

# Determination of cobalt in human liver by atomic absorption spectrometry with electrothermal atomization

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(Received 15th January 1991; revised manuscript received June 7 1991)

## Abstract

A detailed study of the use of electrothermal atomic absorption spectrometry for the determination of cobalt in human liver is described. Comparisons of sample digestion using nitric acid or nitric acid plus perchloric acid, atomization procedures and the application of palladium and magnesium nitrate chemical modifiers were studied using NBS SRM 1577a Bovine Liver. The best results were achieved with sample decomposition in nitric acid, atomization from the tube wall and no chemical modifier. Cobalt was determined in 90 samples of livers from foetuses and deceased newborns using the standard addition method with an average recovery of 99.8%.

*Keywords:* Atomic absorption spectrometry; Electrothermal atomization; Biological samples; Cobalt

Cobalt is an essential trace element that is required in the normal diet of man in the form of vitamin B<sub>12</sub> (cyanocobalamin). No specific deficiency of cobalt has been demonstrated, but a lack of vitamin B<sub>12</sub> in man results in pernicious anaemia [1]. Little is yet known of the forms in which this element occurs in man, other than vitamin B<sub>12</sub>.

Cobalt is widely distributed throughout the body, with the highest concentrations generally occurring in the liver, kidneys and bones. However, the amounts found in these organs and in body fluids are below 0.2  $\mu\text{g g}^{-1}$ , thus requiring the use of a sensitive technique such as electrothermal atomic absorption spectrometry (ET-AAS). This technique has been described for cobalt

in urine, plasma and serum [2–4]. However, the few reports on cobalt determination in liver of animals by ET-AAS [5,6] did not report the optimum experimental conditions.

ET-AAS often suffers from matrix interferences when it is applied to the analysis of biological material. Spectral interferences can be eliminated in most instances by the use of background correction systems. The introduction of chemical modifiers by Ediger [7] and of the L'vov platform [8] helped to eliminate both physical and chemical interferences.

Degradation of biological material in an environment free from contaminants and with complete liberation of metals without loss is the aim of sample decomposition. For oxidation of organic

matter using wet digestion, a single acid such as nitric acid or various acid mixtures have been employed [9,10]. Although the nitric acid followed by perchloric acid is recommended for biological samples [11], according to Wurfels et al. [12] almost all constituents of biological materials are completely mineralized after pressure decomposition with nitric acid.

In this work the optimum conditions for the determination of cobalt in human liver samples were studied, testing nitric acid and nitric acid plus perchloric acid for sample decomposition. The use of two chemical modifiers, magnesium nitrate and palladium [3,4], widely employed with biological samples and the L'vov platform in order to fulfil the requirements for the stabilized temperature platform furnace (STPF) technique [13], was also tested. All tests were run using NBS SRM 1577a Bovine Liver and procedures were rechecked with samples of human liver.

## EXPERIMENTAL

### *Apparatus and chemicals*

An atomic absorption spectrometer (Varian Spectra AA40 system) equipped with a double-beam, DS 15 data station, GTA 96 atomizer, autosampler, deuterium arc background corrector and hollow-cathode lamp as source was used. Pyrolytic graphite-coated graphite tubes and platforms were used. Argon was employed as the purge gas. The parameters were programmed for wavelength (240.7 nm), slit width (0.2 nm) and lamp conditions according to manufacturer's instructions. All experiments were run with 20- $\mu$ l samples and measurements were made based on peak-area absorbance.

The chemicals used were of analytical-reagent grade from Merck, except for nitric acid (Suprapur, Merck) and palladium nitrate (BDH). Working standard solutions in the range 5–50 ng Co ml<sup>-1</sup> were prepared in 0.5% (v/v) nitric acid by appropriate dilutions of 1000  $\mu$ g ml<sup>-1</sup> cobalt stock solution immediately prior to determination. Palladium nitrate (1000  $\mu$ g Pd ml<sup>-1</sup>) and magnesium nitrate (1000  $\mu$ g Mg ml<sup>-1</sup>) solutions were prepared in 0.5% (v/v) nitric acid.

### *Samples*

Ninety samples from the central portion of the right lobe of liver from fetuses and deceased newborn infants were collected in flasks free from metals. Using a surgical lancet, each sample was divided into four portions and two of them were comminuted. Approximately 2 g of these minced portions was weighed in a glass tube and kept frozen at -20°C until analysed.

### *Bovine liver decomposition*

A 3-ml volume of pure concentrated (Suprapur) nitric acid was added to 1 g of bovine liver and the mixture was placed in an autoclave for 15 min and evaporated to dryness. The residue was dissolved in 10 ml of 0.5% (v/v) nitric acid but this solution showed the presence of undissolved solids. A modified procedure was tested by adding 1 ml of perchloric acid after the drying step followed by evaporation to dryness and dissolution of the residue in 10 ml of 0.5% (v/v) nitric acid.

### *Optimization of graphite furnace operation*

The bovine liver digests and 50  $\mu$ g ml<sup>-1</sup> cobalt standard solution were used to establish the optimum temperature and time of drying, charring and atomization.

Cobalt was determined using a calibration graph in the range 5–40 ng Co ml<sup>-1</sup> for tube wall and 10–50 ng Co ml<sup>-1</sup> for platform experiments. Additions of 0.2, 0.4 and 0.6 ng of Co to the 20- $\mu$ l sample were done by adding 5, 10 and 15  $\mu$ l of a solution of 40  $\mu$ g Co ml<sup>-1</sup> and diluting to 40  $\mu$ l with 0.5% (v/v) nitric acid.

## RESULTS AND DISCUSSION

### *Charring and atomization temperatures*

The programmed temperatures and the peak-area absorbances obtained in tube wall and platform experiments are given in Table 1. The nitric acid-perchloric acid digests, completely clear, require a higher charring temperature for both tube wall and platform atomization. The residual perchloric acid probably forms more stable peroxides with cobalt, retaining it at higher temperatures. The analytical signals obtained by processing nitric

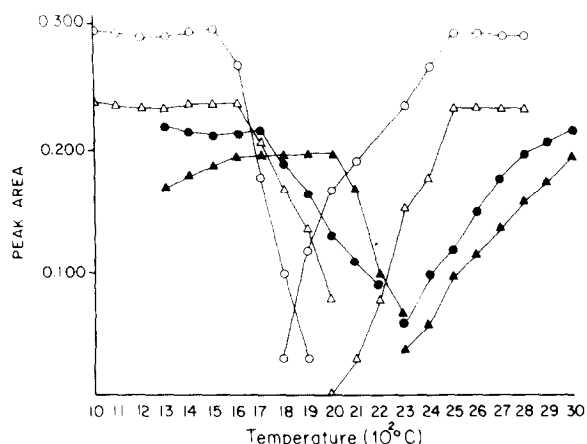


Fig. 1. Plot of cobalt peak area vs. temperature showing the charring and atomization temperatures of NBS bovine liver acid digests. ○, Nitric acid, tube wall atomization; △, nitric acid-perchloric acid, tube wall atomization; ●, nitric acid, platform atomization; ▲, nitric acid-perchloric acid, platform atomization.

acid-perchloric acid digests are lower than those for nitric acid digests. According to Fuller [14] and Welz [15], perchloric acid or its decomposition products react with the graphite to form volatile compounds that volatilize and interfere with the atomization. The tube lifetime is also strongly reduced.

The charring temperatures programmed are higher for samples than for standard solutions. Dissolved and non-dissolved solids in the digests in some way retain the metal. Smeyers-Verbeke et

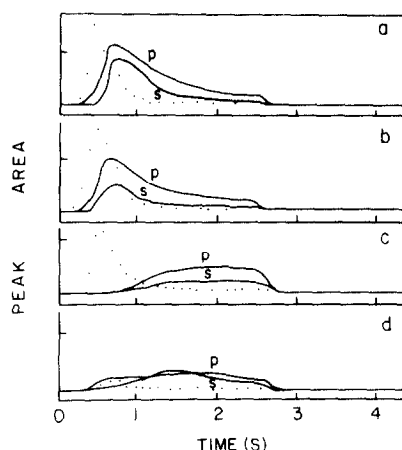


Fig. 2. Cobalt atomization profiles of bovine liver acid digests. (a,b) Tube wall atomization; (c,d) platform atomization. (a,c) Nitric acid digests; (b,d) nitric acid-perchloric acid digests. The dotted line corresponds to background signal. p = Standard; s = sample.

al. [16] reported that matrix effects in the analysis of biological material were caused by the occlusion of the metal in the matrix.

Figure 1 shows the charring and atomization curves for all the experiments. The stairhead temperature was not achieved with the platform even using the maximum programmable temperature of 3000 °C. The atomization temperature of cobalt reported in the literature ranges between 2400 and 2700 °C [3,17–19]. The signals obtained using the platform were lower than with atomization from

TABLE 1

Conditions of operation and tests of accuracy and precision on digests of bovine liver ( $0.21 \pm 0.05 \mu\text{g Co g}^{-1}$ )

Method	Temperature (°C)		Peak area (absorbance s)	Standard addition		Calibration graph	
	Charring	Atomization		[Co] ( $\mu\text{g g}^{-1}$ )	R.S.D. (%) <sup>a</sup>	[Co] ( $\mu\text{g g}^{-1}$ )	R.S.D. (%) <sup>a</sup>
<i>Tube wall</i>							
Standard solution	1300	2500	0.633				
Nitric acid	1500	2500	0.298	0.25	4.0	0.24	4.2
Nitric acid-perchloric acid	1600	2500	0.239	0.22	9.0	0.21	4.7
<i>Platform</i>							
Standard solution	1600	3000	0.384				
Nitric acid	1600	3000	0.215	0.26	3.8	0.23	4.3
Nitric acid-perchloric acid	2000	3000	0.198	0.23	4.3	0.24	8.3

<sup>a</sup> Relative standard deviation (mean of four readings).

the tube wall. This is in agreement with Kimberly et al. [3], who did not achieve good results using a platform for the determination of cobalt in urine.

#### Atomization

Figure 2 compares the atomization profiles of liver acid digests and standard solutions. In Fig. 2a–c, in spite of the background, both solutions atomize in the same way, showing an efficient charring step. For nitric acid–perchloric acid digests using the platform (Fig. 2d) the signal obtained with the standard solution is deformed in comparison with that for the sample digest, probably because the charring temperature used ( $2000^{\circ}\text{C}$ ), although adequate for nitric acid–perchloric acid digests, is higher than that required for a standard solution ( $1600^{\circ}\text{C}$ ). Cobalt atomization from the tube wall (Fig. 2a and b) occurs within a shorter time than that with the platform (Fig. 2c and d). There is a time lag between the heating of the tube wall and the platform [20]. This also explains the increase in the charring and atomization temperatures required with the platform (Table 1) and the broad peak profiles in Fig. 2c and d.

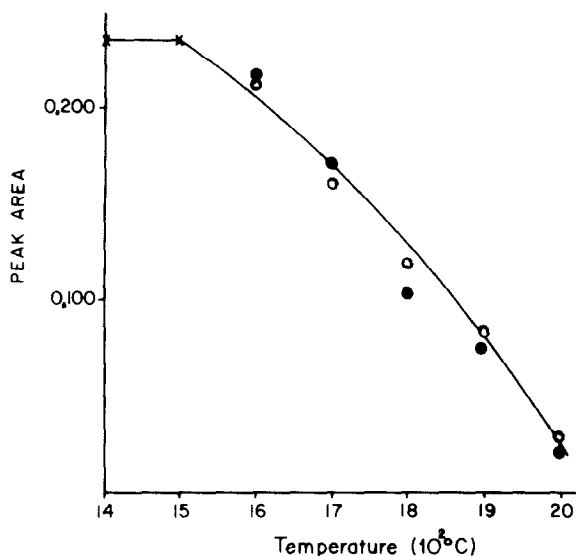


Fig. 3. Plot of peak area vs. charring temperature for Co in NBS SRM 1577a Bovine Liver nitric digests using chemical modifiers. Atomization from the tube wall.  $\times$ , Sample solution;  $\bullet$ , with  $2\ \mu\text{g}$  of Pd;  $\circ$ , with  $2\ \mu\text{g}$  of  $\text{Mg}(\text{NO}_3)_2$ .

#### Chemical modifiers

According to the above, decomposition of samples with nitric acid and atomization from the tube wall seem to give the optimum conditions for the determination of cobalt in liver. The peak-area absorbances obtained with different amounts of magnesium nitrate (2.0, 5.0, 10.0,  $100.0\ \mu\text{g}$ ) and palladium (2.0, 5.0, 10.0,  $20.0\ \mu\text{g}$ ) using a charring temperature of  $1600^{\circ}\text{C}$  were similar. Neither magnesium nitrate nor palladium prevented the loss of metal at a higher temperature and consequently the signal decreased. Figure 3 illustrates the effect of charring temperature on peak-area absorbances when using chemical modifiers. With palladium, this behaviour is probably due to the oxidant nature of the matrix, preventing palladium from remaining reduced and reacting with cobalt [21]. Chemical modifiers are usually employed in samples of fluids (e.g., urine, serum, milk) without digestion.

#### Accuracy and precision

The results for the determination of cobalt in bovine liver were obtained by using the standard addition method and the calibration graph procedure. Accuracy and precision data are given in Table 1. The experimental results are within the range of certified values for NBS SRM 1577a Bovine Liver.

The precision was characterized by a relative standard deviation of about 4% ( $n = 4$ ) for samples digested with nitric acid. The signals corresponding to nitric acid–perchloric acid digests are less affected by the background (Fig. 2).

#### Cobalt in human liver

The procedure for the decomposition of human liver samples was modified for lower dilution as the concentrations in those samples were established by preliminary experiments to be as low as  $0.020\ \mu\text{g}\ \text{g}^{-1}$ . This modification increased the matrix effects, requiring a new set of operation conditions, as shown in Table 2.

Although in determining the cobalt concentration in NBS bovine liver no difference was observed between using the standard addition method and a calibration graph (Table 1), for human liver samples the standard addition method

TABLE 2

Instrumental settings for the determination of cobalt in nitric acid digests of human liver with atomization from the tube wall

Step	Temperature (°C)	Time (s)	Gas flow-rate (l min <sup>-1</sup> )
Dry	90	5.0	3.0
	120	10.0	3.0
	120	40.0	3.0
Char	1700	10.0	3.0
	1700	35.0	3.0
	1700	5.0	0.0
Atomize	2500	0.4	0.0
	2500	2.0	0.0
Clean	2900	2.0	3.0

was necessary. Figure 4 shows the sample profile after applying the standard addition procedure.

A recovery test was made by adding 20 ng of cobalt to three samples with cobalt concentrations of 6, 17 and 38 ng g<sup>-1</sup>; the recoveries were 96.6, 100.0 and 103.0%, respectively. This high recovery demonstrated no losses of cobalt from sample in the overall procedure. The limits of detection and quantification were determined according to Keith et al. [22], and the values found were 0.020 and 0.057 ng, respectively.

The cobalt concentrations found in 90 samples of liver from human foetuses ranged from 6 to 45 (mean 18) ng g<sup>-1</sup> wet tissue. It is difficult to compare these results with others [23–30] owing to the different analytical methodology, sampling procedures and sample processing. However, the present mean values are close to results reported

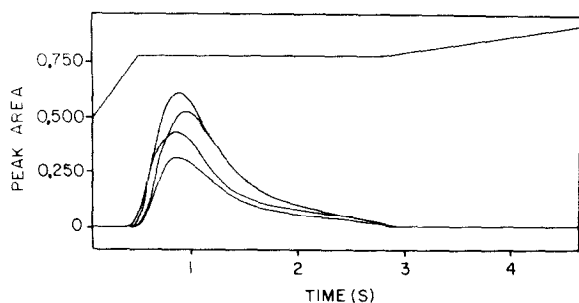


Fig. 4. Atomization signal of a typical standard addition curve in the determination of cobalt in human liver with nitric acid decomposition and atomization from the tube wall. 20  $\mu$ l of sample solution and addition of 0, 0.2, 0.4 and 0.6 ng of Co.

early [23,24] but almost five times lower than the mean value reported by Widdowson et al. [25] using flame spectrophotometry. High values for cobalt in livers of adults have been reported [26] also using AAS. In both papers [25,26] the analytical range was below that recommended for flame techniques. The spectrographic method used by Tipton and Cook [27] has poor sensitivity and therefore gives high values. The results reported by Iyengar [28] are difficult to discuss as no information on sampling was given.

In conclusion, the determination of cobalt in human tissues in the range 6–45 ng g<sup>-1</sup> was possible using ET-AAS and the standard addition method. Nitric acid pressurized digestion of liver samples was efficient and accurate for cobalt determination. Chemical modifiers commonly used in the analysis of biological fluids present no advantages for digested biological tissues.

The authors thank Dr. Maria Ophelia G. Araujo, Dr. Maria Amelia Yunes and Dr. Katia Jacomo, Hospital L-2 Sul, for collecting the autopsy samples and the National Research Council of Brazil for a Postgraduate Scholarship (to E.D.C.) and financial support (CNPq grant 40.5721/87).

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