Atomic Absorption Full Method
Cr in urine

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 levels.

Normal levels of chromium in urine have been shown to be below 1µg/L, while elevated levels in occupationally exposed subjects and patients on chromium supplemented diets can rise to 20 - 30µg/L. 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 essential. Sensitivity and detection limits are such that it is necessary to use solvent extraction procedures, 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 0.08µg/L. Samples containing up to 25µg/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.

Instrumentation
The M Series Atomic Absorption Spectrometers (M6 and MQZ) from Thermo Electron Corporation fitted with a GF95 Zeeman Graphite Furnace and an FS95 Furnace Autosampler were used for all analyses.

Method
Reagents:
Nitric acid (Spectrosol grade).
Chromium master standard (1000mg/L Spectrosol or equivalent).
Methanol (AnaR grade or equivalent).
Magnesium nitrate (Aristar grade or equivalent).

Reference urine samples were obtained from:
Nycomed Pharma AS
PO BOX 4284
Oslo N-0401
Norway

All reagent examples available from:
Merck House, Merck Eurolab Ltd,
DE15 1TD Poole, or
BH15 1TD
UK
UK.

Fisher Scientific
Bishop Meadow Rd
Loughborough,
LE11 5RG
UK.

Application Note:
AN40182_E 10/03
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 5.0µg/L was used.

A pooled sample of natural urine was spiked with 1.0µg/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 5.0µg/L standard using the FS95 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).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chromium found (µg/L)</th>
<th>Recovery (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>1.692</td>
<td>-</td>
</tr>
<tr>
<td>Urine+0.5µg/L</td>
<td>2.056</td>
<td>0.364 (73%)</td>
</tr>
<tr>
<td>Urine+1.0µg/L</td>
<td>2.349</td>
<td>0.675 (66%)</td>
</tr>
</tbody>
</table>

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).
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 1.0µg/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 3.8pg for the area measurement, and 1.5pg for peak height measurement.

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

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

The Seronorm reference urine sample gave a result of 23.8µg/L after taking account of the dilution; the Preliminary Recommended Value taken from the manufactures certificate was 24µg/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.
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 M6 and MQZ 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 FS95 to be utilised.

References

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