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Direct determination of chromium in urine by electrothermal atomic absorption spectrometry

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NISE, G. and VESTERBERG, O. Direct determination of chromium in urine by electrothermal atomic absorption spectrometry. *Scand. j. work environ. & health* 5 (1979) 404—410. A simple, direct method for the determination of chromium levels in urine is described, electrothermal atomic absorption spectrometry having been used after the urine samples had been acidified. The optimal conditions at the ashing and atomization stages was determined. The detection limit of this method is 5 nmol/l, and the coefficient of variation (within day) is about 3% in the concentration range 20—400 nmol/l. Use of a low temperature asher with electrically excited oxygen before analysis gave results similar to those of the direct procedure. Good correlation was obtained from two other laboratories using different instruments and pretreatments.

Key words: chromium, electrothermal atomic absorption spectrometry, urine.

The deleterious effects of chromium on human subjects, especially those of hexavalent chromium compounds, have created increasing concern in recent years (1, 2, 10). The effects include ulcerations on skin and mucous membranes, dermatitis, and allergic skin and asthmatic reactions (2, 12). Hexavalent chromium compounds, mainly chromates, have also been connected with an increased incidence of lung cancer (8, 10).

Chromium is considered to be an essential metal in man, animals, and plants (8). In mammals it plays an important role for, e.g., glucose utilization. Low levels of chromium in the human body have been implicated in cases of increased incidences

of malfunctions, e.g., artery disease (9, 13). It occurs in high concentrations in soil, vegetables, and water, which in certain areas constitute important sources of supply for humans. Extensive reviews about the occurrence and function of chromium in biological systems have been published (8, 10).

Because chromium taken up in the body is excreted predominantly in urine (10), one would expect the analysis of urine to provide an estimate of the absorbed dose. Being able to measure urinary chromium levels would be especially relevant to the evaluation of occupational exposure, but it is also important for the estimation of uptake from food and in studies of dietary chromium deficiency.

Many analytical methods for the determination of chromium in urine have been published, some of which depend on the use of gas chromatography after derivatization (13), while others use atomic absorption spectrometry (AAS) (3, 5). Gener-

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ally the methods described earlier require considerable sample preparation, and they are quite laborious. One recently published, more direct procedure makes use of a one part in ten dilution of urine, which, however, results in a low precision at low concentrations and a detection limit above the normal concentration (17). Other procedures have used a direct injection of urine and calibration against standards prepared in water (14). But due to the variable composition of urine there have been problems with high background absorption, and the results have been unreliable. Furthermore, the use of temperatures above 1250°C at ashing may result in losses of chromium (15, 18). Difficulties with the analysis of chromium in various materials have been illustrated in an international laboratory control program (11).

We have examined possibilities of analyzing chromium in urine with the flameless atomic absorption of acidified samples following a careful optimization of the parameters at ashing and atomization and using strictly controlled conditions.

MATERIAL AND METHODS

Urinary samples were taken from welders and people with no known occupational

exposure to chromium. The samples were stored at -20°C until analysis.

Chemicals

A chromic chloride standard containing 1 mg of chromium per milliliter in 1 mol of nitric acid per liter (BDH, Poole, England) was diluted in 0.01 mol of nitric acid per liter upon use. The nitric acid, Suprapure 85 % (w/v), was from Merck, Darmstadt, the Federal Republic of Germany. The water used was distilled and deionized.

Instrumentation

An atomic absorption spectrophotometer model AA-6 (Varian Tectron, Melbourne, Australia) with a carbon rod CRA-63, a workhead model 90, and a background corrector was used, as well as a temperature control unit according to Lundgren (7), which measures the temperature of the carbon rod with a photodiode. Other details and instrument settings are given in table 1.

A recorder model TE 220/2 (Tekman Electronics Ltd., Oxfordshire, England) with a full-scale deflection response time of less than 0.3 s was used. The recorder was modified so that when no atomizing

Table 1. Instrument characteristics for the determination of chromium in urine.

Instrument	Characteristic
Graphite atomizer	
Drying sequence	100°C, 35 s
Ashing sequence	1050°C, ^a 40 s, ramp rate 0.5 V/s ^a
Atomize sequence	2500°C, 3.5 s, heating rate 10 V/s
Inert gas	Nitrogen with 10 % hydrogen ^b
Spectrophotometer	
Operation mode	Corrected absorbency
Metal lamp (Varian)	4 mA
Hydrogen lamp (Varian)	8.5 arbitrary units
Photomultiplier	435
Spectral band width	0.5 nm
Slit height	5 mm
Wavelength	357.9 nm

^a Settings of the TCH unit: ramp rate 0.5 V/s corresponds to a high heating rate of the tube during ashing, while during the atomizing sequence the maximum heating rate was used.

^b According to recommendations in Varian's analytical manual.

signal was expected it could be short-circuited.

The electronic signals were also evaluated by use of a Peak Reader Module (PRM 6; LL-Electronic, Umeå, Sweden) which gives a digital readout of the absorbency and integrated area of each peak.

When many samples were to be analyzed, an Automatic Sample Dispenser ASD-53 (Varian) was used.

The instrument was modified by changing the damping of the output signal from 0.35 s to 50 ms to obtain a fast response (6). This procedure increased the range of absorbency with a linear response. A digital printer (Kontron, Switzerland) was also used. An electromagnetic high-frequency low-temperature vacuum asher, Typ 500 R (Uppsala Elektronik, Stackmästarv. 4, Uppsala, Sweden) producing excited oxygen (4) was used in some experiments according to instructions from the manufacturer.

Analytical procedures

Test tubes of polystyrene (size 12 ml, Haeger Plastics, Stallarholmen, Sweden and size 4 ml, Nunc, Roskilde, Denmark) were washed in metal-free water containing the detergent RBS-25 (Chem. Prod.,

Brussels, Belgium) and then thoroughly rinsed in metal-free water prior to use. After the addition of 0.10 ml of concentrated nitric acid to 10.0 ml of urine and thorough mixing, the test tubes were allowed to stand for at least 1 h before analysis. From this mixture 1.0 ml portions were pipetted into three test tubes, two of which also received standard additions at different levels. Usually 5.0- μ l samples were pipetted into the carbon tubes either manually with a Micropipette (Oxford Lab. Int. Corp., Ireland) with disposable teflon tips or with an automatic sample dispenser. Three pipettings were made of each solution. In order to obtain the best reproducibility, threaded carbon tubes were used. Urine samples containing a high chromium concentration were diluted with nitric acid (0.01 mol/l) before analysis. The peak heights of the recorded signal or the peak absorbencies from the PRM-6 were used for the calculation of the chromium content in the urine samples.

RESULTS

Various temperatures have been used at the ashing stage. As can be seen in fig. 1, an approximately constant absorbency was obtained for chromium in nitric acid (0.01 mol/l) in the range 600–1500°C. Decreased absorbency was obtained at temperatures above this range, and losses in the ashing sequence were therefore indicated at elevated temperatures. The corresponding studies for urine samples are shown in fig. 2, where it can be seen that temperatures up to 1250°C may be used without significant losses in order to obtain a low background. During the study the use of ashing aids was investigated in order to improve the ratio of the chromium signal to the background noise. Ammonium sulfate, magnesium nitrate, ammonium nitrate, and ascorbic acid were added in different experiments to obtain final concentrations of 0.5 to 5 % (w/v). A somewhat higher sensitivity was achieved with the addition of ammonium sulfate and ammonium nitrate, but the background noise

Fig. 1. Peak absorbency values (A) as a function of ashing temperatures. Samples with a chromium concentration of 385 nmol/l in nitric acid (0.01 mol/l) (5 μ l injection volume).

Fig. 2. Peak absorbency values (A) as a function of ashing temperatures (chromium absorbency for one urine specimen ○—○; the same urine with 385 nmol of chromium added per liter ●—●; the nonspecific absorbency for the same urine □—□) (5 μ l was injected).

Fig. 3. Peak absorbency values (A) as a function of atomizing temperatures for 5 μ l samples with a chromium concentration of 385 nmol/l.

also became higher. Ascorbic acid lowered the background but also gave a reduced chromium signal. Therefore, for the final method we added nitric acid only.

The influence of different atomization temperatures has also been studied in some detail. As can be seen in fig. 3, a satisfactory temperature was obtained at 2500°C. There was no difference when calculations of the chromium concentrations in urine samples were made from peak heights or integrated values. It was very important to know whether different urine samples would give rise to different calibration slopes of their standard curves. For this purpose we analyzed many urine samples with and without additions of chromium at four levels and with three determinations on each level. Most urine samples gave very similar calibration slopes. However, among ten samples there were about three which produced rather aberrant slopes; they give rise to concentration values differing up to 25 % from that obtained with the standard addition method.

To decrease the error of single samples, we therefore used the method of standard addition instead of evaluation against a standard curve.

Table 2. Studies of the recovery of chromic chloride added to urine samples with chromium concentrations ranging between 33 and 175 nmol/l for samples 21—24 and 260 and 550 nmol/l for samples 25—28.

Sample	Added	Found	Recovery (%)
21	170	158	93
22	170	162	95
23	170	172	101
24	170	168	99
25	510	514	101
26	510	498	98
27	510	512	100
28	510	507	99

The precision and reliability of this method was studied by determination of the recovery of additions of chromium as can be seen in table 2.

Precision data are presented in table 3 (within day and between days). The detection limit was 5 nmol/l when calculated with the formula $(2 \times SD \times Cr \text{ concentration})/\bar{x}$, where $\bar{x}$ is the peak height average and SD the standard deviation, from 15 injections of 10 μ l of a urine sample low in chromium. Similar results were obtained with two other urine samples. The blank was mostly negligible.

It is well known that precipitates may occur in urine samples, especially during storage. We therefore determined the chromium in the supernatant after centrifugation and in homogenized samples. For the ten samples examined after storage for three weeks at +4°C, no difference was found; therefore no significant enrichment of chromium in the precipitates was indicated. Nine urine samples were also

analyzed with Perkin-Elmer instruments and different pretreatment procedures in two other laboratories. As can be seen in table 4, good agreement, at least above 300 nmol/l, was obtained between the different laboratories. We also analyzed the urine samples after low temperature ashing at < 200°C by using a method similar to the one described earlier (18). No statistically significant difference between the two methods was obtained, as can be seen in table 4.

Table 3. Data for the precision and variability of the determinations of chromium in urine (nmol/l).

Sample	N	Average concentration	SD	CV %
<i>Within a day</i>				
1	10	1,564	30.1	1.9
2	10	352	11.5	3.3
3	10	18	0.56	3.1
<i>Between days</i>				
4	10	323	23.7	7.4
5	10	64	7.0	11.0
6	10	30	3.8	12.5

DISCUSSION

One of the causes of difficulties encountered in determining chromium in urine may be interference by a rather high and variable concentration of salt. Some workers have tried to circumvent this problem by carrying out extraction prior to analysis. Others have tried to eliminate interference, for example, by the use of ion exchange. However, apart from being time consuming, these procedures impart

Table 4. Results of the analyses of chromium in urine (nmol/l) by electrothermal atomic absorption spectrometry, as obtained by different laboratories. Laboratory A was the Institute of Occupational Health, Oslo (N. Gundersen); a Perkin-Elmer AAS Model 300 with an HGA 76 and direct injection of 50 µl of undiluted urine were used with two standard additions to each sample. (This analytical procedure was found to be critical and required, among other things, a very careful alignment of the lamps.) Laboratory B was the Institute of Occupational Health, Helsinki (J. Kilpiö) a 1/10 dilution of urine was used as described earlier (17). Laboratory C was our laboratory, column I being the method described in this paper and column II the same method after low temperature ashing.

Sample	Laboratory			
	A	B	C (I)	C (II)
1	110	75	85	90
2	640	635	660	505
3	280	290	260	305
4	680	705	695	695
5	645	680	630	585
6	750	720	680	690
7	625	560	535	525
8	45	60	20	25
9	400	415	360	400
<i>Regression equations</i>			<i>Correlation coefficients</i>	
$C_I = 0.991 \cdot A - 23.7$			0.991	
$C_I = 0.996 \cdot B - 22.0$			0.994	
$B = 0.991 \cdot A + 0.2$			0.992	
$C_I = 1.053 \cdot C_{II} - 21.7$			0.992	

the risks of variable recovery and contamination. Pitfalls encountered in flameless atomic absorption have been described in a recently published critical study (16). Explanations for failure using direct injections of urine samples may be that undiluted urine has been used and/or that the parameters for drying, ashing and atomization as well as the background correction have not been evaluated. We have found that these conditions may be critical.²

It has been reported that part of the chromium in urine occurs in the form of a complex, which is said to be much more volatile than chromium ions in water solutions (15). Some earlier published methods can be criticized for not completely measuring total chromium due to losses arising during sample preparation, caused by excessive temperatures being used during the dry ashing or in the flameless atomic absorption. To diminish these possibilities, we used a lower ashing temperature (1050°C) than the ones used in earlier reports [e.g., 1300°C (15) or 1600°C (14)]. Secondly, we treated the samples with nitric acid prior to analysis. We believe that this procedure eliminates the problem of volatile chromium because acidic and oxidizing conditions are known to destroy complexes of most organic compounds and metals.

The concentration levels of chromium found in the urine of persons not occupationally exposed have been reported in the literature to range down to about 20 nmol/l. The detection limit (5 nmol/l) of the method reported in this paper is well below this level, and accordingly we have found some samples with chromium concentrations in this range. Thus, earlier published procedures do not have a sufficiently low detection limit. We can lower

our detection limit somewhat by increasing the sample volume injected into the graphite tube. Furthermore, the precision obtained with our procedure is better than that reported for earlier published methods.

Because no certified method exists for quantifying chromium levels in urine, it is difficult to ascertain the accuracy of the new method. However, the analytical recovery of chromium added to urine was close to 100 %. Furthermore, the good agreement between different laboratories (table 4) using different instruments and procedures implies a good accuracy.

In comparison to other procedures using extraction, ashing and extraction, and dry ashing and dissolution of the ash the procedure described in this report is straightforward and simple, involving less risk of contamination by obviating the use of many chemicals, pipettings, and vessels. When evaluating several urine samples against one standard curve, one person can analyze about 40 urine samples in 8 h, including the calculation of results. When the method involving the addition of standards is used for each sample, at least 20 urine samples may be analyzed. This rate is faster than analysis after extraction and less elaborate than other procedures which depend, for example, on dry or wet ashing. With automatic injection with the auto-sampler, about 30 urine samples may be evaluated in 1 d with the method of added standards.

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