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Short Communication

The use of direct determination of chromium in human urine by electrothermal atomic absorption spectrometry in diabetic patients

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Introduction

Chromium is an essential trace element in man. Evaluation of the nutritional status of this element in humans has traditionally been achieved through biochemical and oral glucose tolerance tests. The former include analytical determination of the chromium concentration in biological specimens such as serum and plasma, urine and hair [1]. In fact, absorbed chromium is excreted mainly in the urine and only small amounts are lost in hair, perspiration and bile. Therefore, chromium excretion can be used as a meaningful indicator of chromium absorption [2].

It has been reported that diabetic patients produce higher levels of urinary chromium than healthy individuals [3, 4]. Anderson [2] indicates that factors that affect glucose metabolism often affect chromium metabolism too. Once chromium is utilized, it does not appear to be reabsorbed by the kidneys but is rapidly excreted in the urine. In addition, the extent of exposure to chromium fumes can be indirectly assessed by measuring urinary chromium excretion. All of the above makes it of interest to determine chromium in urine.

Electrothermal atomic absorption spectrometry is the most commonly used technique for chromium determination in biological material. Determination of this element is difficult because of the low concentrations present in urine and also because of matrix interferences. The sample is usually subjected to pre-treatment which consists of dilution and addition of surfactant or an acid, in order to assure more thorough destruction of the organic matter [5–10].

Direct determination of chromium in urine gives rise to certain background problems that do not seem to be eliminated by using a deuterium lamp. For this reason some authors propose that a tungsten–iodide background correction lamp [7–10] or the Zeeman-effect background correction [11] be used. However, when the assay conditions do not permit use of these corrections, it is possible to eliminate the background by using a deuterium lamp as long as the energy levels of the deuterium and hollow-cathode lamps are balanced [12]. Moreover, Veillon *et al.*, in a proposed selected method [13], showed that use of the method of addition is essential in these determinations because urine samples differ widely in composition. Analytical sensitivity also decreases with tube use, but this is compensated for by the addition method [10].

Halls and Fell [5] propose a direct and faster determination of chromium in urine where the matrix effects are reduced by the addition of Triton X-100.

Because the urine of diabetic patients presents more matrix interferences than the urine of healthy subjects and the sample manipulation involved in pre-treatment implies the use of reagents that increase the risk of contamination, this paper describes a method for chromium determination in urine that is

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useful with diabetic patients, where the sample is directly introduced in the graphic furnace without pre-treatment. The furnace programme is short and background interference is eliminated with a deuterium lamp, the most common accessory to atomic absorption instrumentation. This method therefore permits determination with the atomic absorption instrumentation available in many laboratories. This fact, and the values of analytical parameters that it yields, are the method's main advantages.

Materials and Methods

Apparatus

The measurements were obtained with a Perkin–Elmer Model 2380 atomic absorption spectrophotometer equipped with deuterium lamp background correction, a HGA-400 graphite furnace and a Model 531 recorder. Argon of 99.9998% purity was used as the purging gas through the graphite tube. The instrument settings are given in Table 1.

Table 1
Instrument setting

Wavelength (nm)	357.9
Slit width (nm)	0.7
Hollow-cathode lamp current (mA)	10
Background correction	on
Sample volume (μl)	10

Reagents

Chromium standard solutions were prepared from a 1 g Cr l^{-1} solution (as $CrCl_3$; Titrisol, Merck).

Contamination control

All glass and polyethylene ware were soaked in 20% (v/v) nitric acid for 24 h and rinsed with deionized water before use.

Experimental

Samples

The method was assayed with standard solutions containing 2, 4, 8, 12, 16 and 20 ng Cr ml^{-1} and 24 h urine from diabetic patients and healthy subjects. Urine samples were collected in polyethylene bottles previously treated with 20% nitric acid and rinsed with deionized water.

Instrumental conditions

The furnace conditions were chosen on the basis of drying, charring and atomization profiles obtained by measuring a 10 μl chromium standard solution (containing 2 or 12 ng Cr ml^{-1}) and a 10 μl urine sample to which a 10 μl chromium standard solution was added.

The following factors and conditions were assayed: the use of uncoated or pyrolitically coated tube; the argon flow rate during the atomization step; temperature, ramp and hold time at every step. The experimental conditions are given in Table 2.

Interference assay

The matrix interference study was checked by the method of standard additions which was applied to several urine samples. Interference was observed in some samples and it was therefore necessary to apply the standard additions method for the chromium determination.

Results

The range of standards used in the chromium determination by the addition methods (2 at 20 ng Cr ml^{-1}) had a linear response in the calibration curve.

Sensitivity, expressed as "characteristic concentration" (chromium concentration which increased the absorbance by 0.0044 units), was

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

Cycle	Temperature (°C)	Ramp time (s)	Hold time (s)
Dry	110	10	10
Charring-1	150	10	5
Charring-2	1150	15	10
Atomization*	2400	0	4
Cleaning	2650	1	4

*Mini flow.
Pyrolytic graphite coated tubes.
Peak height measurement.

Table 3
Chromium recovery study

Sample number	ng Cr ml^{-1} (after the addition)	Per cent recovery*
1	16.9	90.9
2	16.5	87.2
3	17.6	97.4
4	16.3	85.3
5	17.4	95.5
6	17.6	97.4

*$R \pm \sigma_{n-1} = 92.3 \pm 4.8$.

0.25–0.5 ng ml^{-1} (depending on which urine sample was analysed).

Precision (relative standard deviation, RSD) was calculated by 20 determinations of one pool of the urine sample of the diabetic patients (chromium concentration = 3.2 ng ml^{-1}). The RSD was 8.1%.

Accuracy was verified by means of recovery studies applied to six aliquots of 100 ml of another pool of the urine sample (chromium concentration = 7.1 ng ml^{-1}). 1.1 μg of chromium was added to these six aliquots (1 ml of 1 μg Cr ml^{-1} solution and 1 ml of 0.1 μg ml^{-1} solution) which is equivalent to 10.78 ng Cr ml^{-1} (1100 ng 102 ml^{-1}). The results are summarized in Table 3.

Discussion

It has been observed that pyrolytically coated tubes enhance signal and measurement precision. Veillon *et al.* [14] ascribe this improvement to the elimination of reabsorption and retention of chromium by the tube wall.

Dry stage

The lowest temperature for obtaining a good degree of dryness was 110°C. When drying was done at this temperature, the fumes produced at the next stage decreased considerably.

Charring stage

Charring had to be carried out in two steps in order to avoid possible analyte loss at temperatures near 180°C and to eliminate a double peak formation, resulting from incomplete ashing at the atomization step. A temperature of 1150°C was found to be the maximum charring temperature that could be used without causing a loss of chromium.

Atomization stage

At 2600°C, non-compensatable interference by the deuterium lamp was observed (recoveries of 143–168% were obtained). When atomization took place at 2400°C the deuterium lamp was able to eliminate the background problems if its energy was balanced with the energy of the hollow-cathode (10 mA). Halls and Fell [12] found interference at 2700°C attributable to the emission of sodium and potassium present in the matrix. This interference did not appear at 2400°C.

Argon flow rate at the atomization stage

When a stop flow was used, a second small peak after atomization appeared. It was eliminated when miniflow was used. In the determination of some urine samples, a second peak was observed during atomization which was eliminated by dilution with deionized water.

Conclusion

We conclude from the present results that the proposed method offers the following advantages:

The method is useful for the urine of healthy, as well as diabetic patients. The latter present more matrix interference than the healthy subjects.

No reagent or sample pre-treatment is necessary, so the risk of contamination is reduced.

The proposed furnace programme is short in comparison with others mentioned in the literature.

The deuterium lamp was able to eliminate the background interference.

The main advantages of this method are its simplicity and the possibility of use with AAS instruments available in most laboratories.

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