AAS
APPLICATION NOTES

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

GBC

Introduction

Chromium, although toxic at high levels, is considered a necessary trace element for humans to maintain normal glucose metabolism. Consequently the analysis of chromium in urine is important at both high and normal levels. Normally chromium will be found in a range of 5 – 10 µg/L[1].

Because of its sensitivity, graphite furnace AAS is the most common method of analysis for Cr determinations in biological matrices. Background correction has been cited as difficult at the chromium wavelength (357.9 nm), where the emission intensity of the deuterium lamp is low[2]. However, the HYPER-PULSE background correction system of the GBC atomic absorption spectrophotometers overcomes this problem[3] and a simplified, straightforward analysis results.

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 system 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 output for the analysis is shown in Figure 1. Neither modifier nor platform were needed for the analysis. The conditions for the drying stage were set to give complete drying of the urine sample without boiling. The ash temperature of 900°C was found to yield the greatest sample absorbance.

Element	Cr
Beam Mode	Double Beam
Wavelength	357.9 nm
Slit Width	0.5 nm
Atomization	Furnace
Lamp Current	5.0 mA
EHT (gain)	– 344 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	10.0	5.0	Inert	No
2	110	40.0	10.0	Inert	No
3	900	10.0	15.0	Inert	No
4	900	0.5	0.5	None	No
5	2400	1.0	3.0	None	Yes
6	2700	0.2	3.0	Inert	No

Autosampler Details	
Sample Volume (µL)	10.0
Modifier Volume (µL)	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	10
Standard 2 (4 µL) Concentration	20
Standard 3 (6 µL) Concentration	30
Recalibration Rate	5
Recalibration Standard	2

Figure 1: Operating parameters

For the normal calibration, 10, 20 and 30 ppb Cr standards were prepared automatically by the autosampler. For the standards additions calibration, additions of 10 and 20 ppb Cr were made automatically by the PAL autosampler.

Sample preparation

A Seronorm™ standard with a known amount of chromium was solvated in 10 mL of distilled water and diluted 1:1 with distilled water. The dilution was necessary to counter an apparent suppression of the atomic signal that was occurring with undiluted urine. No matrix modifier was required.

The standards were prepared by the autosampler injecting a varying amount of 50 ppb chromium in 0.5% v/v nitric acid. A constant injection volume of 10 µL was used.

To achieve this, blank solution was added to the appropriate standard volume to a total of 10 µL. For standard additions a 5 µL sample was used and to this was added 0, 2 and 4 µL of 50 ppb chromium in 0.5% v/v nitric acid. The balance to 10 µL was made up with distilled water.

Nitrogen was the only gas used and then at a flow rate of 3.5 L/min.

Results

The analyses were performed by two different operators at different times, using the same operational parameters, but using a different furnace and hollow cathode lamp. As well, both standard additions and normal calibration method were used. Figure 2 shows an output of results obtained using peak area measurements and normal calibration. The following table shows results obtained on the Seronorm™ standard by the various techniques used. The certified concentration of chromium was 22 ± 2 µg/L.

Measurement Technique	Calibration Technique	Results (µg/L)
Peak Height	Standard Additions	24.4
Peak Height	Direct Calibration	22.8
Peak Area	Standard Additions	22.2
Peak Area	Direct Calibration	21.2, 20.2

Table 1: Concentration of chromium found in Seronorm™ urine standard batch No. 108 (certified value 22 ± 2 µg/L Cr)

Calibration					
Standard/Blank	**Reading 1**	**Reading 2**	**Reading 3**	**Mean**	**RSD (%)**
Blank 1	0.014	0.013	0.015	0.014	–
Standard 1	0.192	0.200	0.207	0.200	3.89
Standard 2	0.363	0.362	0.362	0.363	0.19
Standard 3	0.514	0.514	0.514	0.514	0.02

Concentration					
Standard/Blank	**Reading 1**	**Reading 2**	**Reading 3**	**Mean**	**RSD (%)**
Sample 1	10.64	10.52	10.69	10.62	0.79

Figure 2: Results output of direct calibration using peak area

As can be seen from the table, all the results agree with the certified value and with each other. Examples of calibration curve graphs for both standard additions and direct calibration are shown in Figures 3 and 4 respectively. Figure 5 shows an absorbance vs time peak height graph. Both types of calibration graphs are quite linear over the range measured and there is very little difference in the precision of the two measurement techniques.

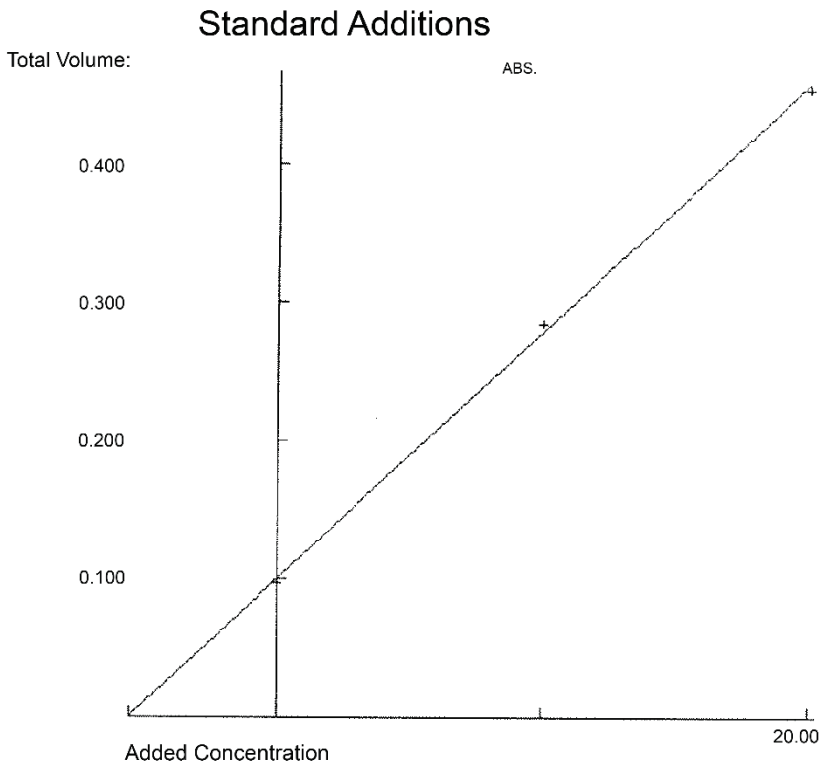
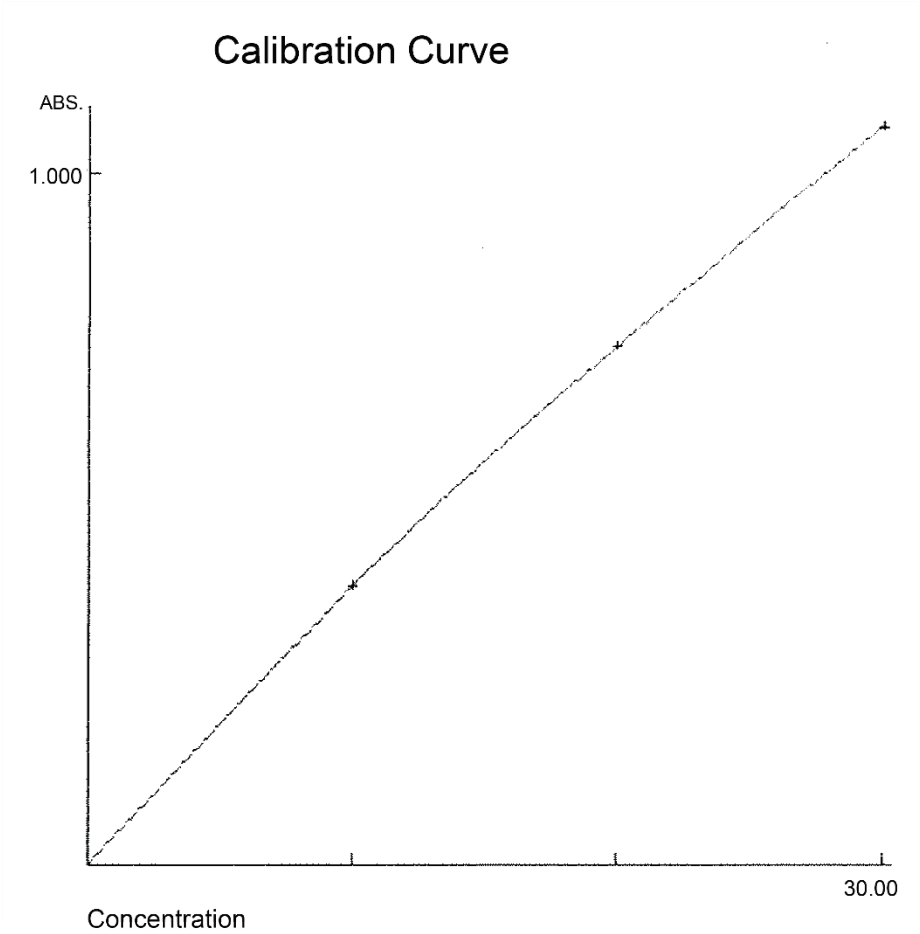


Figure 3: Standard additions graph



Calibration Curve		
Standard	**Absorbance**	**Concentration**
1	0.403	10.00
2	0.746	20.00
3	1.062	30.00

Figure 4: Calibration curve

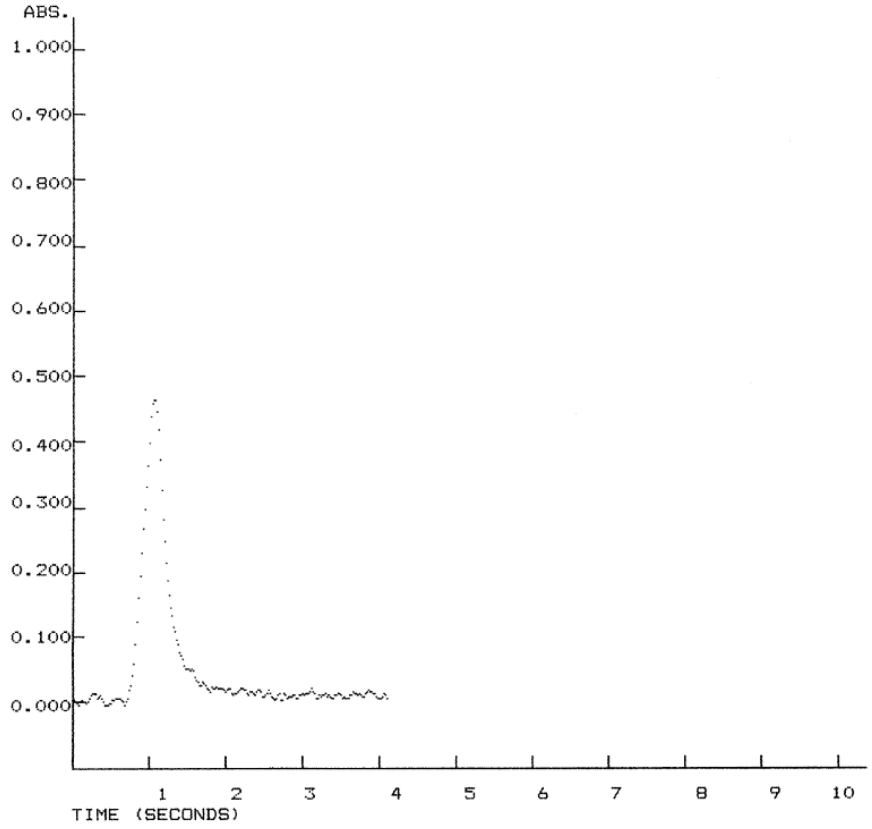


Figure 5: Peak height graph (absorbance vs. time)

Obviously, the simplest method is one that involves doing a direct calibration with either the peak height or peak area measurement technique.

Conclusion

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

The sample size was only 10 µL and 20 ppb levels of chromium were measured easily. Lower concentrations of chromium could be measured without altering the method. Levels below those normally found in urine could be measured by using 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 and either peak height or peak area measurement of absorbance signal were used. No problems were encountered with background correction, due to the unique HYPER-PULSE background correction system of the GBC atomic absorption spectrophotometer. As a result excellent results were obtained for a hard to analyse element in a complex matrix.

References

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Authors

Robert Peile, GBC Scientific Equipment Pty Ltd, Australia.

Bettina Oelke, Applied Research Inc., 9545 Wentworth Street, Sunland, California, 91040, USA.

GBC Scientific Equipment Pty Ltd
A.B.N. 30 005 472 686
4 Lakewood Boulevard, Braeside Victoria 3195, Australia
Phone: 61 3 9588 6666 Fax: 61 3 9588 6677
e-mail: gbc@gbcsci.com Website: www.gbcsci.com

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