this study (Table 1), the HNO$_3$ based solutions were more efficient and from these solvents, 2 M HNO$_3$ was selected, because, it was organic free eluent and can prevent co-elution of organic compounds possibly present in the real samples as well as reducing exposure to such evaporative and hazardous compounds. Moreover, maximum recovery has been achieved using this eluent.

As Table 1 shows, the smallest satisfactory volume for 2 M HNO$_3$ from Chromosorb 102 sorbent was 15 ml giving a suitable concentration factor of 3.33. Therefore, the volume required to elute the analyte from the sorbent, depends on two important parameters. First, the strength of its retention, a solvent with greater elution strength can be used to elute an analyte in less volume, but may incorporate undesirable contaminants into the eluted fractions; secondly, the sorbent mass used in SPE, in which, using a larger sorbent mass cartridges requires an increase elution volume to be applied. Moreover, the faster elution of 15 ml eluent can affect on the whole analysis time when numerous samples is going to be applied. Therefore, through this experiment, the reduced eluent flow rate of 5 ml/min was enough to reduce the elution time to one third.

An accurate measurement as low as 0.04 µg/ml (0.04 ppm) of cobalt when a large sample volume (500 ml) is applied on the column, resulting in a possible trace enrichment of the analyte with an appropriate concentration factor of 33.3 which was compatible to the current atomic absorption spectrometry detection system. As the high volume of sample is applicable with an efficient recovery, it would be of favorite if high sample flow rate can be applied. In this investigation, sample flow rate of up to 9 ml/min were applied with acceptable recovery of 96% and more (Table 2). Therefore, to be confident, the sample flow rate of 5 ml/min was selected, providing a reduced extraction time for as large as 500 ml sample volume.

It was seen that, 100 mg sorbent was not appropriate amount as breakthrough was happened through the experiment, so, a non efficient amount of 62% of the retained compound was recovered which is not acceptable in our optimized method. By using the sorbent mass as large as 500 mg, it allowed that a longer interaction to be taken place, causing retention of significant amount of cobalt on the sorbent and subsequent efficient recovery of 98%. However, using large amount of sorbent mass needs a large volume of washing solvent and eluent to be applied for the efficient removal of possible interferences.

In order to show effect of possible matrix components on the optimized method, the similar ions illustrated in Table 3, having three different concentrations were added to the sample. The ions added to the samples are mostly present in the real environmental samples and can be used as closely related interferences present in matrices. The results clearly showing the non-effectiveness of the all of the added components for each concentration on the recoveries obtained from optimized method. As it can be seen, the recoveries are 94% or greater which is promising either no cross-reactivity is taken place between added interferences and the Chromosorb 102 or no co-elution is happened.

In this study, reproducibility of the optimized method was performed for day-to-day and within-day experiments. A linear standard curve (for extracted sample) over the range concentrations of 1, 1.5, and 2 µg/ml was obtained every day for 6 consecutive days (n=6) with the correlation coefficient of 0.995 or grater. In within-day experiments evaluation, six experiments were performed per day for three consecutive days. The extraction procedure was reliable and reproducible from day-to-day and within-day. Coefficient of variations (CV%) of 1.13, 1.70, and 0.42 were obtained for 1, 1.5, and 2 µg/ml respectively for day-to-day and 1.13, 1.55, and 1.57 at the same concentrations respectively for within-day, showing suitable accuracy and precision (Table 4).

From 20 urine samples, it was found that, no Co (II) was detected in all samples, showing that workers, in their relevant jobs, have been under exposed to this element compare to the standard value of BEI developed by ACGIH [30]. Regarding to hair, from 14 obtained hair samples, Co were detected in just one sample (29.24 ppm) and this element was not detectable in the range of our method detectability. That is why the SD is ±00.00. For the nails samples, workers showed mean value of 2.71±1.01 ppm (1.03 -4.77 ppm). It seems that, the natural type of sample, compare to the urine sample, can play a main role for this amount of cobalt in hair and nail. Urine samples can be considered as diluted
solutions for such analyte of interest as the urine is excreted daily compare to the hair and nail, in which, they are removed at least every two months. This process can cause cobalt to be accumulated in hair and nail. However, for such evaluation, urine samples still are appropriate samples as their BEIs are available for evaluation of human occupational and environmental exposures.

A recently reported method [15] has used liquid liquid extraction (LLE) for some heavy metals. Although the technique may be useful in some conditions, however, there are still no basic rules for selection of a solvent system for extraction of given analyte, therefore, selection of a solvent is still empirical and of course time consuming step as well as a tedious stage. Sometimes, emulsion formation of the sample makes the analyte extraction too hard as such solutions are extremely difficult to be broken and often cannot be isolated by either centrifugation or ultra-sonication. Other problems associated with LLE include: the use of large volumes of toxic and sometimes inflammable solvents, contamination of extracts from solvents and glassware, low recovery due to degradation by heat, and volatilization or adsorption to glassware. Therefore, due to such problems, nowadays, there is a strong trend towards replacing LLE by SPE. Based on reported methods [20, 22-24, 26], for optimizing SPE, authors generally have used 5-6 factors to optimize the method for environmental samples [23, 31], while, in this study, 9 parameters were screened, including significant factors of sorbent mass, eluent flow rate, sample matrix interferences, and also ligand concentrations. This allows that a robust and more reliable method is introduced for biological monitoring of Co (II). Therefore, to make an advantage from this study compare to the other studies [18-24], further experiments of reproducibility of the method were carried out on spiked urine samples to validate the possible use of the optimized SPE for measuring Co (II) when an environmental study and biological monitoring of worker exposed to such pollutant are required. Although the concentration factor obtained from this study is high, however, the relatively low sensitivity of the AAS did not allowed the authors to get even more concentration factor. Applicability of the optimized method for real samples of urine, hair, and nail can be considered as another advantage of this method.

**CONCLUSION**

Through this study, factors influencing SPE were optimized, showing an efficient sample preparation procedure for cobalt (II) as a solid phase extraction method has more advantages than liquid liquid extraction. Depending on the chemical and physical properties of the analyte, manipulating factors including sample pH, ligand concentration (APDC), loading flow rate, elution solvent, sample volume (up to 500 ml), elution volume, amount of resin (Chromosorb 102), and sample matrix interferences can play essential roles in optimizing the method, providing reliable, easy to use, and cost effective procedure to overcome difficulties associated with other sample preparation techniques. The concentration factor was 33.3 and the resin can be used several times. Applications of real samples, including urine, hair, and nail obtained from relevant industries showed that, the method is efficient enough when a trace analysis of such metal in occupational and environmental exposures assessment are of interest.

**ACKNOWLEDGMENTS**

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