Determination of Chromium in Human Urine by Graphite Furnace Atomic Absorption Spectrometry with Zeeman-effect Background Correction

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A rapid and direct method for the determination of chromium in human urine using graphite furnace atomic absorption spectrometry with Zeeman-effect background correction is described. No reagent or sample pre-treatment (except for straightforward dilution of concentrated samples with distilled water) was necessary, thereby reducing the risk of contamination. The concentration of chromium in urine was evaluated directly from a calibration graph constructed from a metal-spiked human urine pool. Hence, the time consuming method of standard additions was avoided, which permitted an increased sample throughput (100–120 samples per day) with minimum attention of the analyst. In routine use, the reproducibility (both within-day and day-to-day) and limit of detection were in the order of ±10% and 0.09 μg l^{-1} of Cr, respectively. With a minor alteration in charring temperature the method may also be used for the determination of chromium in whole blood and serum. The method described is suitable for the biological monitoring of chromium in the general population or in occupationally exposed persons.

Keywords: *Chromium determination; graphite furnace atomic absorption spectrometry; Zeeman-effect background correction; biological monitoring*

Chromium is an essential human micronutrient involved in the metabolism of carbohydrate and fat. However, at higher concentrations it presents severe health hazards. There is sufficient evidence, both from animal experiments and epidemiological studies, that chromium and some of its compounds are carcinogenic,[1] although the specific carcinogenic compounds have not been identified.[2]

As chromium enters the blood stream, it is reduced to the Cr^{III} state in red blood cells. It rapidly disappears from blood and is taken up by other tissues. The major pathway of elimination (about 80%) of absorbed chromium is excretion in urine. It has been suggested[3] that urinary excretion predominantly reflects recent exposure and because a greater chromium burden is also associated with a greater excretion level, urinary chromium levels can be used as an indicator of the total body burden. Recently, McAughey and Smith[4] have suggested that blood chromium reflects long term exposure to Cr^{VI}, whereas urinary chromium reflects recent uptake.

Among the techniques available for the determination of chromium in biological fluids, graphite furnace atomic absorption spectrometry (GFAAS) is used by most analysts because of its speed, minimum need for sample preparation, possibility of automation and good sensitivity. However, the determination of chromium by GFAAS has until recently been difficult, especially determination in urine where the influence of the matrix greatly affects the analytical results. Previous studies have shown that an improvement in analytical sensitivity has been helpful in reducing the previously reported "normal" urinary values (2–20 μg l^{-1} of Cr) almost ten-fold.[5–7] This demonstrates that special care should be taken to reduce matrix interferences and effective background correction should be employed during analysis. Gutherie *et al.*[8] have shown that the conventional deuterium arc background correction system is inadequate for urinary chromium determinations owing to the low intensity of the deuterium lamp at the optimum chromium wavelength. Later, these workers used wavelength modulated GFAAS to overcome background interferences.[5] A satisfactory background correction in serum or urinary chromium determination was also achieved by employing a quartz halogen light source for background absorption.[6,9] Halls and Fell,[10] however, demonstrated that chromium in urine can be determined without significant interferences using deuterium arc background correction if the atomisation temperature is reduced from 2700 to 2400 °C and the intensities of the emission and deuterium lamps are properly adjusted. In this laboratory, satisfactory results were not obtained for the determination of chromium in urine using deuterium arc background correction (Perkin-Elmer, Model 5000) and depending on the urine sample, matrix over-estimation was noted in spite of employing the suggested instrumental settings and reduced atomisation temperatures.

For nationwide biological monitoring of trace metals, this laboratory required an accurate and direct method with negligible background interferences for the determination of urinary and whole blood chromium levels. This paper describes a method which is direct, in that no sample pre-treatment is necessary and chromium concentrations in urine samples are evaluated directly from a standard calibration graph constructed from a metal-spiked human-urine pool. The background corrections were carried out by applying the Zeeman-effect background correction directly to the atomic vapour. The reliability of the method was verified by internal and external quality controls and by interlaboratory comparisons. An over-all reproducibility of ±10% and a detection limit of 0.09 μg l^{-1} of Cr allows the routine use of this method for determining normal or exposed levels of urinary chromium. The introduction of an additional charring step in the presence of a compressed air flow allowed the determination of chromium in whole blood samples with an over-all analytical performance of the same order as for urine samples. The automated sampling and on-line data collection system allowed unattended operation and about 100 analyses per day.

Experimental

Instrumentation

Chromium determinations were performed using a Zeeman-effect background correcting (modulated, transverse field)[11] atomic absorption spectrometer (Model Z-3030 from Perkin-Elmer, Überlingen, FRG). The instrument included computerised graphic signal processing and was equipped with an AS-60 autosampler, an HGA-600 furnace controller and a PR-100 printer. An Intensitron[11] hollow-cathode lamp as the emission source and pyrolytic graphite coated tubes, both from Perkin-Elmer, were used. Disposable autosampler cups

(Polystyrol, 2-ml capacity) and disposable pipette tips for Eppendorf fixed-volume pipettes (plastic exposed internal parts) were obtained from Sarstedt (Nümbrecht, FRG) and were used to prepare calibration solutions and samples. These were regularly checked for contamination and it was noted that one rinse with 1% nitric acid was sufficient for cleaning. All precautions were taken to avoid extraneous chromium contamination.

Materials and Standards

Chromium standard solutions (20–500 ng ml^{-1}) were prepared from a 1 g l^{-1} Cr (as $CrCl_3$) solution (Titrisol, Merck, FRG). Lanonorm, a synthetic urine matrix for metal levels I (normal) and II (elevated), was obtained from Behring Institute, (Marburg, FRG) and in conjunction with a human urine pool (a mixture of more than 100 different samples from persons not occupationally exposed), was utilised as an internal quality assurance material.

Procedure

For the development of the method, six urine samples were selected to provide variable matrices. This selection was qualitative and was based on the visual appearance of the samples. As these samples were stored frozen, a preliminary check revealed that there was no loss in urinary chromium by multiple freeze-thawing or prolonged storage at −18 °C. Aliquots of 1 ml of the selected urine were spiked with chromium by adding 10 µl of a standard solution containing 50, 100, 200 or 500 ng ml^{-1} of Cr. The small change in the volume was ignored. With these spiked samples, the ashing and atomisation temperatures were optimised.

A calibration range of chromium concentrations (0, 0.2, 0.5, 1.0, 2.0 and 5.0 µg l^{-1} of Cr) was prepared by spiking 1-ml aliquots of the human urine pool (a mixture of about 100 urine samples, unacidified) with 10 µl of standard solutions containing a chromium gradient of 20–500 ng ml^{-1}. The urine pool contained 0.18 ± 0.03 µg l^{-1} of Cr as determined by the proposed method. A calibration graph was constructed at least three times during the daily analytical run.

The well mixed and undiluted urine samples were pipetted directly into the autosampler cups and chromium was determined according to the instrumental conditions shown in Tables 1–3. Duplicate determinations of each sample were made and the chromium concentration was evaluated from the calibration graph (linear regression) prepared as described under Procedure. Samples which had a chromium concentration higher than 5.0 µg l^{-1} were appropriately diluted with distilled water and then re-analysed.

Table 1. Instrument settings

Wavelength/nm	357.9
Slit width/nm	0.7
Lamp current/mA	25.0
Integration time/s	2.5 (peak height measurement every 0.02 s)
Background correction	Zeeman effect (at the atomic vapour, modulated, transverse field)
Sample volume/µl	40.0 (urine); 20.0 (blood)

Results and Discussion

Temperature Programme Optimisation

It has been demonstrated that for the determination of chromium in urine, ashing temperatures below 1200 °C are not sufficient.[9] Also, in our attempt to use 1100 °C for as long as 120 s the urine matrix was not completely burnt in any of the six test urine samples and during the atomisation step the background correction system failed to compensate for this, owing to the smoke from the fast burning of the remaining urine matrix. Therefore, it was decided to use 1200 °C as the starting point. Fig. 1(*a*) shows the results of the optimisation of the ashing stage. The ramp time required to reach the charring temperature was 10 s and the atomisation temperature was held constant at 2300 °C during these experiments. Each point represents the average of several determinations. The urine matrix burns only after 30 s at 1200 °C, as is evident from the drop in the background absorbance to a stable level of about 0.3 *A*, which is well below the background correction capacity of the instrument.[11] The atomic signal of chromium from urine is significantly increased at a 40-s hold time; however, longer hold times result in a drop both for chromium in urine and the aqueous chromium standard. Increasing the charring temperature in the hope of burning out the matrix quickly results in a loss of chromium from the standard solution [Fig. 1(*a*)] with the absorbance profile of urinary chromium becoming asymmetrical and flattened [see peak shapes in Fig. 2(*e–g*)]. At this stage, it was apparent that an ashing temperature of 1200 °C for 40 s was appropriate for urinary chromium determination. This was later confirmed during optimisation of the atomisation temperature [Fig. 1(*b*)]. The atomic signals obtained under the optimum temperature programme (Table 2) are shown in Fig. 3. Using uncoated graphite tubes, we noted that the chromium signal, from aqueous standards or spiked urine, was slow in appearing during the atomisation period and that the analyte peaks were relatively flat. We used pyrolytic graphite coated tubes for about 200–250 firings without any loss in sensitivity (reduced absorbance of standard solutions) or deterioration of pyrolytic coating. Veillon *et al.*[9] have reported a sensitivity loss with tubes used for about 75 firings which may be due to the higher atomisation temperatures (2700 °C) used by these workers. In routine use we noted that the life of the coated tube can be

Table 2. Furnace conditions used for determination of chromium in urine

	Dry		Char	Atomise	Clean out
Temperature/°C	100	180	1200	2400	2650
Ramp time/s	1	25	10	0	1
Hold time/s	10	15	40	3	2
Argon flow-rate/ml min^{-1}	300	300	300	0	300
Read cycle				*	

Table 3. Furnace conditions used for determination of chromium in blood

	Dry			Char		Atomise	Clean out
Temperature/°C	80	90	120	500	1200	2400	2650
Ramp time/s	1	1	10	10	10	0	1
Hold time/s	20	10	20	20	10	3	2
Argon flow-rate/ml min^{-1}	300	300	300	0	300	0	300
Compressed air flow-rate/ml min^{-1}				300			
Read cycle						*	

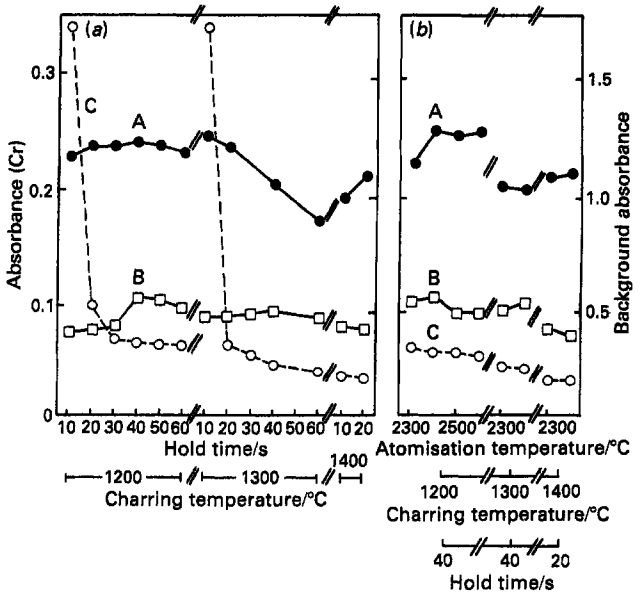

Fig. 1. (*a*) Char and (*b*) atomisation curves for (A) a 5 μg l^{-1} Cr standard solution, (B) urine spiked with 1 μg l^{-1} of Cr and (C) background signal. The ramp time for both panels was 10 s

extended up to 400–500 firings if the tube and the furnace cones are cleaned between runs with a cotton swab to remove the build-up of ash and fine carbon. McAughey and Smith[4] have recently reported that reducing the atomisation temperature from 2700 to 2500 °C improved the reproducibility and gave a 2–3 fold increase in tube lifetime. This improved reproducibility may be due to reduced interference from the matrix which remains unburnt during the ashing stage and appears in the atomisation stage if temperatures above 2400 °C are used.[10] We, however, did not observe such a phenomenon up to an atomisation temperature of 2600 °C and the background signal also remained stable at about 0.3 *A* [see Fig. 1(*b*)].

Evaluation of the atomic signal in the peak-height or peak-area mode had no influence on the analytical results. We preferred to use the peak-height absorbance mode to evaluate our results. Spiking the test urine samples with either Cr^{III} or Cr^{VI} salts gave atomic signals which were identical in shape, height and appearance time.

Analytical Performance

The limit of detection for this method was found to be 0.09 μg l^{-1} of Cr for undiluted urine samples. This value was calculated as three times the standard deviation[12] of multiple measurements of the urine pool (used for preparing matrix-matched calibration) made on five different days. Using a 40-μl sample volume this value gives an absolute detection limit of 3.6 pg. Other workers have reported an absolute detection limit of 4.0 pg for undiluted urine.[4] The detection limit observed using the proposed method is low enough to determine the normal levels (up to 2 μg l^{-1} of Cr) and is adequate for general biological monitoring and the determination of occupational exposure.

The precision of the method was checked by determining chromium in six spiked urine specimens, differing in their appearance (colour and sediment), several times per day for six days. The within-day variation in results was ±10% and the day-to-day variation ranged from ±7 to ±12%, giving an over-all reproducibility of *ca.* 10%.

To minimise the matrix interferences and the effect of graphite tube ageing in order to improve the results, the method of standard additions has been recommended for determining chromium in urine.[9] This procedure, however,

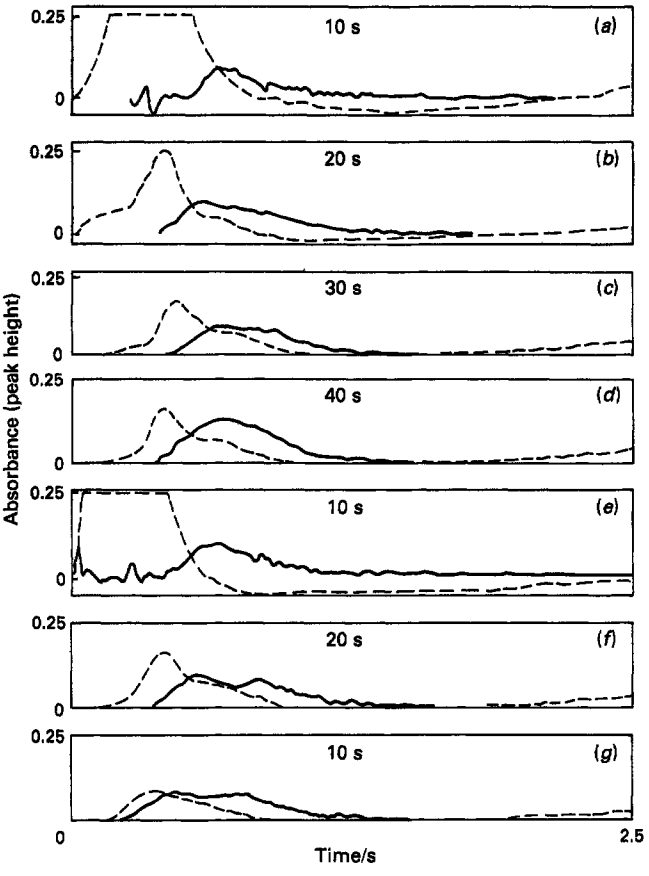

Fig. 2. Absorbance profile of a urine sample spiked with 1 μg l^{-1} of Cr using Zeeman-effect background correction. The broken line shows the background signal. Charring temperatures: 1200 (*a*–*d*), 1300 (*e*, *f*) and 1400 °C (*g*). The ramp time was 10 s and hold times are given in the figure

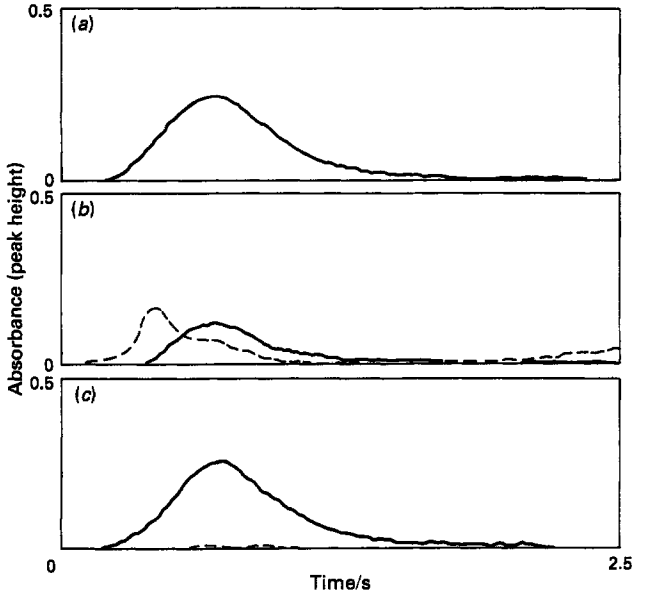

Fig. 3. Absorbance signals obtained using the proposed method. (*a*) 5 μg l^{-1} Cr standard solution, (*b*) urine spiked with 1 μg l^{-1} of Cr and (*c*) blood (diluted 1 + 5) spiked with 4.0 μg l^{-1} of Cr

requires careful sample preparation and the time of analysis is increased 6-fold. In our experience with urinary chromium determination, the effect of tube age was not observed up to 250 firings. Regarding the suitability of the analyte addition technique, Welz[13] has recently reported that this technique is

Table 4. Variation in calibration slope of Cr-spiked urine samples

Sample No.	Slope/ $(\mu g\,l^{-1})^{-1}$	r
1	0.58	0.9998
2	0.59	0.9999
3	0.72	0.9999
4	0.71	0.9830
5	0.65	0.9992
6	0.65	0.9993
7	0.60	0.9990
8	0.62	0.9993
9	0.57	0.9964
10	0.67	0.9959
	$\bar{X} = 0.641$	
	S.D. = 0.054	

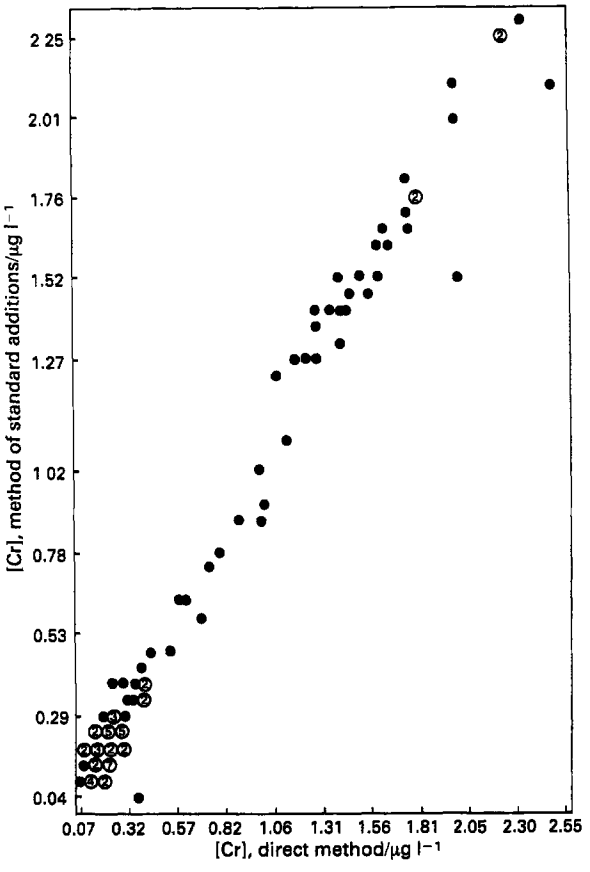

Fig. 4. Computer scattergram showing correlation between the urinary Cr values determined by the method of standard additions and the direct method. Enclosed numbers denote the number of points falling on the same place. $n = 96$; $r = 0.939$; slope = 0.980; and intercept = 0.057

not always suitable as it can only correct non-spectral rather than spectral interferences. We decided to investigate whether the method of standard additions can be avoided in urinary chromium determination in order to reduce the analysis time without compromising the analytical quality. For this, ten urine samples were spiked with various concentrations of $CrCl_3$ and multiple determinations were made. Table 4 lists the slopes of the calibration graphs of these ten urine matrices, which varied by only 8.5%. We extended this work by determining chromium in 100 urine samples from persons not occupationally exposed by the direct (matrix-matched calibration) method and by the standard additions method. Some of the urine samples were also spiked with 1.0 $\mu g\,l^{-1}$ of Cr so as to obtain a good concentration range for this comparison. The regression line was $Y_{addition} = 0.057 + 0.95X_{direct}$ ($r = 0.9$),

Table 5. Interlaboratory comparison. Results for the determination of Cr in urine. Results given as $\mu g\,l^{-1}$

	Sample No.	Mean ± SD of all laboratories	This laboratory
Comparison I*	1	0.20 ± 0	0.20
	2	0.21 ± 0.03	0.25
	3	0.29 ± 0.08	0.27
	4	0.18 ± 0.05	0.20
	5	1.30 ± 0.18	1.40
	6	1.15 ± 0.10	1.20
	7	1.12 ± 0.10	1.20
	8	1.06 ± 0.10	1.15
Comparison II†	1	7.39 (5.41–9.37)‡	7.30
	2	25.95 (20.37–34.53)	24.00
Comparison III†	1	41.10 (28.74–53.46)	40.60
	2	104.20 (81.94–126.46)	104.70

* A comparison among four trace analysis laboratories.
† A national quality control scheme conducted by German Society for Occupational Medicine in 1986 and 1987.
‡ Target value with 95% confidence range.

showing a good agreement. In about 90 urine samples the values obtained (duplicate determinations) by both methods varied by less than ±10% (see Fig. 4).

To further test the accuracy of the proposed method we selected eight urine samples and sent them to three trace metal analysis laboratories for the determination of chromium. Our results did not deviate by more than 10% from the mean of the reported values. Additionally, a satisfactory agreement was shown between our results of urinary chromium and the mean of the reported results in recent nationwide interlaboratory comparisons (see Table 5).

A good recovery of added chromium(III) as determined by the proposed method was obtained. The results are presented in Table 6.

The main advantages of the method are decreased sample handling, shorter analysis time (about 3.5 min for duplicate determinations) and the absence of sample preparation. Concentrations are directly evaluated by using a spiked "low-Cr" human urine as matrix-matched calibration. With these advantages, about 100–120 samples (excluding inter-run recalibration) can be analysed in an 8-h working day with a minimum of the analyst's attention—an optimum condition required in epidemiological surveys related to trace metal biological monitoring.

Blood Chromium Determination

Initially, the temperature programme employed for urine samples was used for blood samples diluted 1 + 5 *V/V* with water, except for a longer drying time. We observed that the hold time of 60 s for the charring temperature can be reduced to 10 s without any change in analyte or background signals. However, after a few determinations the build-up of carbonaceous material inside the graphite tube was noted, probably due to incomplete burning of the protein matrix. Therefore, we included an intermediate charring stage at 500 °C in the presence of a compressed air flow which assisted in burning away most of the protein matrix before the final charring stage at 1200 °C.

The calibration graph was prepared by appropriate dilution of a reference blood standard (Kontrol Blut 1; Behring Institute) or by spiking a diluted blood sample containing low chromium levels. The percentage recovery of added chromium (see Table 6) and the limit of detection for blood chromium determination were the same as those observed in urine analysis.

During the routine use of the above method we observed that very often the uneven spreading of the blood samples led to incomplete drying and during the 10 s ramp to 500 °C the

Table 6. Recovery study of added Cr^{III}. Results given as $\mu g\ l^{-1}$

Matrix	Cr in sample	Cr added	Cr found	Recovery, %
Urine 1	0.45	0.5	1.02	107.4
		1.0	1.48	104.8
		2.0	2.51	102.4
		3.0	3.37	97.7
Urine 2	0.39	0.5	0.94	105.6
		1.0	1.48	106.5
		2.0	2.47	103.3
		3.0	3.45	101.8
Urine 3	0.42	0.5	0.94	104.4
		1.0	1.47	103.5
		2.0	2.44	100.8
		3.0	3.39	99.1
Blood 1	0.72	0.5	1.31	107.4
		1.0	1.88	109.3
		2.0	2.68	98.5
		3.0	3.80	102.1
Blood 2	0.96	0.5	1.48	101.4
		1.0	2.0	102.0
		2.0	2.9	98.0
		3.0	3.8	96.0

sample often boiled out of the delivery hole of the graphite tube. This was avoided by using a solution of 0.1% EDTA and 5% isopropanol as a diluent. A 3% ammonia solution, which gives a very homogeneous dilution and an even distribution of the sample in the graphite tube, could not be used due to an increased background signal.

We found the proposed method very suitable for the determination of blood chromium when compared with other reported methods, which use nitric acid deproteinisation[14] or pre-ashing at 600 °C in the presence of oxygen,[15] causing a rapid deterioration of the pyrolytic coating and chromium contamination through nitric acid.

As the intensity of the background absorption during the determination of blood chromium is very small (mostly below 0.1 *A*), the determinations can also be carried out using instruments equipped with a deuterium arc background correction system.

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References

1. International Agency for Research on Cancer, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans," Volumes 1–20 (Suppl. 1), IARC, Lyon, 1979, p. 29.
2. International Agency for Research on Cancer, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans," Volume 23, IARC, Lyon, 1980, pp. 205–323.
3. Rinehart, W. E., and Gad, S. C., *Am. Ind. Hyg. Assoc. J.*, 1986, **47**, 696.
4. McAughey, J. J., and Smith, N. J., *Anal. Chim. Acta*, 1987, **193**, 137.
5. Gutherie, B. E., Wolf, W. R., Veillon, C., and Mertz, W., *in* Hemphill, D. D., *Editor*, "Trace Substances in Environmental Health-XII," University of Missouri, Columbia, 1978, p. 490.
6. Kayne, F. J., Komar, G., Laboda, H., and Vanderlinde, R. E., *Clin. Chem.*, 1978, **24**, 2151.
7. Routh, M. W., *Anal. Chem.*, 1980, **52**, 182.
8. Gutherie, B. E., Wolf, W. R., and Veillon, C., *Anal. Chem.*, 1978, **50**, 1900.
9. Veillon, C., Patterson, K. Y., and Bryden, N. A., *Anal. Chim. Acta*, 1982, **136**, 233.
10. Halls, D. J., and Fell, G. S., *J. Anal. At. Spectrom.*, 1986, **1**, 135.
11. Welz, B., "Atomic Absorption Spectrometry," VCH Verlagsgesellschaft, Weinheim, 1985, p. 140.
12. Analytical Methods Committee, *Analyst*, 1987, **112**, 199.
13. Welz, B., *Fresenius Z. Anal. Chem.*, 1986, **325**, 95.
14. Stoeppler, M., and Brandt, K., *Fresenius Z. Anal. Chem.*, 1982, **300**, 372.
15. Delves, H. T., and Woodword, J., *At. Spectrosc.*, 1981, **2**, 65.

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