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APPLICATION NOTES

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The determination of cobalt in urine using the GBC automated graphite furnace system

GBC

Introduction

Exposure to cobalt in the workplace can occur via inhalation of dusts used in the manufacture of superalloys and tungsten carbides[1]. Exposure can also occur in the nuclear technology field[2].

As cobalt is excreted mainly through the kidneys[3], it makes sense to analyse urine for cobalt content. Normal levels of cobalt in urine are less than 1.3 µg/L[4], a concentration so low that graphite furnace AAS is required for analysis. In this study, several methods were investigated. Atomization off the furnace wall was compared with atomization off the platform, the method of standard additions was compared with direct calibration, and peak area measurement was compared with peak height.

Experimental

Instrumentation

The GBC atomic absorption spectrophotometer and the GBC automated graphite furnace system was used. The GBC graphite furnace system comprises the graphite furnace (GF) and programmable automatic sample loader (PAL). This combination of equipment is particularly suited to trace biological analysis since the GBC graphite furnace can automatically prepare a range of standards from a single stock solution, inject chemical modifier and even automatically prepare a series of standard additions. The complete parameter outputs for platform and wall analysis are shown in Figure 1 and 2. The programs differ because of the different heating characteristics of the platform. As the platform is heated by radiation and not by current passing through it, no ramping is necessary in the drying stage because the temperature will increase naturally.

Element	Co
Beam Mode	Double Beam
Wavelength	240.7 nm
Slit Width	0.2 nm
Atomization	Furnace
Lamp Current	10.0 mA
EHT (gain)	– 420 V
Scale Expansion	1.000
Integration Time	0.02 s

Graphite Furnace Parameters					
Step No.	**Final Temp °C**	**Ramp Time (sec)**	**Hold Time (sec)**	**Gas Type**	**Read On**
1	160	1.0	60.0	Inert	No
2	250	1.0	10.0	Inert	No
3	1400	2.0	30.0	Inert	No
4	1400	0.5	0.5	None	No
5	2600	0.6	4.0	None	Yes
6	2700	0.3	2.0	Inert	No

Autosampler Details	
Sample Volume (µL)	10.0
Modifier Volume (µL)	10.0
No. of Multiple Injections	1
No. of Sample Repeats	3
Dry Steps for Multiple Injections	1
Inject on Step Number	1

Standards Details	
No. of Standards	2
Standard 1 (2 µL) Concentration	10
Standard 2 (4 µL) Concentration	20
Recalibration Rate	5
Recalibration Standard	2

Figure 1: Program for platform atomization

Element	Co
Beam Mode	Double Beam
Wavelength	240.7 nm
Slit Width	0.2 nm
Atomization	Furnace
Lamp Current	10.0 mA
EHT (gain)	– 412 V
Scale Expansion	1.000
Integration Time	0.02 s

Graphite Furnace Parameters					
Step No.	**Final Temp °C**	**Ramp Time (sec)**	**Hold Time (sec)**	**Gas Type**	**Read On**
1	80	5.0	1.0	Inert	No
2	120	30.0	10.0	Inert	No
3	1000	20.0	10.0	Inert	No
4	1000	0.5	5.0	Auxiliary	No
5	2400	0.7	3.0	Auxiliary	Yes
6	2700	0.3	2.0	Inert	No

Autosampler Details	
Sample Volume (µL)	10.0
Modifier Volume (µL)	10.0
No. of Multiple Injections	1
No. of Sample Repeats	3
Dry Steps for Multiple Injections	1
Inject on Step Number	1

Standards Details	
No. of Standards	3
Standard 1 (2 µL) Concentration	5
Standard 2 (4 µL) Concentration	10
Standard 3 (6 µL) Concentration	15
Recalibration Rate	5
Recalibration Standard	2

Figure 2: Program for wall atomization

Similarly, higher temperatures are required because the temperature of the platform lags that of the furnace by up to two hundred degrees. A GBC graphite furnace system with GBC software was also used to collect and store data as well as to aid in method development through the real-time graphics display of absorbance peaks.

Sample preparation

A Seronorm™ standard with a known amount of cobalt was solvated in 10 mL of distilled water and diluted 1:1 with distilled water. The dilution was necessary to decrease the background signal and to counter an apparent suppression of the atomic signal that was occurring with undiluted urine. A matrix modifier solution of 1% w/v magnesium nitrate anhydrous and 4% v/v nitric acid in distilled water was used[4]. The autosampler automatically added 10 µL sample volume.

The standards were prepared by the autosampler injecting a varying amount of a 25 ppb cobalt solution in 0.5% v/v nitric acid. A constant injection volume of 20 µL was used. To achieve this, 10 µL of matrix modifier was added to the standard volume and distilled water made up the balance. For standard additions, a 5 µL sample was used and to this was added 0, 2 and 4 µL of 50 ppb cobalt solution in 0.5% v/v nitric acid. Ten microlitres of matrix modifier was added and distilled water made up the balance of 20 µL.

Gas flow rates were:

Inert (N_2) 3.3 L/min
Auxiliary (Ar) 0.1 L/min

The argon flow is kept on during the atomization step for the wall because it depresses the background signal without affecting the atomic signal significantly. For platform work the background signal is depressed sufficiently with an argon flow.

Results

Table 1 shows results obtained on the Seronorm™ standard by the various techniques used. The certified concentration of cobalt was 11 ± 2 µg/L.

As can be seen from the Table, all the results agree extremely well with the certified value and each other. Examples of calibration curve graphs for both standards additions and direction calibration are shown in Figures 3 and 4 respectively. These graphs are linear over the range measured. Comparison of peak height measurement results with peak area measurements results showed that peak area measurement gave better precision. Obviously, the simplest method is one that involves doing a direct calibration and by atomizing off the furnace wall. As peak area measurements gave better precision than peak height measurements, peak area was chosen as the measuring mode. Figure 5 shows a graph, as it appeared on the screen, of the background only peak and the background corrected peak of a typical urine sample spiked to 20 ppb cobalt content. These peaks were used to optimize the temperature programs and gas flow rates for wall and platform atomization.

Measurement Technique	Atomization Technique	Calibration Technique	Results (µg/L)
Peak Area	Furnace Wall	Standard Additions	12.2
Peak Area	Platform	Standard Additions	10.0
Peak Area	Platform	Direct Calibration	9.9
Peak Area	Furnace Wall	Direct Calibration	10.5
Peak Height	Furnace Wall	Direct Calibration	9.9

Table 1: Concentration of cobalt found in Seronorm™ urine standard batch No. 108 (Certified value 11 ± 2 µg/L Co)

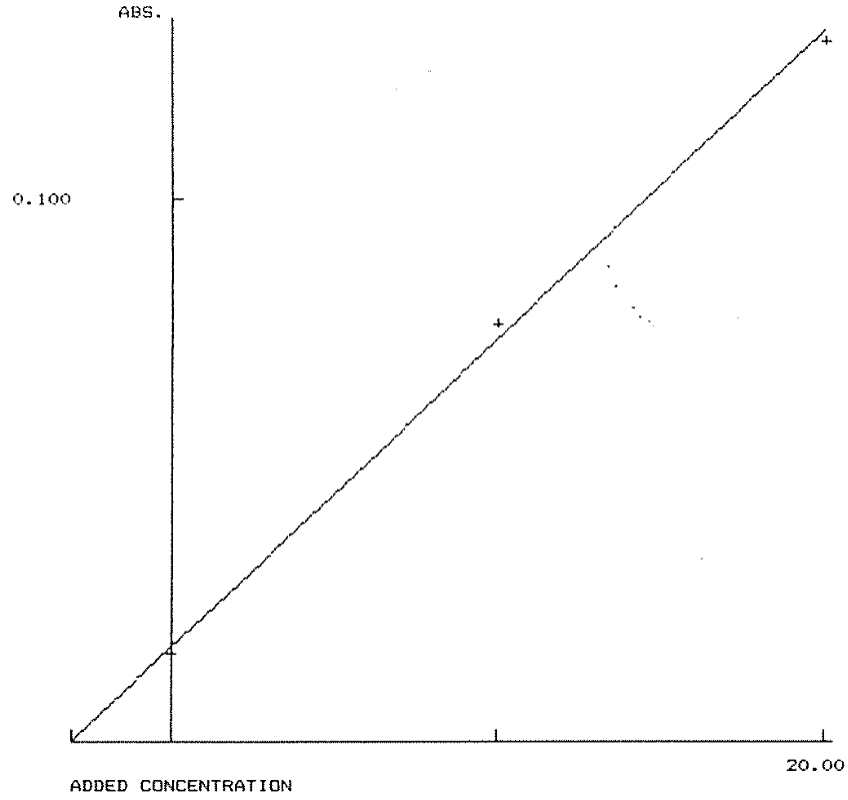

Figure 3: Standard additions graph using peak area measurements and atomization off the furnace wall

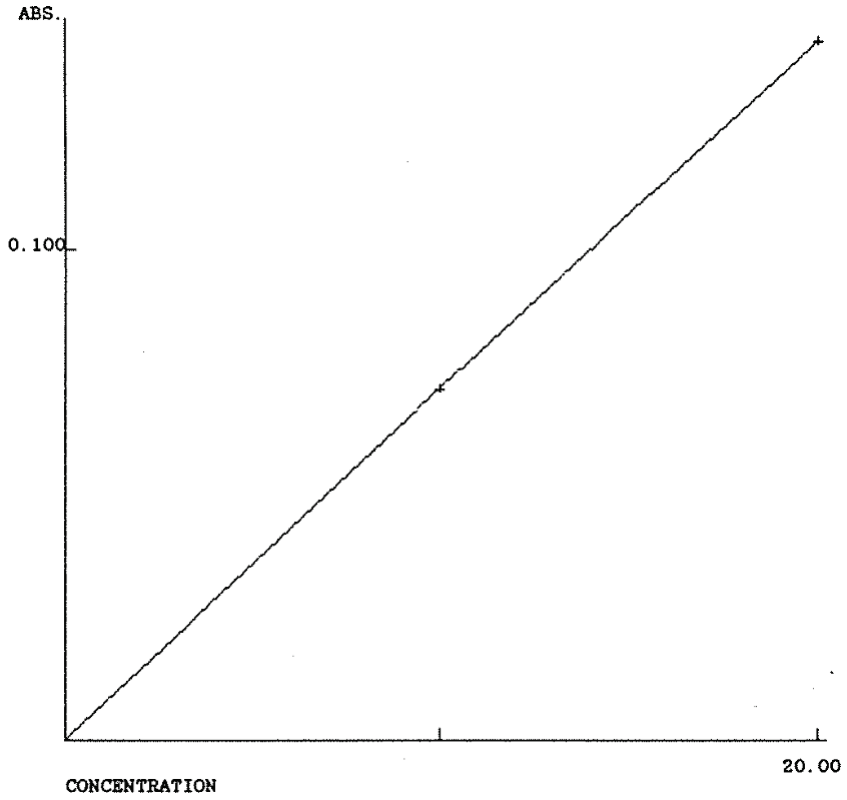

Figure 4: Calibration graph for platform atomization using peak area measurement

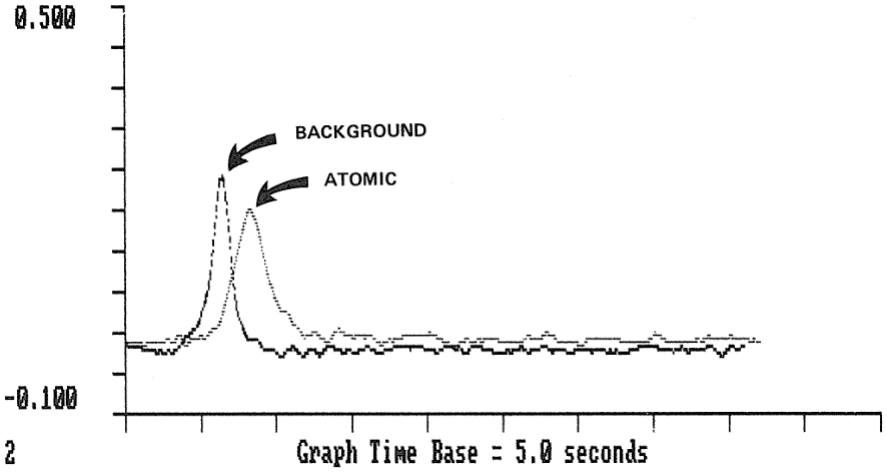

Figure 5: Peak graph from GBC graphite furnace system software of background and background corrected peaks (Abs. vs time). Urine diluted 1:1 with distilled water and spiked to 20 ppb cobalt content

Conclusion

A simple method for determining cobalt in urine was developed. It involved diluting the urine 1:1 with distilled water and utilizing the GBC atomic absorption spectrometer and the GBC automated graphite furnace system to automate the entire analysis. This was achieved by the programming the system to automatically prepare a series of calibration standards from a single standard stock solution, add modifier and load the sample.

The sample size was 10 µL and 11 ppb levels of cobalt were measured easily. Lower concentrations of cobalt could be measured without altering the method. Levels below those normally found in urine could be measured by the GBC automated graphite furnace system to make larger injections (up to 100 µL), or by making up to 255 multiple injections. With this technique an injection is made, dried and then a further injection is made.

A three point calibration curve with peak area measurement of absorbance signals was used. This calibration was then simplified to two points because of the linearity of the curve. The method was further simplified by atomizing directly off the furnace wall. However any of the techniques discussed would give excellent results.

References

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3. Venugopal B., and Luckey T.D., "Metal Toxicity in Mammals", Volume 2, Plenum Press, New York, 1978 p 284.

4. Kimberley, M.M., Bailey G.G., and Paschal D.C., Analyst, 1987, 112, 287-290.

5. Seronorm™ Urine Batch No. 108. Produced by: Nycomed AS Diagnostics P.I. Box 4284 Torshov 0401 OSLO 4 Norway.

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