*Technical Note*

# Determination of Cobalt in Urine by FI-ICP-AES with Online Preconcentration

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## Abstract

**A method for the preconcentration and determination of cobalt in human urine samples was developed. The online preconcentration and determination were attained using inductively coupled plasma atomic emission spectromety (ICP-AES) coupled to a flow injection (FI) method. Cobalt was retained on an Amberlite XAD-7 resin as cobalt-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complex at pH 9.5. Cobalt was removed from the microcolumn with perchloric acid. A sensitivity enhancement factor of 90 was obtained with respect to cobalt determination by ICP-AES without preconcentration. The value of detection limit for the preconcentration method proposed was 25 ng/L. The precision for 10 replicate determinations at the 5 mg/L (mean ± SD, 5.1 ± 0.14) Co level was 2.7% relative standard deviation, calculated from the peak heights obtained. The calibration graph preconcentration method for cobalt was linear with a correlation coefficient of 0.9994 from approximately 0.25 mg/L up to at least 100 mg/L. The method was successfully applied to the determination of cobalt in human urine samples.**

## Introduction

Cobalt is considered an essential trace element that is required in the normal diet of humans in the form of vitamin $B_{12}$ (cyanocobalamin). For this reason cobalt has been used in the treatment of anemia (1). However, the use of cobalt supplementation has been associated with toxic side effects such as cardiomyopathy (1). On the other hand, occupational exposure to cobalt represents a continuing problem of considerable magnitude.

As cobalt often occurs in water and tissues in complicated matrices at extremely low levels, analyte preconcentration and/or matrix separation is required prior to most analytical techniques (2–4).

Atomic absorption spectrometry with electrothermal atomization (ETAAS) has become the most appropriate technique for its determination (5,6). However, ETAAS often suffers from matrix interferences when it is applied to the analysis of biological materials (3). Besides this technique is very well suited to determine cobalt levels found in urines of occupationally exposed persons, but for measuring normal urine levels preconcentration steps are required.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is widely recognized as a suitable technique for the determination of trace elements, the particular advantages being the multi-element capability, large dynamic range, and effective background correction.

On the other hand, if conventional ICP-AES is used, the low level of cobalt in urine is not compatible with the detection limit of this technique. In order to achieve accurate, reliable, and sensitive results, preconcentration and separation are needed when the concentrations of analyte elements in the original material or the prepared solution are too low to be determined directly by ICP-AES.

López-Artiguez et al. (4) have obtained good results for the determination of some heavy metal in urine using a preconcentration method. The method consists of a complexing of metals with ammonium pyrrolidine dithiocarbamate (APDC) and subsequent liquid–liquid extraction with methyl isobutyl ketone (MIBK). However, the detection limit obtained for Co was not compatible with the concentration of the element in urine.

Preconcentration is an effective means for extending the detection limits of ICP-AES methods. However, when practiced manually in the batch mode, the operations are usually too tedious to be compatible with the ICP-AES measurements. Stringent control of the laboratory environment is also required to avoid sample contamination if ultra-trace determinations in the nanogram-per-milliliter range are to be attempted. This situation has been improved significantly by utilizing flow injection (FI) coupled with ICP-AES (7,8), such that the general drawbacks of batch preconcentration procedures have been

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largely eliminated, and currently online preconcentrations could be achieved almost as efficiently as a simple ICP-AES determination.

In order to be used as packing in preconcentration columns for FI (9), materials have to meet several requirements. XAD resins meet many of these requirements and because they have good physical properties such as porosity, uniform pore size distribution, high surface area as a chemical homogeneous non-ionic structure, and good adsorbent properties for great amounts of uncharged compounds, they have been used as supports for immobilization of chelating agents and metal complexes (10–12). 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) forms stable complexes with numerous metal ions (13,14), and is therefore a suitable reagent for cobalt preconcentration on an XAD resin (15).

In the present work, a method for preconcentration and determination of cobalt using a microcolumn filled with a macroporous resin Amberlite XAD-7 is proposed. Cobalt was retained under the form of cobalt-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol [Co-(5-Br-PADAP)] complex. The determination was performed using ICP-AES associated with a FI methodology.

## Experimental

### Reagents

A solution of 5-Br-PADAP (Aldrich, Milwaukee, WI) $10^{-2}$ mol/L was prepared by dissolution in ethanol. Lower concentrations were prepared by serial dilution.

Working standard solutions were prepared by stepwise dilution from 1000-mg/L Co stock standard solution (Merck, Darmstadt, Germany) immediately before use.

A 3.0-mol/L buffer solution (pH 9.5) was prepared by diluting ammonium hydroxide solution with a hydrochloric acid solution.

Chloroform and a 1% aqueous solution of APDC were used.

The Amberlite XAD-7 resin (Rohm & Haas, Philadelphia, PA) was used. The particle size was between 20 and 50 mesh with a surface area of 450 $m^2/g$. Before using it, the surface of the resin was activated by immersion in 4 mol/L methanol/hydrochloric acid (1:1). Subsequently, metal impurities were removed by further washing by immersion with 2 mol/L hydrochloric acid.

**Table I. ICP Instrumental Parameters***

| | |
|---|---|
| RF generator power | 0.8 kW |
| Frecuency of RF generator | 40.68 MHz |
| Plasma gas flow rate | 8.5 L/min |
| Auxiliary gas flow rate | 1 L/min |
| Carrier gas flow rate | 0.5 L/min |
| Observation height (above load coil) | 15 mm |
| Analytical line: Co | 228.616 nm |

* A concentric glass nebulizer was used.

### Apparatus

The measurements were performed with a sequential ICP spectrometer (ICP2070, Baird, Bedford, MA). The 1-m Czerny-Turner monochromator had a holographic grating with 1800 grooves/mm. The ICP conditions are listed in Table I. The FI system used is shown in Figure 1. A Minipuls 3 peristaltic pump (Gilson, Villiers-le-Bel, France) was used. Sample injection was achieved using a Rheodyne (Cotati, CA) model 50, four-way rotary valve. A microbore glass column (50-mm length, 3-mm i.d.) fitted with porous 25-mm glass frits was used as the resin holder. The 228.616-nm spectral line was used and FI system measurements were expressed as peak height emission, which was corrected againts the reagent blank.

### Procedure

*Separation of Co from urine matrix.* Prior to the preconcentration step, the metal was released by means of the following procedure described by López-Artiguez et al. (4).

One-hundred milliliters of urine was brought to pH 1.0–1.1 with concentrated $HNO_3$, and 0.5 g activated charcoal was added. The mixture was heated and kept boiling for 5 min. Once cold, it was filtered through Whatman no. 1 paper and the charcoal was washed with 5 mL of deionized water. The resulting solution was adjusted with pH meter to pH 3.0 with 1 mol/L ammonium hydroxide. In separating funnels, 1 mL of 1% APDC was added to the purified urine, and the formed complexes were extracted twice with 15 mL chloroform. The organic extracts were mixed together and evaporated to dryness; 20 mL absolute ethanol was added, which, by coevaporation, contributed to the elimination of the chloroform.

The residue was digested with 3 mL concentrated $HNO_3$, followed by 3 mL 30% (v/v) $H_2O_2$. The digested solution was made up to 10 mL with deionized water.

*Preconcentration step.* Before loading the column, it was conditioned for the preconcentration at the correct pH (pH 9.5) running a $NH_4^+/NH_3$ 0.1 mol/L buffer solution through the microcolumn for 0.2 min., valve $V_1$ in position B (Figure 1).

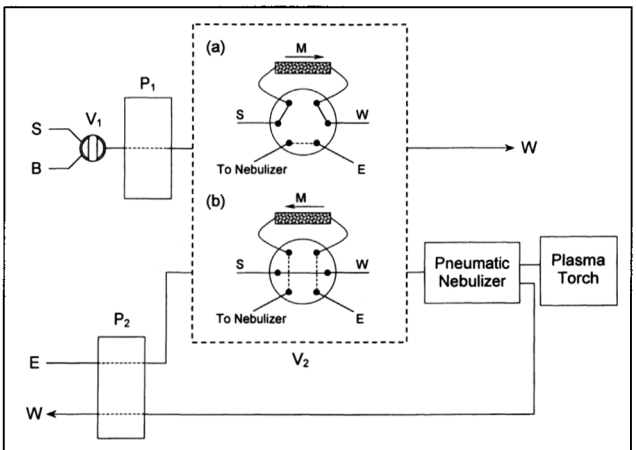


**Figure 1.** Schematic diagram of the instrumental setup. S: sample; B: buffer diluted; E: eluent; W: waste; P1 and P2: peristaltic pump; M: microcolumn; V1: two-way valve; V2: load-injection valve ((a) Load position; (b) Injection position).

To 10 mL of the solution from the separation step were added 0.1 mL 5-Br-PADAP of $10^{-2}$ mol/L, 0.4 mL ethyl alcohol, and 0.1 mL Triton 5% (v/v). The resultant solution was conditioned with 1 mL of 3 mol/L $NH_4^+/NH_3$ buffer to pH 9.5.

The complex was then loaded on the XAD-7 resin at a flow rate of 8 mL/min, with valve $V_1$ in position S and valve $V_2$ in load position (a). After the loading time, the sample still present in the lines and the column was removed with a further washing with buffer diluted solution, with valve $V_1$ again position B. Finally valve $V_2$ was switched to the injection position (b), and the retained metal complex was eluted in countercurrent (i.e., reversal of the flow direction through the column during elution in respect to sample loading) with 75% (v/v) $HClO_4$ at flow rate of 1.5 mL/min directly into the concentric nebulizer and subsequently into the plasma.

Because the metal complex Co-5-Br-PADAP is not totally retained on the resin (approximately 95%) the standard solution must also be passed through the microcolumn. The operating conditions were established, and the determinaton was carried out.

### Method validation

In order to demonstrate the validity of this method, the following experience was realized: 1 L of urine sample was collected and divided into 10 portions of 100 mL each. The proposed method was applied to six portions, and the average quantity of cobalt obtained was taken as a base value. Then, increasing quantities of cobalt were added to the other aliquots of sample and cobalt was determined by the same method (Table II).

## Results and Discussion

### Purification by activated charcoal

Generally, upon mixing the urine with immiscible organic solvents, persistent emulsions are formed; these are sometimes reduced to a layer at the interface, which introduces considerable error in retaining the organic compounds present in the urine.

To avoid emulsions and facilitate extraction, the samples were treated with activated charcoal, heated to the boiling point, and filtered.

**Table II. Method Validation***

| Aliquots | Base value (µg/L) | Quantity of Co added (µg/L) | Quantity of Co found (µg/L) | Recovery (%)† |
|---|---|---|---|---|
| 1 | – | 0.00 | 0.52 ± 0.02 | – |
| 2 | 0.52 | 0.20 | 0.71 | 95.0 |
| 3 | 0.52 | 0.40 | 0.92 | 100.0 |
| 4 | 0.52 | 0.80 | 1.30 | 97.5 |
| 5 | 0.52 | 1.20 | 1.71 | 99.1 |

* 95% confidence interval; $n = 6$.
† 100 × [(found-base)/added]

### Optimization of preconcentration system

The preconcentration of cobalt from urine samples was necessary because its concentration is too low to be compatible with ICP-AES detection limits. This preconcentration, performed prior to the ICP-AES measurement, permitted accurate and precise analytical results.

The retention conditions of the metal complexes were optimized, and the cobalt signal was monitored by measuring it with ICP-AES while changing the pH of the solution that passed through the sorption microcolumn. Figure 2 shows that the optimal pH values were in the range of 8–10.5. This phenomenon is understandable because better complexation occurs within this range. Considering these results, the selected pH was 9.5.

In the present work, a bead size of resin of 20–50 mesh was considered adequate for the preconcentration procedure in the microcolumn. Smaller resin particles could have improved retention capacity because of an increase in surface area. However, a decrease in particle size would cause an increase in the microcolumn back-pressure, with the consequent risks of tubing uncoupling.

It is well known that complex retention on XAD resins is modified by the concentration of organic solvents (15). Furthermore, the formation of metal complexes with 5-Br-PADAP is also affected by the solvent. Higher retention was observed for lower ethanol percentages. The value selected was 5% because it was the lowest value compatible with the complex stability. An increase in retention could be expected when using methanol as solvent (15). However, the toxicity of methanol can be considered a disadvantage for its use in routine analysis.

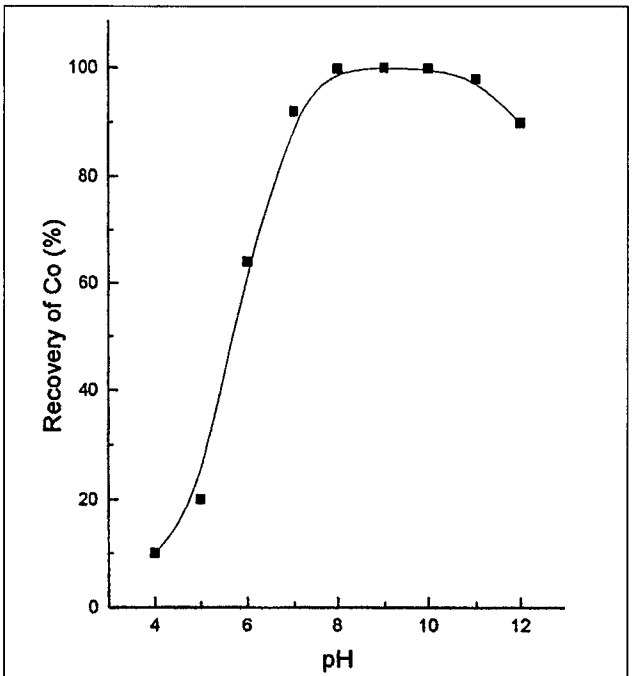


**Figure 2.** Dependence of retention of Co-5-Br-PADAP complex on pH of loading solutions. Preconcentration of 10 mL of Co-5-Br-PADAP complex. Cobalt concentration was 25 mg/L; ethanol concentration was 5% (v/v); and 5-Br-PADAP concentration was 10–5 mol/L.

In regards to the variation of response with the molar relation between reagent 5-Br-PADAP and cobalt, the signal remained constant between 10:1 and at least 100:1.

The flow rate sample through the microcolumn is a very important parameter because it is one of the steps that controls the time analysis. It could be verified that with flow rates up to 8 mL/min, there is no effect on the analyte recovery, which in optimum conditions is approximately 95%. At higher flow rates, recovery decreases.

The selection of eluent for the complex was critical. In most of the reported work, determination is effected using ETAAS and, therefore, the use of an organic solvent does not cause any diffilcuties. This was not possible in this case, however, because organic solvents generate strong turbulence in the ICP, which can eventually lead to its extinction. Therefore, in order to release the free metal it is necessary to develop a complex decomposition procedure. However, the decomposition of pyridylazo-Co complex is difficult because of its high stability. Consequently, concentrated strong acid were used.

Perchloric acid turned out to be a good eluent for the Co with 70% (v/v) as the minimun concentration necessary to obtain the best response. A concentration of 75% (v/v) percloric acid was adopted for the remainder of this work. Cobalt was completely eluted from the resin with 1.0 mL of 75% (v/v) $HClO_4$. The optimum eluent flow rate was 1.5 mL/min. All other conditions as an Table I.

### Interferences

Using the system described, experiments were performed to discover the degree to which the proposed method is susceptible to the effects of representative potential interferant species were tested. Thus, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Mn^{2+}$, and $Fe^{3+}$ could be tolerated up to at least 2500 mg/L. Commonly encountered matrix components such as alkali and alkaline earth elements generally do not form stable complexes and are not retained on the resin. The value of the reagent blank signal was not modified by the presence of the potentially interfering ions assayed.

### Performance of the preconcentration and determination system

The overall time required for preconcentration of 10 mL of sample (1.25 min, at flow rate of 8 mL/min), washing (0.2 min), elution (0.5 min, at flow rate of 1.5 mL/min) and conditioning (0.2 min) was about 2.15 min; hence, the throughput was approximately 27 samples/h.

A total enhancement factor of 90 was obtained with respect to the cobalt determination by ICP-AES without preconcentration.

The relative standard deviation (RSD) for 10 replicates containing 5 µg/L (mean ± SD: 5.1 ± 0.14) of Co was 2.7%. The calibration graph was linear with a correlation coefficient of 0.9994 from approximately 0.25 mg/L up to at least 100 mg/L. The detection limit, calculated as the amount of Co required to yield a net peak that was equal to three times the standard deviation of the background signal (3s), was 25 ng/L. Finally, the results of the method applied to cobalt determination in urine samples are shown in Table III. The concentrations were in the range of 0.27–0.52 mg/L for cobalt. The results obtained are in good agreement with those reported by Seiler et al. (1).

**Table III. Concentrations of Co Urine Samples***

| Sample Co | Concentration (mg/L) |
|---|---|
| 1 | 0.27 ± 0.03 |
| 2 | 0.47 ± 0.02 |
| 3 | 0.38 ± 0.02 |
| 4 | 0.52 ± 0.02 |
| 5 | 0.30 ± 0.03 |
| 6 | 0.46 ± 0.03 |
| 7 | 0.50 ± 0.02 |

* 95% confidence interval; $n = 6$.

## Conclusions

The main difficulty in the determination of cobalt in urine is its low concentration level. The work described in this paper using online preconcentration system with an FI-ICP-AES method has shown adequate sensitivity.

The coupling of an online preconcentration system with FI-ICP-AES increases the speed of the preconcentration and analysis process, and reduces sample consumption and contamination risks.

The recovery studies using the standard addition method showed that the proposed method can be used for the determination of cobalt in urine samples.

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