P2-25

CHROMIUM DETERMINATION IN URINE AND SERUM BY ETAAS

Nadica Todorovska[1], Irina Karadjova[2], Sonja Arpadjan[2], Trajče Stafilov[3]

[1] Institute of Preventive Medical Care and Toxicology, Military Health Institution Center, 1000 Skopje, REPUBLIC OF MACEDONIA*
[2] Faculty of Chemistry, University of Sofia, 1126 Sofia, BULGARIA
[3] Institute of Chemistry, Faculty of Science, St. Cyril and Methodius University, POB 162, 1001 Skopje, REPUBLIC OF MACEDONIA*

Introduction

Chromium is one of the essential trace elements in the human body as it appears to play a role in the metabolism of glucose and some lipids (mainly cholesterol)[1]. Excessive amounts of the element, particularly in the more toxic Cr(VI) valence state, are detrimental to health as it may be involved in the pathogenesis of some diseases like lung and gastrointestinal cancers. The routes by which chromium enters the body are the digestive tract, respiratory system and skin. Chromium is excreted principally in the urine, and in small amounts in the hair. Therefore urinary excretion values are a good indicator of the ingestion of chromium, but not necessarily of its body burden. More accurate information can be obtained by analyzing blood, serum and other tissue samples. Electrothermal atomic absorption spectrometry (ETAAS) is probably one of the most selective and sensitive techniques used for chromium determination in body fluids[2-5]. Accurate chromium determination depends on two very important points: (i) – strict contamination/ blank control and (ii) – reliable background correction. The purpose of this investigation is to develop and evaluate parameters suitable for the direct determination of chromium in serum and urine by ETAAS. The parameters studied include ashing and atomization temperature, heating rate, suitable modifiers and atomizers, method of calibration. The matrix interference study is carried out. Method developed is applied for the analysis of serum and urine samples from healthy persons and serum samples from patients on dialysis.

Experimental

Instrumentation

The atomic absorption spectrometer Varian Spectra AA 640Z Zeeman AAS equipped with GTA 100 graphite furnace (Varian, USA) and PSD-100 autosampler (Varian, USA) was used. Argon was applied as protective gas and 10 µl serum or urine was injected into the graphite furnace.

Reagents and Samples

The working standard solutions were prepared by dissolving the Merck stock solution containing 1 g L^{-1} chromium in nitrate form. Hydrogen peroxide is additionally purified by ion exchange, produced in the Laboratory for High Purity Substances, University of Sofia, Bulgaria. $Mg(NO_3)_2$ p.a. Merck was additionally purified by recrystallization.

All disposable devices were rigorously cleaned shortly before use by immersion in hot concentrated nitric acid and rinsing with doubly distilled water.

Serum and urine samples were obtained from 20 presumable healthy volunteers and additionally serum samples were obtained from 15 dialysing patients and were transferred to plastic tubes. The serum samples were collected with plastic iv kanula No. 24 (TIK, Slovenia) with injection valve. Urine samples were taken as spot samples. The samples were acidified till pH 2 and kept frozen until analysis.

---

* Editorial note: Recognized by Greece as FYROM.

Procedures

The serum or urine samples of 10 µl were directly introduced into the graphite furnace with equal volume of hydrogen peroxide (30 %, 10 µl as matrix modifier).

The calibration curves (1-5 µg l^{-1} Cr) were prepared by using aqueous standard solutions of Cr.

Results and Discussion

Selection of chemical modifier: Several preliminary experiments were carried out to select a valid chemical modifier for the measurement of Cr in urine and serum. The modifiers tested include commonly used: H_2O_2, HNO_3 and $Mg(NO_3)_2$. Normally relatively high pyrolysis temperatures could be used for chromium determination, so the action of the modifier in this case is to assist complete matrix mineralization and removal during ashing step. From this point of H_2O_2 is very useful due to the high oxidative properties of this compound. In the presence of H_2O_2 the build up of carboneous residue is also avoided and finally this modifier is selected for all further investigations.

Optimization of ETAAS conditions: Serum and urine samples were spiked with 4 µg L^{-1} Cr. To establish optimum temperature programs for the determination of Cr in urine and serum using wall (pyrolytically graphite coated) and platform (tubes with centre fixed platforms) atomization, the characteristic ashing-atomization curves were obtained and plotted in each case. Ashing temperatures (from 400 °C to 1700 °C) and atomization temperatures (from 2100 °C to 2700 °C) were assayed with different atomization systems. Results obtained are summarized in Fig. 1. Unexpectedly with ashing temperature between 700 to 800 °C better shaped absorbance signals as well as lower values of nonspecific absorption were observed. The measurement reproducibily in this case is much better than when higher pyrolysis temperatures were used. This conclusion is valid for both matrices investigated and for both atomizers – even for center fixed platform this relatively low ash temperatures are preferable. Atomization curves (Fig. 1) showed that, as higher is the atomization temperature as higher is the measurement sensitivity. As can be seen from Fig. 1 even for the center fixed platform the plateau region in the atomization curve is not achieved and that is way this atomizer is not suitable for chromium determination in serum and urine samples – measurement sensitivity and reproducibility are lower, nonspecific absorption values are higher in comparison with wall atomization.

As a main conclusion wall atomization is recommended for Cr ETAAS determination in serum and urine samples and optimal instrumental paarmeters are summarized in Table 1.

Calibration: The degree of matrix interferences on the atomization of Cr was evaluated by the ratio of the slopes of calibration curves obtained in the presence of serum or urine and in the presence of aqueous standard solution. Results obtained for serum and urine samples previously spiked and spiked directly in the graphite furnace are presented in Table 1 and clearly illustrate strong matrix depression. The presence of matrix modifier does not significantly decrease matrix intereferences. Once more it is confirmed that center fixed platform is not useful atomizer in these analysis. The depression effect is much more pronounced for previously spiked samples than for samples spiked in the graphite furnace.

Obviously, method of standard addition should be used for calibration and standard additions should be performed preliminary to the sample solutions. Experiments provided showed that for different type of serum samples with different Cr content statistically equal slopes of calibration curves were obtained which means that at least one standard addition to one of the samples is enough.

Accuracy and precision The accuracy of the proposed procedure for ETAAS determination of Cr in serum and urine samples was checked by analyzing certified reference materials for serum and urine. Results obtained (Table 3) are in very good agreement with certified values (t-test, 95 %).

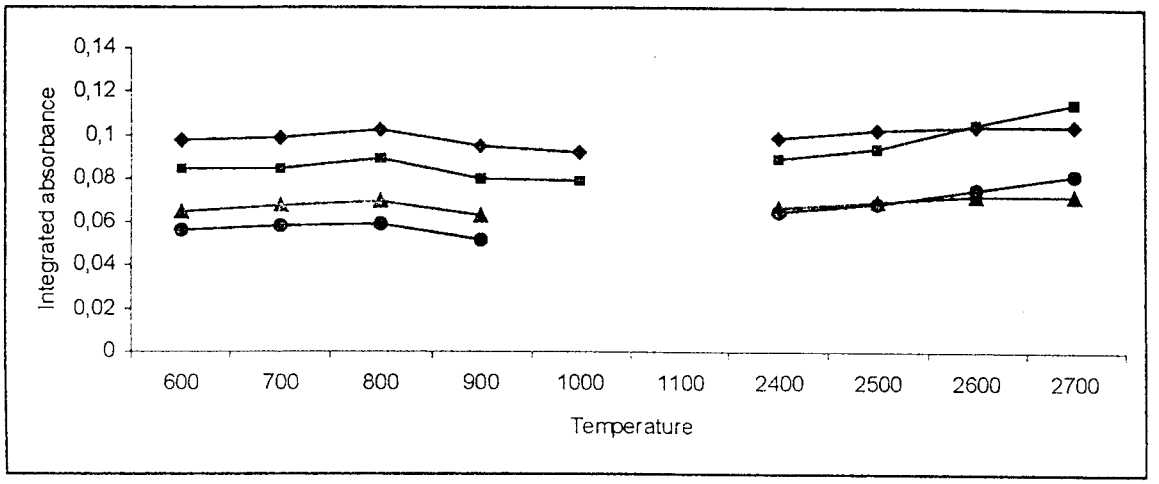

Figure 1. Pretreatment and atomization curves for 4 μg L^{-1}Cr in urine: (◆) wall atomization, (■) center fixed platform and in serum: (▲) wall atomization, (•) center fixed platform. Modifier H_2O_2.

Table 1. Temperature program for ETAAS determination of Cr in serum and urine.

Step		Temperature, °C		Time, s	Argon flow, l min^{-1}
		Serum	Urine		
Drying	1	85	85	5	3
	2	95	95	40	3
	3	120	120	10	3
Pyrolysis	4	700	800	5	3
	5	700	800	10	3
	6	700	800	2	0
Atomization	7	2600	2600	1.2	0
	8	2600	2600	2	0
Cleaning	9	2700	2700	2	3

Table 2. The ration of calibration curves in the presence of matrix (b_m) and for aqueous standard solution (b_o)

Sample	Wall atomization premixed	Wall atomization mixed in the furnace	Center fixed platform
	b_m/b_o [mean ± s]	b_m/b_o [mean ± s]	b_m/b_o [mean ± s]
Serum	0.49 ± 0.13	0.79 ± 0.13	0.30 ± 0.15
Serum + H_2O_2	0.61 ± 0.03	0.82 ± 0.03	0.32 ± 0.12
Urine	0.51 ± 0.15	0.82 ± 0.15	0.28 ± 0.16
Urine + H_2O_2	0.69 ± 0.02	0.93 ± 0.02	0.41 ± 0.12

Table 3. Analysis of reference materials.

Sample	Determined [mean ± s]), μg L^{-1}	Certified, μg L^{-1}
Serum, Clin Rep 1	9.43 ± 0.01	9.6 (7.1-12.2)
Lypochek Urine, level 1	1.52 ± 0.01	1.6 (1.1-2.1)

The limits of detection (LOD) and limits of quantification (LOQ) were calculated as the three-fold respectively six-fold standard deviation of replicate measurements (n = 10) of the blanks of the analytical procedure. For both serum and urine samples LOD is 0.07 μg L^{-1} and LOQ is 0.15 μg L^{-1}. Relative standard deviation for serum and urine sample with Cr contents 0.08-0.12 μg L^{-1} is 15-18 % and for Cr content 0.2-2 μg L^{-1} is 4-7 %. Between-batch precision (calculated as the standard deviation for results obtained for parallel samples analysed during different days) is 8-10%.

Application of the method. The method developed was applied for the determination of Cr in serum and urine samples obtained from 21 presumed healthy volunteers, and in serum samples from 10 patients on dialysis. For 21 non-exposed persons the value of Cr concentration in serum ranges from 0.08-0.25 μg L^{-1} and in urine this range is 0.08-0.92 μg L^{-1}. Chromium content in the serum obtained from patients on dialysis is statically higher with the range 2.2-4.5 μg L^{-1}.

Conclusions

Two atomizers wall and centre fixed platform and several modifiers have been tested and critically compared for the determination of low levels of Cr in serum and urine samples without any sample pretreatment by using ETAAS. Wall atomization (H_2O_2 as modifier) was found to be the most convenient technique, providing the best analytical performance under optimal instrumental parameters. Detection limits achieved by the proposed procedure are very close to the detection limits obtained by the SF ICP-MS [6,7].

References

1. Ducros, V., Biol. Trace Elem. Res., 32, 4-15, 1992.
2. Subramanian, K., Prog. Anal. Chem., 11, 513-521, 1988.
3. Gong, B., Liu, Y.-m., At. Spectrosc. 11, 229-234, 1990.
4. Routh, M., Anal. Chem.,52, 182-185, 1980.
5. Cimadevilla, E.,Wrobel, K., Gayon, J., Sanz-Medel, A., J. Anal. Atom. Spetrom., 9, 117-123, 1994.
6. Begerow, J., Turfeld, M., Dunemann, L., J. Anal. Atom. Spetrom., 15, 347-352, 2000.
7. Rodushkin, I., Odman, F., Oloofsson, R., Axelsson, M., J. Anal. Atom. Spetrom., 15, 937-944, 2000.