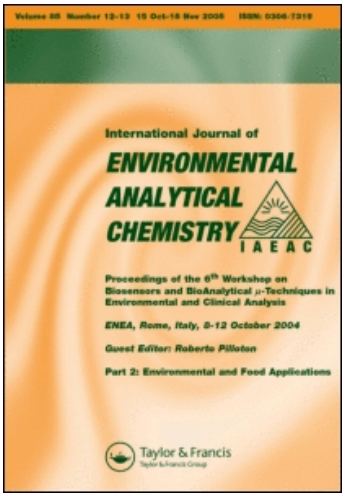

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Determination of Ultra Trace Amounts of Cobalt in Fish by Graphite Furnace Zeeman Effect Atomic Absorption Spectrometry

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A method is described for determining stable cobalt concentrations in fish flesh and bone using polarized Zeeman effect graphite furnace atomic absorption spectrometry (ZAAS). Cobalt analysis on freshwater fish flesh samples (10 g dry weight) required predigestion and wet-ashing at 70–80°C. Cobalt is chelated with ammonium pyrrolidine dithiocarbamate (APDC) extracted with methyl isobutyl ketone (MIBK) and analysed by ZAAS. The mean cobalt content calculated from the standard additions method using three replicate fish flesh samples was $4.23 \pm 1.0\,\mu g\,Co.\,Kg^{-1}$ (dry weight) Analyses were also carried out on flesh and bone samples from similar sized fish, of the same species, taken from three area lakes.

KEY WORDS: Stable cobalt, fish, Zeeman, graphite furnace AAS, trace element.

INTRODUCTION

Trace amounts of cobalt are essential to the metabolism of both terrestrial and aquatic biota.[1] However, the study of cobalt uptake by biota, particularly in freshwater ecosystems, has been limited mainly because the sensitivity of instrumentation for analysis of cobalt has been inadequate to measure the low concentrations

usually encountered in environmental samples. Radiotracer studies have reported ^{60}Co uptake by freshwater fish[2,3,4] but in order to relate ^{60}Co accumulation to that of the stable element, knowledge of specific activity is required. This requires determination of the ratio of stable cobalt to radioactive ^{60}Co. Limited analysis for trace quantities of cobalt, using flame atomic absorption spectroscopy, has been carried out on freshwater,[5] and on marine fish.[6]

Recent development of a simple method for direct determination of trace levels of cobalt in lake water, using graphite furnace Zeeman effect atomic absorption spectrometry,[7] prompted extension of this technique to measuring cobalt in fish tissue and bone. This paper reports a relatively simple, accurate procedure for determination of μg quantities of cobalt in fish. The precision of the method, determined by % relative standard deviation (% RSD) obtained from analysis of several "spiked" samples is noted. A 40 g flesh sample (10 g dry weight) was the minimum amount necessary for determination of cobalt at the concentrations we encountered in freshwater fish.

EXPERIMENTAL

Sample preparation

A simplified flowchart for cobalt extraction is shown in Figure 1. Flesh and bone samples were pre-digested overnight at room temperature using 50 mL and 25 mL of concentrated double distilled nitric acid ($DDHNO_3$) respectively. Each sample was then preheated to initiate fuming and removed from heat until fuming subsided. Wet ashing was continued, using a low heat (70–80°C), until all yellow-orange fumes dissipated. Sample volume was maintained ($\simeq$30 mL vol.) using deionized distilled water (DDW). The samples were then cooled in an ice bath to solidify the fat, which could be substantial in some samples. The waxy deposits were then removed using a flattened glass rod. This step minimised the use of MIBK in fat extraction. Heating was then continued, following addition of H_2O_2 (20 mL), until sample volume evaporated to $\simeq$5 mL. The solution was transferred to a 100 mL volumetric flask, using a minimum three rinses with DDW. Five millilitres concentrated double distilled hydrochloric acid was added and sample volume adjusted to 100 mL with DDW.

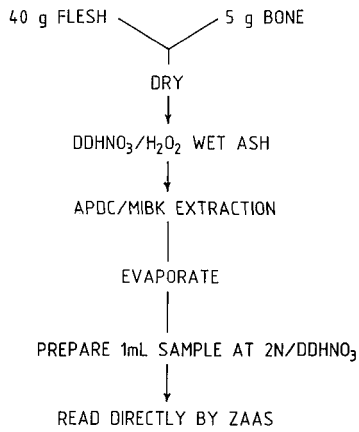

FIGURE 1 Simplified schematic for cobalt determination in fish flesh and bone.

Remaining fat was removed by transferring the 100 mL solution to a 250 mL volumetric flask containing 20 mL methyl isobutyl ketone (MIBK-Merck) and shaking for ten minutes using a mechanical shaker. The mixed solution was transferred back to the 100 mL volumetric flask, the MIBK removed by pipetting and discarded. This step was repeated three times using fresh MIBK and 10 minute shaking periods. After final MIBK removal the solution was returned to the 250 mL flask for cobalt extraction.

Cobalt extraction

One millilitre 0.1% bromphenol blue indicator and 30 mL 5% ammonium citrate in 6N NH_4OH was slowly added to the fat-free solution. Sample was adjusted to pH 3.5 by addition of 6N NH_4OH until the colour was greenish-blue. Sample volume was then increased to $\sim$220 mL using MIBK saturated DDW. The pH was readjusted, if necessary. Two millilitres of freshly prepared 2.5% ammonium pyrrolidine dithiocarbamate (APDC, Merck) in MIBK saturated DDW was then added and the solution shaken for 30 seconds. Four millilitres MIBK was then added and the sample shaken vigorously for 10 minutes. Sample volume was brought up into the neck of the flask with MIBK saturated DDW and allowed to stand for five minutes to complete extraction and separation.

The MIBK layer, containing the extracted cobalt, was carefully pipetted out of the neck of the flask, measured in a 10 mL cylinder and stored in an airtight glass vial.

Samples for injection were prepared for the graphite furnace by mixing four parts of the above MIBK solution with one part concentrated glacial acetic acid as recommended in the manual on Zeeman AAS operation. Ten μl of this was injected into the graphite cuvette using an Eppendorf pipette (Type 3130).

Instrumentation

Cobalt was determined using an Hitachi Model 170-70 graphite furnace polarized ZAAS equipped with a standard non-pyrolytic tube-type cuvette and Hitachi 561 recorder. The operating parameters for cobalt analysis are shown in Table I. The dry/ash/atomize parameters were optimized to yield maximum reproducible peak height for cobalt at the atomize phase of the furnace cycle for lake water samples.

TABLE I
Instrumental parameters

Lamp	Hollow cathode Tube Instrumentation Laboratory catalogue No. 62928	Carrier Gas Argon	0.2 L min^{-1}
Lamp current	10 mA	Sheath Gas Argon	3.0 L min^{-1}
Wavelength	240.7 nm	Dry	21 A, 60 s
Slit	1	Ash	115 A, 60 s
Expansion	$\times 3$	Atomize	300 A, 6 s
Response	3	Chart Range	10 mV
Sample volume	10 μL	Chart speed	5 mm min^{-1}
Cuvette	Tube-type Hitachi PT No. 170-5100		

RESULTS AND DISCUSSION

The standard addition method (SAM)[8] was used on fish flesh samples to compensate for possible errors in absorption response

produced by interfering substances in the sample matrix. Twelve 40 g flesh samples from a Northern Pike (*Esox lucius*) were divided into three groups of four samples. Standard additions of 50, 100 and 150 ng of cobalt were added to the first three samples in each group. The fourth sample received no cobalt addition. After extraction the absorption response of the Co was determined by the ZAAS. Figure 2 shows a calibration curve where peak height (mm) is plotted against concentration for cobalt standards added to flesh samples. The curve is based on a plot of mean values for three samples with no cobalt addition and three each of 50, 100 and 150 ng cobalt additions. The line was fitted by linear regression. The content in ng of the O addition sample may be read at the point

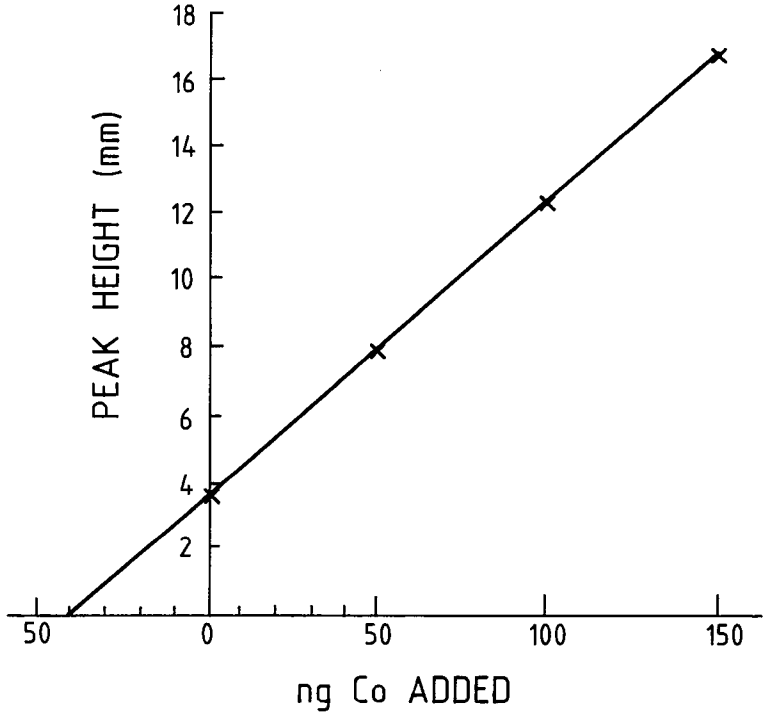

FIGURE 2 Standard addition method applied to analysis of fish flesh. Results show a linear response with concentration over the range 50 to 150 ng cobalt per 40 g wet flesh with a slope of $y=3.66+0.087x$ ($r=0.99$) where y=peak height in mm and x=ng Co. Extrapolation of line to zero yields total amount of Co in sample.

where the line intersects the concentration axis or may be calculated from the regression equation. The linearity was excellent, the correlation coefficient being $r=0.999$. The mean Co concentration of the zero addition sample of pike flesh, calculated from the SAM, using three replicate samples, was 4.23 g Co kg^{-1} (dry weight). The precision based on incremental standard additions of 50 ng Co, was 8% RSD ($n=12$).

Cobalt recovery

Percent recovery of cobalt with this method was determined using three replicate samples of fish flesh "spiked" before digestion with measured quantities of a standard cobalt solution. Cobalt concentrations added were 0.25 and 0.5 μg. A third sample received no cobalt. Results were compared against a 0.1 $\mu g.mL^{-1}$ standard solution of cobalt in the same MIBK-acetic acid matrix. Cobalt recovery, after background correction, for the two spiked samples was 85 and 82% respectively.

Cobalt analyses were carried out on flesh and bone samples from similar sized Yellow Perch (*Perca flavesens*) taken from three area lakes. Results (Table II) shows mean cobalt values (μg/kg dry weight) and standard deviations for perch flesh and bone from the

TABLE II
Cobalt content of flesh and bone samples from Yellow Perch (*Perca flavesens*) living in three different lakes (see text)

	μg Co/kg dry weight $\pm$SD	
Lake	Flesh	Bone
Perch	8.49 $\pm$ 2.72 (8)[a]	19.67 $\pm$ 5.15 (6)
Rutherford	8.54 $\pm$ 4.08 (9)	14.27 $\pm$ 7.87 (9)
Muskrat	10.16 $\pm$ 5.7 (9)	40.58 $\pm$ 8.59 (6)

[a](n) = no. of samples.

three lakes. The RSD for cobalt content in natural populations of perch average 46%.

The observed differences in cobalt content between flesh and bone samples, between fish from different lakes and between fish species (pike flesh—4.23 μg/kg and perch flesh—8.5–10 μg/kg) are subjects of an ongoing study.

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