

# Determination of cobalt in urine by gas chromatography–mass spectrometry employing nickel as an internal standard

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## ABSTRACT

A gas chromatographic–mass spectrometric method for the determination of cobalt in biological materials employing stable enriched  $^{62}\text{Ni}$  as an internal standard and using lithium bis(trifluoroethyl)dithiocarbamate as a chelating agent is described. The method involves the addition of a known amount (1  $\mu\text{g}$ ) of  $^{62}\text{Ni}$  to the sample, the formation of the chelate and the determination by selected-ion monitoring of the  $m/z$  571/574 ratio, which corresponds to  $^{59}\text{Co}/^{62}\text{Ni}$ . No appreciable memory effect was observed, and an acceptable dynamic range of 100 was found. There was good agreement between the cobalt concentration values determined by gas chromatography–mass spectrometry and electrothermal atomic absorption spectrometry. The present method has high sensitivity and can be used for the quantitation of cobalt at concentrations as low as 1  $\mu\text{g/l}$ . The use of enriched  $^{62}\text{Ni}$  circumvents the problem caused by endogenous nickel and simultaneously provides data on the nickel concentration in the biological sample without any additional experimental effort.

## INTRODUCTION

Cobalt chloride has been used for its erythropoietic effect to treat anemic anephric patients [1], but acute ingestion of several grams has been reported to have toxic manifestations. The most widely noted toxic effects were the results of beer drinkers' cobalt cardiomyopathy where 48 cases with 20 death were recognized in Quebec. Subsequently, further cases were reported in the USA and Belgium. Cobalt salts, added for froth stability, were implicated in the acute myocardial damage, although compromised nutritional status

due to ethanol abuse may have contributed by increasing sensitivity to cobalt [2–4].

Studies by Alexandersson and Atterhog [5] have established that some cobalt-exposed workers had as much as 700 times the urinary cobalt concentration as did non-exposed individuals. The levels at which toxicity occurs are not well defined [6]. Further, cobalt toxicity is potentiated by other factors such as diet, alcohol consumption and tungsten carbide. This gives important implications to the development of methods to monitor biological fluids to determine cobalt's effect on health and safety.

A number of techniques have been developed and used for the determination of cobalt in biological samples. These techniques include atomic absorption spectrometry [7], neutron activation analysis [8], chemiluminescence [9], colorimetry [10] and catalytic methods [11]. These tech-

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niques have different shortcomings such as the necessity for separation of cobalt and/or poor reproducibility due to matrix effects or variable recovery. Schumacher-Wittkopf and Angerer [12], have improved the atomic absorption methodology by a chelation extraction step, but without an internal standard problems of variable recovery will continue to limit the methodology. As a result, there is no standard method for the determination of cobalt in biological samples such as urine, blood and serum. Consequently, there is no agreement about normal or toxic cobalt levels.

We have previously developed and reported stable isotope dilution gas chromatography-mass spectrometry (GC-MS) methods for the determination of nickel and chromium in urine using lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), as a chelating agent [13,14]. Isotope dilution depends upon the use of an enriched stable isotope (spike) of the element of interest. Enriched stable isotopes are ideal internal standards because quantitative recovery is not essential. However, cobalt is a monoisotopic element and does not even have a useful long-lived radioactive isotope that can be used as a spike for isotope dilution. The radioactive isotope,  $^{60}\text{Co}$ , is readily available and has a sufficiently long half-life ( $t_{1/2} = 5.3$  years), but it is inappropriate for isotope dilution mass spectrometry because of its high specific activity and high-energy gamma rays. We, therefore, explored GC-MS analysis of cobalt using another element as the internal standard. The major problem with this approach would be variations in the relative recoveries of the two elements during the separation and purification steps and during chelate formation prior to analysis. This variable recovery could lead to inaccurate quantitation because of the resulting inconsistency in relative responses. However, if this error can be minimized by ensuring quantitative and reproducible yield for both the internal standard and the monoisotopic element of interest, then monoisotopic elements could be analyzed in conjunction with the isotope dilution analysis of other elements.

The use of another element as an internal standard has been advocated [15] and a few studies have been published [16-18]. For example, nickel has been used as an internal standard for the determination of cobalt in human tissue samples using sodium diethyldithiocarbamate as a chelating agent [16]. However, the internal standard was added in the form of the nickel chelate just prior to the injection into the gas chromatography column but after the sample digestion and chelate formation. In another study on the determination of platinum by high-performance liquid chromatography in plasma ultrafiltrate and urine, nickel has been used as an internal standard [17]. In environmental studies,  $^{243}\text{Am}$  is commonly used as a chemical yield determinator for the recovery of curium. In an application to nuclear technology,  $^{233}\text{U}$  has been used as a non-isotopic diluent for the determination of plutonium [18]. Lastly, the use of non-endogenous isomeric analogues and homologues has long been a powerful tool in the development of quantitative GC-MS methods of organic compounds.

In this work, nickel was selected as an internal standard based on our previous studies which showed the absence of any appreciable memory effect (cross-contamination) in the sequential analyses of samples with different isotope ratios by GC-MS [13]. Further, the chelate formation using Li(FDEDTC) as a chelating agent, in the case of nickel, is quantitative over a wide pH range of 3-8 [19]. It is likely that the use of Li(FDEDTC) as a chelating agent is responsible for the absence of memory and the success of the method. The use of an enriched isotope ( $^{62}\text{Ni}$  used in the present work) as the internal standard circumvents possible contamination problems caused by endogenous element. The natural isotope contribution to the internal standard peak can be accounted for by simultaneously monitoring the highest abundant isotope ( $^{58}\text{Ni}$ ) of the natural element. Further, the determination of the concentration of the monoisotopic element can be performed during isotope dilution experiments for nickel without involving any additional experimental effort.

## EXPERIMENTAL

### Instrumentation

The GC-MS system consisted of a double-focusing, reverse geometry mass spectrometer (Model 8230, Finnigan MAT, San Jose, CA, USA) coupled to a gas chromatograph (Varian 3700). The mass spectrometer is equipped with a SpectroSystem 300 data system for on-line data acquisition and processing. The instrument was operated in the electron ionization (EI) mode using 70-eV electrons with a source temperature of 200°C, the conversion dynode at -5000 V and the secondary electron multiplier at 2500 V. Data for isotope ratio measurements were acquired in the selected-ion monitoring (SIM) mode using voltage peak switching.

The gas chromatograph was equipped with a DB-1 (J. & W. Scientific, Rancho Cordova, CA, USA) poly(dimethylsiloxane) bonded-phase fused-silica capillary column (10 m × 0.32 mm I.D., 0.25  $\mu$ m film thickness). Samples were injected by using an on-column injector (OCI-3, Scientific Glass Engineering, Austin, TX, USA) at an oven temperature of 100°C followed by a 15°C/min ramp to 300°C. The GC-MS interface was maintained at a temperature of 220°C. High-purity helium was used as a carrier gas.

### Reagents

$^{62}\text{Ni}$  (>98% enriched) used as an internal standard was obtained from Oak Ridge National Laboratory (Oak Ridge, TN, USA). Certified Atomic Absorption Standards of nickel and cobalt purchased from Fisher Scientific (Fairlawn, NJ, USA) were used as the primary standards. Double sub-boiling quartz-distilled nitric acid in PTFE bottles was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Ultrex-grade ammonium hydroxide solution (30%) was purchased from J. T. Baker (Phillipsburg, NJ, USA); stabilized hydrogen peroxide (50%) was obtained from Fisher Scientific. The standard reference material, freeze-dried urine SRM 2670 (normal and elevated levels of toxic metals), was purchased from the NIST and prepared according to

their directions. Li(FDEDTC) was synthesized in an inert atmosphere at -70°C using bis(trifluoroethyl)amine from PCR Research Chemicals (Gainesville, FL, USA), *n*-butyllithium and carbon disulfide from Aldrich (Milwaukee, WI, USA) [20].

Contamination control, always important in trace metals analysis, would not be considered a major problem when working with cobalt. Tap water has been reported to contain amounts of cobalt undetectable by electrothermal atomic absorption spectrometry (EAAS). Blank values were determined to be less than 0.1 ng for the reagent used for each specimen. Nonetheless, to eliminate the possibility of adventitious cobalt becoming a serious problem at the low levels present in biological materials and to minimize the correction of the  $^{62}\text{Ni}$  peak due to contamination from natural nickel, normal trace metal procedures were followed. Polypropylene/PTFE labware were leached with 5% (v/v) nitric acid and washed several times with doubly deionized water (DDW) before use. The entire procedure was carried out in a clean hood with laminar flow to prevent any contamination from dust. Powder-free gloves were worn to avoid contamination from hands. All dilutions were performed using DDW with a specific resistance of *ca.* 18 M $\Omega$ /cm at 25°C. The acetic acid-sodium acetate buffer (pH 3) was treated with 0.1 M solution of sodium diethyldithiocarbamate and extracted with dichloromethane to remove any contamination of nickel and cobalt.

### Preparation and standardization of internal standard solution

$^{62}\text{Ni}$  solution to be used as an internal standard was prepared and standardized as discussed previously [13]. Briefly, the  $^{62}\text{Ni}$  solution was calibrated by reverse isotope dilution GC-MS using a natural nickel primary standard. Weighed aliquots of the primary standard solution were mixed with weighed aliquots of the internal standard solution, in amounts which yielded an optimum standard-to-spike ratio. Chelates were prepared from the spiked samples and were used for MS determination of isotope ratios. Concentra-

TABLE I

ISOTOPE AND ION PEAK ABUNDANCES IN NATURAL NICKEL AND  $^{62}\text{Ni}$  INTERNAL STANDARD

| Natural nickel |                     |           |                                | $^{62}\text{Ni}$ internal standard |                     |           |                                |
|----------------|---------------------|-----------|--------------------------------|------------------------------------|---------------------|-----------|--------------------------------|
| Atomic mass    | Atom % <sup>a</sup> | Ion $m/z$ | Ion abundance (%) <sup>b</sup> | Atomic mass                        | Atom % <sup>a</sup> | Ion $m/z$ | Ion abundance (%) <sup>b</sup> |
| 58             | 68.27               | 570       | 54.47                          | 58                                 | 0.98                | 570       | 0.82                           |
| 60             | 26.10               | 572       | 30.97                          | 60                                 | 0.83                | 572       | 0.85                           |
| 61             | 1.13                | 573       | 5.30                           | 61                                 | 0.12                | 573       | 0.12                           |
| 62             | 3.59                | 574       | 7.66                           | 62                                 | 98.07               | 574       | 82.54                          |
| 64             | 0.91                | 576       | 1.60                           | 64                                 | <0.10               | 576       | 15.56                          |

<sup>a</sup> Values given by Oak Ridge National Laboratory.<sup>b</sup> Calculated abundances considering the contributions of C, N and S isotopes.

tion of nickel in the spike solution was calculated [21] from the experimentally determined isotope ratios, the weights of the standard and spike solutions taken for mixing, the ion abundances of nickel in the standard and spike, the average atomic weights of the nickel in the standard and the spike, and the concentration of nickel in the primary standard solution. Table I gives the data on the isotopic composition of natural nickel in the standard solution and  $^{62}\text{Ni}$ -enriched internal standard.

#### *Digestion of the urine sample and chelate formation*

A known volume (1 ml) of the reconstituted urine reference material or unknown urine sample was mixed with internal standard solution of  $^{62}\text{Ni}$  containing 1  $\mu\text{g}$  of nickel. The mixture was treated with 1 ml of concentrated nitric acid and was allowed to stand for at least 1–2 h, but preferably overnight, to allow the partial digestion of the organic matter and reduce foaming during subsequent heating. The partially digested solution was heated gently on a hot plate at 50°C to reduce the volume to *ca.* 100  $\mu\text{l}$ . At this point, 100  $\mu\text{l}$  of 50% hydrogen peroxide were added. The heating was continued and the solution inspected periodically. At each inspection, the contents were mixed and the beaker tapped gently to disperse frothing. The digestion with hydrogen peroxide was repeated four to five times until a white residue remained on complete evaporation of the

solution. The entire procedure required *ca.* 500  $\mu\text{l}$  of hydrogen peroxide and took 3–4 h. The residue was suspended in 1 ml dilute nitric acid, warmed slightly and the solution left at room temperature for 1–2 h to allow complete dissolution. The solution was then transferred to another polypropylene tube, washing the PTFE beaker at least two times with 500  $\mu\text{l}$  of DDW. The pH of the solution was adjusted to 3 using a 4% solution of ammonium hydroxide. Subsequently, 500  $\mu\text{l}$  of pH 3 acetate buffer was added and the metal chelates were formed by addition of 200  $\mu\text{l}$  of a 20 mM solution of the chelating agent. The solution was shaken for 2 min and the chelates extracted with two equal volumes of 500  $\mu\text{l}$  of dichloromethane. The organic extract containing the chelates was stored in the laminar flow hood and used for GC-MS analysis.

#### *Gas chromatography-mass spectrometry*

Prior to carrying out the isotope ratio measurements, the focusing conditions of the mass spectrometer were optimized and mass calibration was established using perfluorokerosene (PFK). The fragmentation pattern of  $\text{Co}(\text{FDEDTC})_3$ , given in Fig. 1, contained an ion at  $m/z$  571 formed by the loss of one ligand from the chelate. Of the cobalt-containing ions, this was the highest intensity ion and would provide the highest sensitivity for cobalt determination. The fragmentation pattern of  $^{62}\text{Ni}(\text{FDEDTC})_2$  is shown in Fig. 2. Our previous studies of nickel have

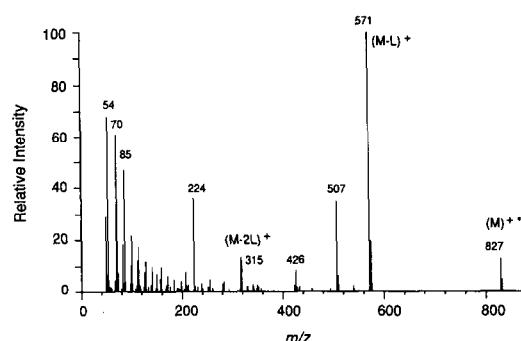


Fig. 1. Electron-ionization mass spectrum of  $\text{Co}(\text{FDEDTC})_3$ .

shown that the isotopic cluster of peaks in the  $m/z$  range of 570–576 correspond to the molecular ion for the nickel chelate [22]. Therefore, the  $m/z$  571/574 corresponding to  $^{59}\text{Co}/^{62}\text{Ni}$  in the cobalt and nickel chelates present in the solution was determined by SIM. The peak with  $m/z$  value of 570, corresponding to  $^{58}\text{Ni}$ , was also monitored to account for the contribution of any natural nickel. The software parameters for the mass spectrometer operation were defined to perform voltage peak switching for SIM at a rate of 2 Hz, yielding twenty measurement cycles across the *ca.* 10-s chromatographic peaks. The measurements were performed with flat peak tops at a mass resolution of *ca.* 1000. Data were obtained in a SIM experiment by integrating the chromatographic peak areas. Details of the measurement methodology have been published earlier [13]. All determinations represent at least duplicate injections.

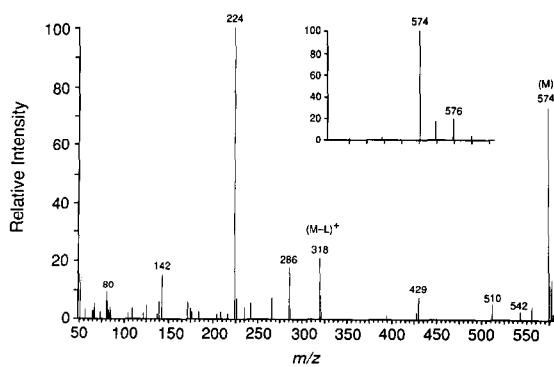


Fig. 2. Electron-ionization mass spectrum of  $^{62}\text{Ni}(\text{FDEDTC})_2$ .

### Memory effect evaluation

The memory effect was evaluated by preparing two synthetic mixtures of standard solution of cobalt and spike solution of  $^{62}\text{Ni}$ . The amount of cobalt in the two mixtures was different by a factor of 100 and the  $^{62}\text{Ni}$  solution added contained 1  $\mu\text{g}$  of nickel. These two mixtures were sequentially analyzed by GC-MS for the determination of  $m/z$  571/574 ratio, corresponding to  $^{59}\text{Co}/^{62}\text{Ni}$ .

## RESULTS AND DISCUSSION

### Evaluation of the memory effect

Memory effect refers to cross-contamination during sequential analyses of samples with different isotope ratios and can be a limiting factor in the GC-MS determination of metals. In fact, the choice of a suitable chelating agent which gives stable and volatile chelates at nanogram levels, without any appreciable memory effect, is crucial for the success of GC-MS method. The results of the memory effect evaluation are shown in Fig. 3. The “y”-axis in this figure is non-linear to clearly show the data. It can be seen that there is no appreciable memory effect during the GC-MS analyses of solutions differing in cobalt amounts by a factor of 100.

### Determination of the linearity range

The linearity of dynamic range of the method was established by taking different amounts of

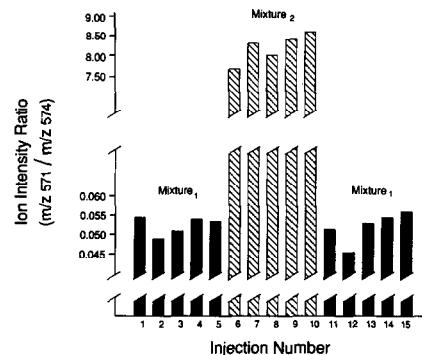


Fig. 3. Evaluation of cross-contamination between samples differing in cobalt amounts by a factor of 100 and 1  $\mu\text{g}$  of  $^{62}\text{Ni}$  internal standard.

the cobalt standard solution and mixing them with  $^{62}\text{Ni}$  internal standard solution containing  $\mu\text{g}$  of nickel. Eleven such mixtures containing  $\text{Co}/\text{Ni}$  amounts over a range of  $10^5$  were prepared and used. These mixtures were used to determine  $m/z$  571/574 isotope ratios. The contribution of natural contamination of Ni at  $m/z$  574 was accounted for by monitoring the  $m/z$  570 peak due to  $^{58}\text{Ni}$ . A least-squares analysis of the data obtained for the corrected  $\log(m/z 571/574$  ratio) and the  $\log(\text{amount of Co present in the sample})$  gives the equation:

$$\log(\text{ion ratio}) = 1.1 \pm 0.03 \log(\text{Co}) + 0.23 \pm 0.04 (n = 11, r = 0.997)$$

#### Determination of cobalt in the urine samples

To evaluate the accuracy of this GC-MS method for cobalt determination, a normal urine pool found to contain cobalt below the detection limit of EAAS ( $< 1 \mu\text{g/l}$ ) was taken and used for the preparation of synthetic urine samples containing known amounts of cobalt. A number of urine samples were prepared by adding known amounts of the primary standard, on a weight basis, to a known volume of urine. Known volumes (0.5 or 1 ml) of these urine samples were taken and a solution of  $^{62}\text{Ni}$  containing  $1 \mu\text{g}$  of nickel was added. The spiked urine samples were subjected to the treatment discussed above for digestion and chelate formation. About  $1 \mu\text{l}$  of this solution, containing 100 pg of cobalt, was used for injection into the GC-MS system. The

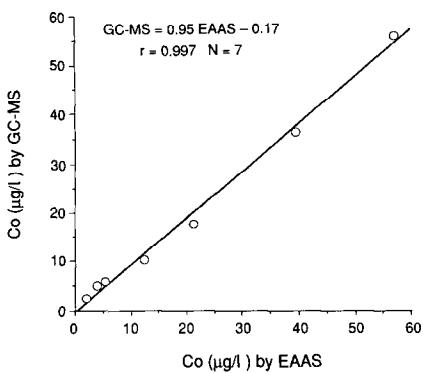


Fig. 4. Comparison of GC-MS and EAAS values for cobalt concentration.

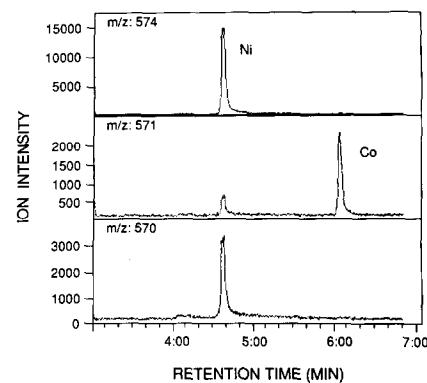


Fig. 5. Reconstructed ion chromatogram for the determination of cobalt in the urine sample using  $^{62}\text{Ni}$  as an internal standard.

results obtained for cobalt determination in these urine samples, using the calibration curve established (as discussed above), were compared with the values obtained by EAAS. As shown in Fig. 4, the cobalt concentration values were in the range  $1.8\text{--}58 \mu\text{g/l}$  with  $\text{GC-MS} = 0.95 \text{ EAAS} - 0.17$ , and correlation coefficient  $r = 0.997 (n = 7)$ . Fig. 5 shows a typical reconstructed ion chromatogram obtained for the GC-MS analysis of the urine sample containing  $2 \mu\text{g/l}$  cobalt. The injection of  $1 \mu\text{l}$  of the chelate solution would contain *ca.* 100 pg of cobalt. A high signal-to-noise ratio shows that the method has the potential to be very sensitive, down to sub parts-per-billion levels.

We also determined cobalt in the urine reference material, SRM-2670 (normal levels and elevated levels of toxic metals), available from NIST. The cobalt concentrations in these reference materials were also determined by EAAS to compare the results since the cobalt concentrations in these materials are not given by NIST. It is seen, from the results given in Table II, that the cobalt concentrations determined by GC-MS using  $^{62}\text{Ni}$  as the internal standard are in good agreement with the values obtained by EAAS.

We also evaluated  $^{50}\text{Cr}$  as an internal standard. It was rejected based on the imprecise and inaccurate results obtained in similar experiments. These problems were attributed to the complexity of the chromium chelate formation. Quantitative chelate formation requires the  $\text{Cr}^{VI}$

TABLE II  
DETERMINATION OF COBALT IN URINE (SRM-2670)

| Urine references material | Cobalt concentration (mean $\pm$ S.D.) ( $\mu\text{g/l}$ ) |                             |
|---------------------------|--|-----------------------------|
|                           | EAAS   | GC-MS                       |
| Normal level              | 1.37 $\pm$ 0.60 ( $n = 4$ )                                | 2.12 $\pm$ 0.24 ( $n = 4$ ) |
| Elevated level            | 105 $\pm$ 6 ( $n = 2$ )                                    | 104 $\pm$ 14 ( $n = 6$ )    |

oxidation state, and also the pH of the solution needs to be strictly controlled (pH 2.5–3.5) [23].

#### CONCLUSIONS

A GC-MS method using  $^{62}\text{Ni}$  as the internal standard and Li(FDEDTC) as the chelating agent is shown to provide good precision and accuracy for determining cobalt in urine samples. When the nickel concentration is determined by stable isotope dilution GC-MS, there is no additional experimental effort required for the cobalt determination. The method involves the addition of a known amount of  $^{62}\text{Ni}$  to the urine sample followed by the determination of  $m/z$  571/574 ratio by SIM using GC-MS. Since the method involves the chemistry of two different elements (cobalt and nickel), it is recommended to periodically run a synthetic mixture of the two to confirm the validity of the predetermined calibration curve. In view of the non-availability of a suitable isotope of cobalt to be used for isotope dilution, we believe that the present method provides a good solution to this problem. Though we have used a double-focusing mass spectrometer in the present work, the method can also be employed in the mass spectrometers equipped with a quadrupole analyzer. Since the chelate formation provides a pre-concentration as well as purification step, this GC-MS method gives a high sensitivity, down to sub part-per-billion levels. The GC-MS method provides additional selectivity by monitoring a specific  $m/z$  and thereby rejecting other interfering substances co-eluting with cobalt or nickel chelates. Further, this GC-MS

approach could be used for other monoisotopic elements, e.g. beryllium, aluminium and manganese.

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