Method Guide: 40182

Atomic Absorption Full Method Cr in Urine

Key Words

- Chromium
- Urine
- Zeeman Background Correction
- Graphite Furnace
- Atomic Absorption
- Extended Lifetime Cuvettes
- Matrix Modification

Introduction

The toxic effects of certain chromium compounds are well known. Chromium is not easily absorbed in the intestinal tract and is mainly excreted in the urine, and so urinary chromium output is frequently used as an indirect reflection of occupational chromium exposure.

The metal has also been shown to be biologically active in trace amounts, and the chromium containing molecule known as the "glucose tolerance factor" is thought to be an insulin co-factor, and hence has been implicated in glucose metabolism. Chromium has been used as a dietary supplement for some diabetic patients, and the uptake of the metal has been assessed by monitoring urinary chromium levels1.

Normal levels of chromium in urine have been shown to be below 1 µg/L, while elevated levels in occupationally exposed subjects and patients on chromium supplemented diets can rise to 20 - 30 µg/L2. Chromium is most usually determined in urine by Graphite Furnace Atomic Absorption Spectrometry, although it has been shown that an accurate background correction system that is free of emission breakthrough effects at the high wavelength normally used for chromium is essential3. Sensitivity and detection limits are such that it is necessary to use solvent extraction procedures4, or to analyse undiluted urine to accurately measure normal levels, although samples from exposed subjects can be diluted as necessary.

Analytical Range

A method for the determination of chromium in urine samples is presented. The 3 sigma method detection limit is 0.08 µg/L. Samples containing up to 25 µg/L of chromium could be analysed after dilution.

Principle

Chromium is determined in undiluted natural urine by direct calibration against aqueous standards using Graphite Furnace Atomic Absorption Spectrometry. Zeeman background correction is used throughout. Magnesium nitrate is used as a matrix modifier with conventional “off-the-wall” atomisation from an Extended Lifetime Cuvette.

Method

Reagents:

Nitric acid (Spectrosol grade).

Chromium master standard (1000 mg/L Spectrosol or equivalent).

Methanol (AnalaR grade or equivalent).

Magnesium nitrate (Aristar grade or equivalent).

All reagent examples available from:

Fisher Scientific
Bishop Meadow Rd
Loughborough,
LE11 5RG
UK

Reference urine samples were obtained from:

Nycomed Pharma AS
PO BOX 4284
Oslo N-0401
Norway

Thermo SCIENTIFIC

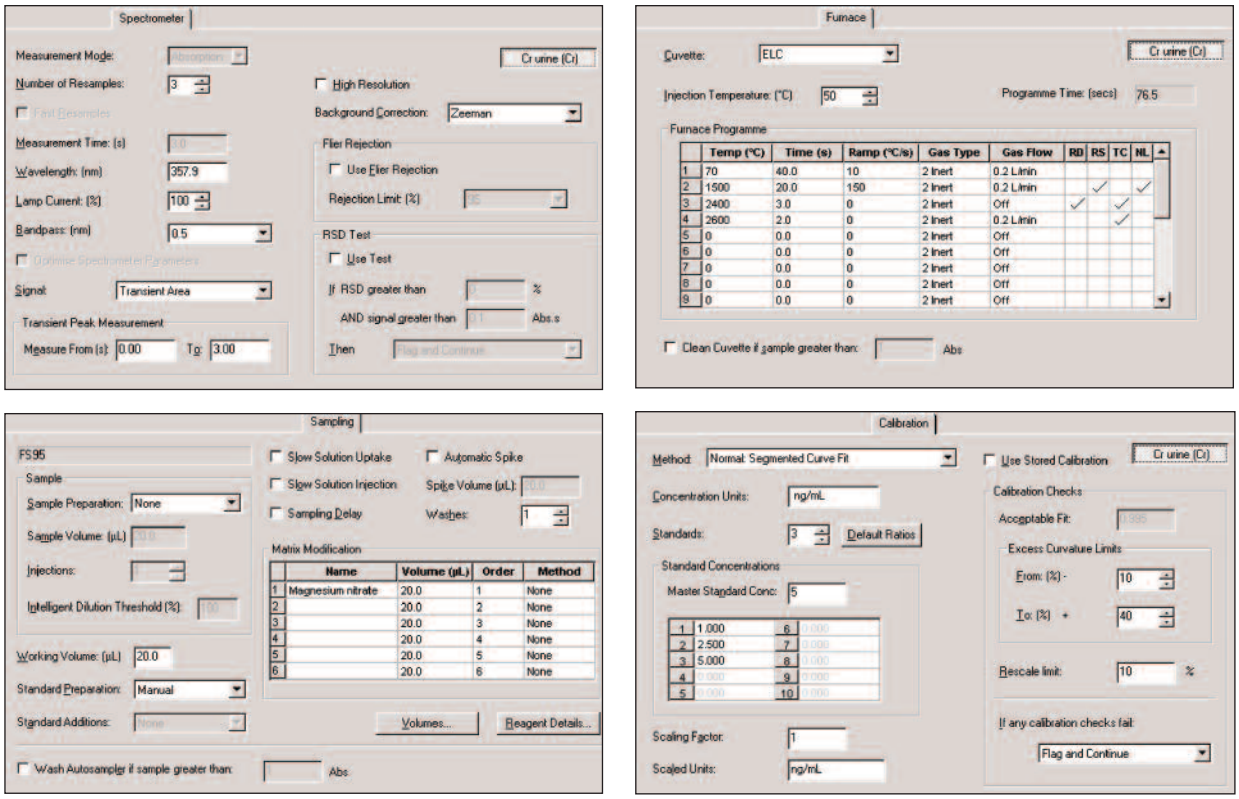

Figure 1: Analysis parameters

Sample collection

Samples were collected from subjects who had not knowingly been exposed to chromium. Metal-free plastic or glass containers were used, and the samples were acidified to a pH <2 with nitric acid immediately after collection. Samples were stored at 4 °C and analysed as soon as possible after collection.

The reference urine was re-constituted following the manufacturer's instructions, and acidified and stored with the natural samples.

Method development

Standard solutions were prepared by dilution of the master chromium standard, and contained 0.1 % v/v nitric acid. A working standard of 5.0 µg/L was used.

A pooled sample of natural urine was spiked with 1.0 µg/L of chromium and was used for the method development experiments. Ridged Extended Lifetime Cuvettes were used throughout.

Default values of most instrumental parameters were used, as shown above. Peak area measurements were used exclusively.

The Normal calibration method, using three standards was used. The two lower standards were automatically prepared from the 5.0 µg/L standard using the furnace autosampler facilities.

It was found that it was beneficial to inject the urine samples into a pre-warmed cuvette, and then dry them at a relatively low temperature of 70 °C. This significantly improved the precision of the measurements.

An autosampler wash solution containing 0.1 % v/v of nitric acid and 5 % v/v of methanol was used to prevent memory and organic deposits on the autosampler capillary tip.

An ash plot for the spiked urine was automatically generated, and showed that the chromium in the urine sample was stable up to 1400 °C. However, when the experiment was repeated with the aqueous standard, significant chromium losses occurred above 1200 °C.

Using an ash phase of 1200 °C, recovery of chromium spikes was low and variable (table 1).

Sample	Chromium found (µg/L)	Recovery (µg/L)
Urine	1.692	-
Urine+0.5 µg/L	2.056	0.364 (73 %)
Urine+1.0 µg/L	2.349	0.675 (66 %)

Table 1. Recovery with 1200 °C ash phase.

Adding 5mL of a 2.5 % m/v magnesium nitrate solution as a matrix modifier stabilised the chromium in the aqueous solution to at least 1600 °C, and stabilised it in the urine up to 1500 °C (figure 2).

With an ash temperature of 1500 °C and the matrix modifier, good recoveries of the spikes were obtained (table 2), with clean, well shaped signals (figure 3).

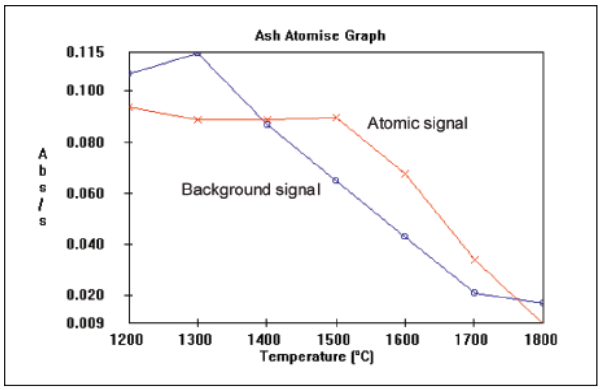

Figure 2: Ash plot of urine with matrix modifier

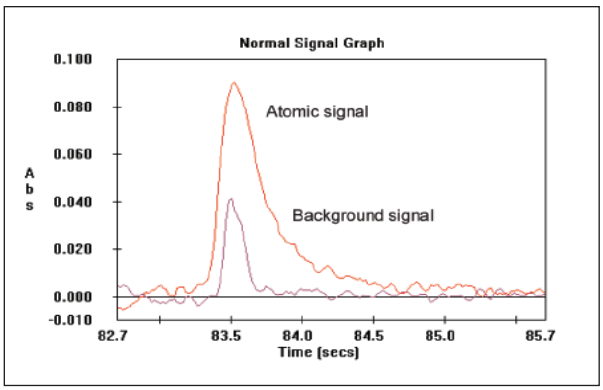

Figure 3: Optimised chromium signal from spiked urine sample

Sample	Chromium found (µg/L)	Recovery (µg/L)
Urine	0.373	-
Urine + 0.2 µg/L	0.565	0.192 (96 %)
Urine + 1.0 µg/L	1.372	0.999 (99 %)

Table 2: Recovery with 1500 °C ash phase

Method validation

Twelve replicate measurements of the Seronorm sample diluted 1+ 9 with water, a single urine sample, and that urine spiked with 0.2 and 1.0 µg/L of chromium were performed in a single run. Each sample result was the mean of 3 resamples.

Results

The results from the validation experiment are summarised in figure 4 and table 3.

The characteristic mass (the mass required to give a signal 0.0044 absorbance.seconds in area or 0.0044 absorbance units high) for chromium measured under these conditions was calculated from the calibration data to be 3.8 pg for the area measurement, and 1.5 pg for peak height measurement.

Sample	Chronium found (µg/L)	s.d (µg/L)	R.S.D. (n=12)
Seronorm(1+9)	2.38	0.043	1.8 %
Urine	0.373	0.027	7.3 %
Urine+0.2 µg/L	0.565	0.026	4.6 %
Urine+1.0 µg/L	1.372	0.062	4.5 %

Table 3: Validation experiment results

The natural urine sample was shown to have a chromium content of 0.373 µg/L, with a standard deviation of 0.027 µg/L. The 3 sigma detection limit for the method is therefore 0.08 µg/L.

The mean recovery of the 1.00 µg/L spike was 0.99 µg/L (100 %), with a standard deviation of 0.06 µg/L. Recovery of the 0.2 mg/L spike was 0.19 µg/L (96 %) with a standard deviation of 0.03 µg/L.

The Seronorm reference urine sample gave a result of 23.8 µg/L after taking account of the dilution; the Preliminary Recommended Value taken from the manufactures certificate was 24 µg/L.

No significant change in sensitivity was noted for at least 500 atomisations; a single ELC cuvette was used throughout the method development and validation experiments.

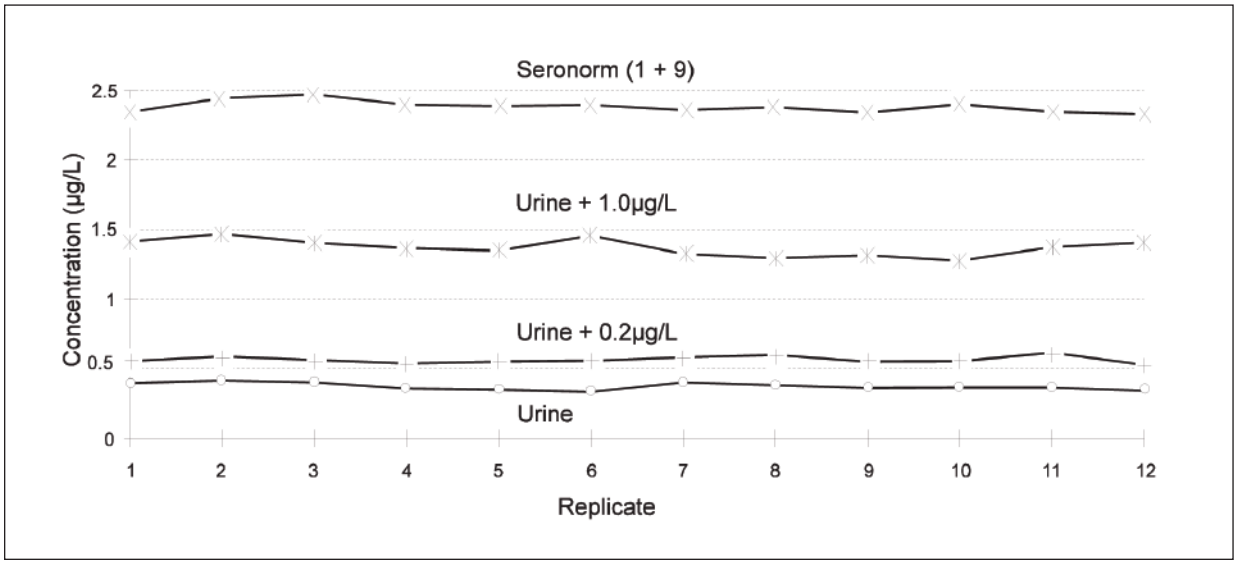

Figure 2: Validation experiment results

Conclusions

A simple method suitable for the routine determination of normal levels of chromium in urine samples is presented. Use of matrix modification with magnesium nitrate allows simple aqueous standards to be used for calibration, and the Zeeman background correction system compensates accurately for the residual background signal without introducing further errors due to emission breakthrough effects. "Off the wall" atomisation was shown to be entirely suitable for this analysis, resulting in excellent sensitivity and detection limits, while the Extended Life Cuvettes allow the full capacity of the furnace autosampler to be utilised.

References

(1) Mertz, W., Physiol. Rev., 1969. 49, 136

(2) Egila, J. N., Littlejohn, D., Ottaway, J.M., and Xiao-Quan, S., Anal. Proc., 1986, 23, 426

(3) Halls, D. J., and Fells, G. S., J.A.A.S., 1986, 1, 135

(4) Xiao-Quan, S., Yan, Z., Zhe-Ming, N., Atomic Spectroscopy, 1990, 11, 116

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa
+43 1 333 5034 127

Australia
+61 2 8844 9500

Austria
+43 1 333 50340

Belgium
+32 2 482 30 30

Canada
+1 800 530 8447

China
+86 10 8419 3588

Denmark
+45 70 23 62 60

Europe-Other
+43 1 333 5034 127

France
+33 1 60 92 48 00

Germany
+49 6103 408 1014

India
+91 22 6742 9434

Italy
+39 02 950 591

Japan
+81 45 453 9100

Latin America
+1 608 276 5659

Middle East
+43 1 333 5034 127

Netherlands
+31 76 579 55 55

South Africa
+27 11 570 1840

Spain
+34 914 845 965

Sweden/Norway/ Finland
+46 8 556 468 00

Switzerland
+41 61 48784 00

UK
+44 1442 233555

USA
+1 800 532 4752

www.thermo.com

The method of sample treatment described in this publication should be performed only by a competent chemist or technician trained in the use of safe techniques in analytical chemistry. Users should acquaint themselves with particular hazards which may be incurred when toxic materials are being analysed and handled in the instruments, and the instrument must be used in accordance with the operating and safety instructions given in the Operators manual.

The exact model of instrument on which this analysis was performed may differ from that stated. Although the contents have been checked and tested, this document is supplied for guidance on the strict understanding that neither Thermo Fisher Scientific, nor any other person, firm, or company shall be responsible for the accuracy or reliability of the contents thereof, nor shall they be liable for any loss or damage to property or any injury to persons whatsoever arising out of the use or application of this method.

FM 09032

Thermo Electron Manufacturing Ltd (Cambridge) is ISO Certified.

AN40182_E 06/08C