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Determination of manganese in human serum and urine by electrothermal atomic absorption spectrometry

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The present paper describes methods for direct determination of manganese in human serum and urine by electrothermal atomic absorption spectrometry (ETAAS) with both deuterium and Zeeman background corrections and with nitric acid as matrix modifier. In both deuterium and Zeeman correction methods, the presence of nitric acid significantly reduces the background signal and direct determination of manganese is possible. Pyrolysis temperature of 900 °C was found optimal for deuterium and 1000 °C for Zeeman correction, while 1800 °C and 2100 °C were found to be optimal atomizing temperatures, respectively. Calibration curves were prepared by the standard addition method. The limit of detection of manganese in serum and urine was 0.2 $\mu g\ L^{-1}$ for the method with deuterium correction and 0.1 $\mu g\ L^{-1}$ for the method with Zeeman correction. The values for manganese in sera of 142 occupationally exposed workers ranged between 0.80 and 7.20 $\mu g\ L^{-1}$ (mean ± SD 2.16 ± 1.18 $\mu g\ L^{-1}$) while for 31 nonexposed subjects the value ranged from 0.75 to 2.30 $\mu g\ L^{-1}$ (mean ± SD 1.73 ± 0.77 $\mu g\ L^{-1}$).

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The importance of the knowledge of the level of toxic metals in human organs and body fluids, their accumulation and level in the food chains and environment has been generally recognized. They are a matter of great concern for national and international authorities responsible for environmental protection. Determination of toxic metals in body fluids is desirable from the toxicological viewpoint.

Determination of manganese in biological material is also important, because manganese is involved in bone and tissue formation and in carbohydrate and lipid metabolism (1–5). Manganese is an essential trace element in humans, but it can elicit a variety of serious toxic responses upon prolonged exposure to elevated concentrations, either oral or by inhalation. Chronic manganese poisoning is present in mining and processing of

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manganese ores, the manganese alloys production, dry-cell battery industries and chemical manufacturing (2, 6, 7).

Chronic exposure to inhalation of high levels of manganese in humans results primarily in central nervous system effects, with a disease called manganism. Other chronic effects from inhalation exposure are respiratory effects and an increased susceptibility to infectious lung disease. Adverse reproductive effects have been noted in male workers. Many workers are occupationally exposed to the dust and fumes of manganese and its compounds. In contrast to inhaled manganese, ingested manganese has rarely been associated with toxicity because of the first-pass hepatic clearance (8).

Different analytical methods are available for manganese determination in biological materials, especially in blood and urine samples, such as neutron activation analysis (NAA), mass spectrometry (MS), flame atomic-absorption spectrometry (FAAS), electrothermal atomic-absorption spectrometry (ETAAS), atomic emission spectrometry with inductive coupled plasma (AES-ICP) or catalytic methods. However, ETAAS, with Zeeman or D_2 background correction, is commonly used for manganese determination because serum and urine manganese concentrations are in the range of $\mu g\ L^{-1}$. There are different approaches to manganese determination in serum and urine – direct introduction of samples into a graphite tube (9–16) or previous dilution with water (17–19), Triton X–100 (20–23) or acids (24). However, matrix interferences, contamination and reagent blank values were seen as limiting factors. Most methods described in the literature involve sample ashing, separation and/or concentration steps (25–28), but these operations are time consuming and may involve contamination and serious systematic errors. In many papers, some of these problems are overcome by the use of chemical modifiers such as $Mg(NO_3)_2$ (29), Pd (30, 31), *etc.* (32).

The purpose of the present study was to examine the improvement of the method for the determination of manganese by ETAAS (either with Zeeman or D_2 corrector) in human serum and urine, previously treated with nitric acid. By applying these methods, data for serum manganese in occupationally exposed and »normal« subjects from Macedonia were obtained.

EXPERIMENTAL

Instrumentation

Two atomic absorption spectrophotometers: ATI Unicam 929 atomic absorption spectrometer (Unicam, UK) equipped with GF90 graphite furnace with deuterium background correction and Varian Spectra AA 640Z Zeeman AAS equipped with GTA100 graphite furnace (Varian, USA) and PSD-100 autosampler (Varian) were used in this investigation. Nitrogen or argon was used as protective gas and 10 μL solution volumes were injected into the graphite furnace. Operating conditions are summarized in Table I.

Table I. Instrumental parameters for determination of manganese in serum and urine by ETAAS

		ATI Unicam 929	Varian SpectrAA 640Z
Wavelength (nm)		279.5	279.5
Spectral bandwidth (nm)		1.0	0.2
Lamp current (mA)		10	4
Graphite furnace step			
Drying	Temperature (°C)	120	120
	Ramp time (s)	10	40
	Hold time (s)	20	10
Pyrolysis	Temperature (°C)	900	1000
	Ramp time (s)	15	5
	Hold time (s)	5	10
Atomization	Temperature (°C)	1800	2100
	Ramp time (s)	0	1
	Hold time (s)	3	2
Gas		Nitrogen	Argon
Injection volume		20 μL	10 μL
Signal mode		Peak area	Peak area
Integration time (s)		3	3

Reagent and samples

The working standard solutions were prepared by dissolving the Merck (Germany) stock solution containing 1 g L^{-1} manganese in nitrate form. Nitric acid was of Tracepur® (Merck) quality. All dilutions were prepared fresh daily.

All disposable devices were rigorously cleaned shortly before use by immersion into concentrated nitric acid and rinsing with redistilled water.

Serum and urine samples were obtained from 31 non exposed persons (men between 30 and 59) and additional serum samples were obtained from 142 occupationally exposed workers from a ferromanganese factory in Skopje (Macedonia) and were transferred to plastic tubes. All occupationally exposed workers were men, aged from 31 to 56, with discontinuous exposure to manganese for 7.7 ± 1.1 years. All patients gave agreement for manganese testing of their serum during the systematic health control. Clinical experiments were performed according to the Ethics Committee Regulations of the Institute of Preventive Medical Care and Toxicology of the Military Health Institution Center.

Serum samples were collected with plastic *i.v.* kanula No. 24 (TIK, Slovenia) with an injection valve. Urine samples were taken as spot samples. The samples were kept at –18 °C until analysis.

Procedures

Serum. – Serum samples were treated with equal volumes of 3% nitric acid (m/V) for deproteinization. After elimination of the precipitate by centrifuging (5000 rpm, 3 min), the obtained solutions were introduced into a graphite furnace.

Urine. – Urine samples were diluted by adding an equal volume of 3% HNO_3 (m/V) and, after mixing, the solutions were introduced into the graphite furnace.

Standard addition were prepared by addition of manganese standards (up to 10 ng mL^{-1}) into either the serum or the urine sample.

RESULTS AND DISCUSSION

Different types of interferences can influence the determination of metals in biological fluids by ETAAS. Physical interferences can effect the accuracy and precision of dispensing microvolumes of sample solution into a graphite tube. Chemical interferences may affect the sensitivity with enhancement, by organic constituents, and suppression by inorganic constituents of the matrix present during atomization.

Interferences will depend on the adequate chemical and thermal pretreatment of the sample prior to atomization and they can be eliminated by special chemical pretreatment of the samples and by applying optimal instrumental conditions for the determination. Some authors suggested direct determination (9–19), but in such cases, neither Zeeman nor deuterium correction eliminates the background absorption effectively. The effects of matrix constituents on the manganese signals and background absorption signals are shown in Fig. 1 for serum, and in Fig. 2 for urine.

This is why nitric acid was used as matrix modifier and for serum deproteinization. In both deuterium and Zeeman correction methods, the presence of nitric acid significantly reduced the background signal and direct determination of manganese was possible. It was found that the solution of 3% HNO_3 was sufficient to eliminate background absorption.

The effect of nitric acid is also evident in increasing pyrolysis and atomizing temperatures (Figs. 3 and 4). Fig. 3 shows the effect of pyrolysis temperature on the manganese signal. Clearly, manganese in serum and urine is not appreciably volatilized up to 900 °C in deuterium correction and 1000 °C in Zeeman correction, with 15 s ramp and hold time in both cases. Although a wide range (1900–2400 °C) of atomization temperatures can be used, the 1800 °C and 2100 °C were chosen. It is evident that nitric acid ensures complete ashing of the organic matter and eliminates any carbonaceous residue buildup inside the graphite tube (24). Optimal instrumental conditions are given in Table I.

Calibration curves were prepared by the standard addition method. The limit of detection of manganese (determined as 3 standard deviations of the blank) in serum and urine was 0.2 $\mu g\ L^{-1}$ with D_2 correction and 0.1 $\mu g\ L^{-1}$ with Zeeman correction. The linear concentration range for the Zeeman correction method was 0.1 to 10 $\mu g\ L^{-1}$ and for the D_2 correction method from 0.2 to 10 $\mu g\ L^{-1}$. The accuracy of the measures obtained from recovery values ranged from 93.8 to 97.0% for the Zeeman correction method and from 90.0 to 95.5% for the D_2 correction method. Repeatability (relative standard deviation) of the

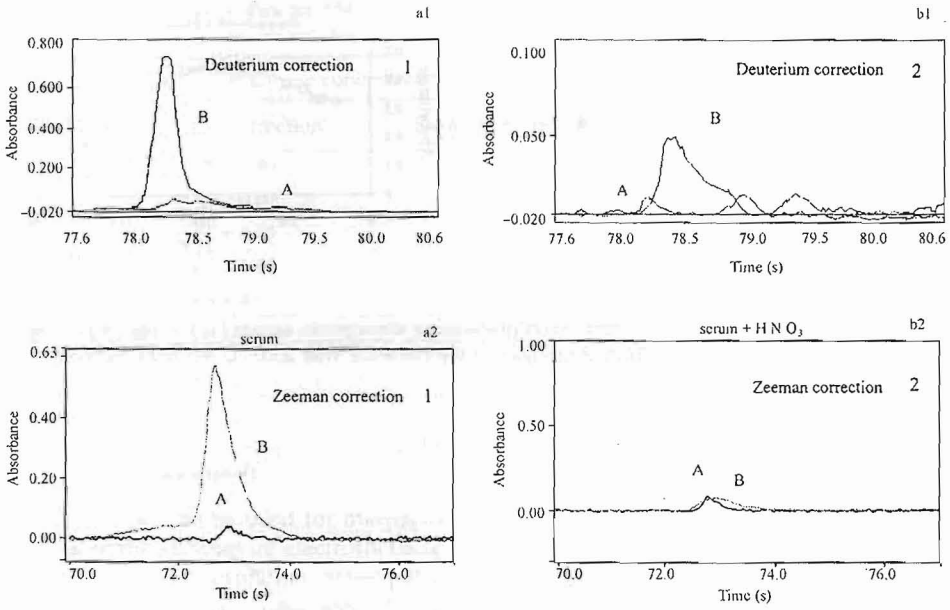

Fig. 1. Manganese absorption (A) and background (B) signals in serum (diluted with water 1:1) in the a) absence and b) presence of nitric acid.

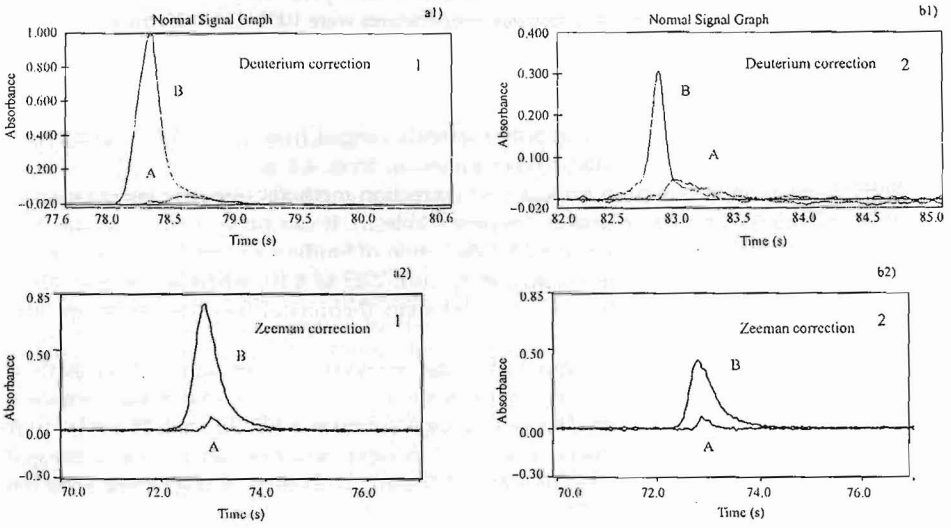

Fig. 2. Manganese absorption (A) and background (B) signals in urine in the a) absence and b) presence of nitric acid.

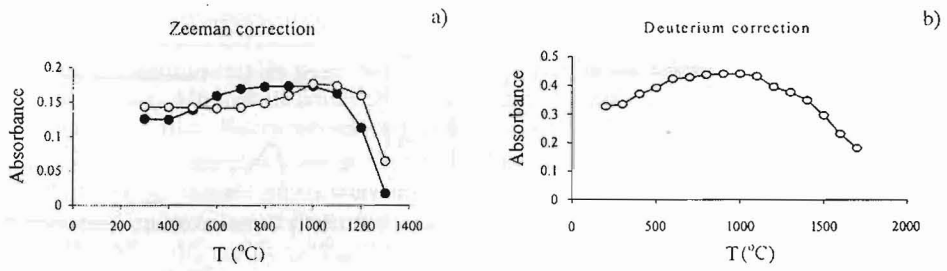

Fig. 3. Effect of the pyrolysis temperature on manganese absorption: serum (o), urine (•) using: a) Zeeman or b) deuterium correction. Atomization temperature was 2000 °C for both methods.

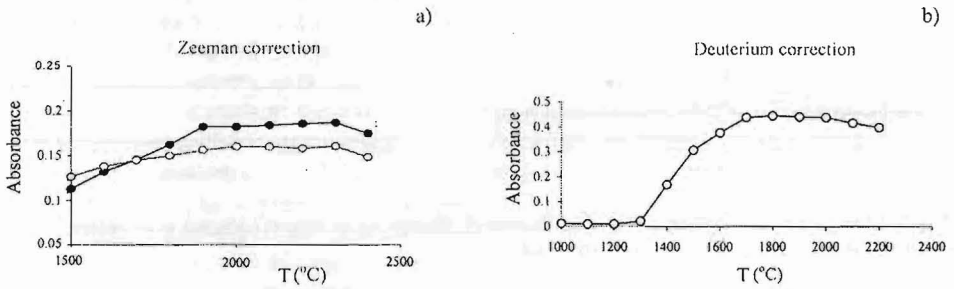

Fig. 4. Effect of the atomization temperature on manganese absorption: serum (o), urine (•) using: a) Zeeman or b) deuterium correction. Pyrolysis temperatures were 1000 °C for Zeeman and 900 °C for deuterium correction method.

data obtained for the same sample in both methods ranged from 2.2 to 5.3%, within day precision from 3.5 to 6.4% and day-to-day precision from 4.2 to 7.5%.

Both Zeeman and deuterium background correction methods were applied to manganese determination in the same serum samples (Table II). It can be seen that the obtained concentrations have no high differences. A calculation of Student's *t*-test for the results obtained by both methods gave the values range from 2.45 to 4.10, whereas the theoretical value (95%) of *t* was 2.78. *t*-Values are smaller than theoretical for three samples, and higher for two samples.

Because of a lower detection limit and better recovery, the Zeeman ETAAS method was applied to analyze serum samples of nonexposed and occupationally exposed workers. Manganese varied from 0.80 to 7.20 $\mu g\ L^{-1}$ (mean ± SD 2.16 ± 1.18 $\mu g\ L^{-1}$) for 142 occupationally exposed workers, and for 31 nonexposed persons the value ranged from 0.75 to 2.30 $\mu g\ L^{-1}$ (mean ± SD of 1.73 ± 0.77 $\mu g\ L^{-1}$). This is in agreement with the literature data (1, 3, 4, 14, 18, 22–24).

Table II. Comparison of data for Mn concentration obtained by Zeeman and deuterium corrections

Serum sample	Manganese concentration ($\mu g\ L^{-1}$)		*t*-test (statistical influence)
	Zeeman correction	Deuterium correction	
1	0.35 ± 0.02	0.40 ± 0.02	4.10 (S^a)
2	0.45 ± 0.02	0.41 ± 0.02	3.26 (S^b)
3	0.57 ± 0.02	0.60 ± 0.02	2.68 (NS)
4	0.65 ± 0.02	0.69 ± 0.02	2.98 (S^b)
5	0.69 ± 0.02	0.73 ± 0.02	2.45 (NS)

S, SN – statistically significant / not significant differences, df = 4.
[a] $p < 0.02$,
[b] $p < 0.02$

CONCLUSIONS

Nitric acid can be used for matrix modification in manganese determination in serum and urine samples by electrothermal atomic absorption spectrometry in both deuterium and Zeeman correction techniques.

Pyrolysis temperature of 900 °C was found optimal for D_2 and 1000 °C for Zeeman correction technique, while 1800 °C and 2100 °C were found to be optimal atomizing temperatures, respectively.

The limit of manganese detection in serum and urine was 0.2 $\mu g\ L^{-1}$ for the D_2 correction method and 0.1 $\mu g\ L^{-1}$ for the Zeeman correction method.

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SAŽETAK

Određivanje mangana u serumu i urinu elektrotermičkom atomskom apsorpcijskom spektrometrijom

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U radu je opisana metoda za izravno određivanje mangana u ljudskom serumu i urinu elektrotermičkom atomskom apsorpcijskom spektrometrijom (ETAAS) s deuterijskom i Zeemanovom korekcijom te s nitratnom kiselinom kao modifikatorom matriksa. U obje metode korekcije, prisutnost nitratne kiseline značajno je smanjila pozadinski signal te je bilo moguće izravno određivanje iona mangana. Optimalna temperatura za pirolizu uz deuterijsku korekciju bila je 900 °C, a 1000 °C za Zeemanovu korekciju, dok su 1800 °C i 2100 °C bile optimalne temperature atomizacije. Kalibracijske krivulje su pripravljene standardnom adicijskom metodom. Granica detekcije mangana u serumu i urinu bila je 0.2 $\mu g\ L^{-1}$ za metodu s deuterijskom korekcijom i 0.1 $\mu g\ L^{-1}$ za metodu sa Zeemanovom korekcijom. Vrijednosti mangana u serumu u 142 profesionalno izloženih osoba bila je između 0.80 i 7.20 $\mu g\ L^{-1}$ (prosječna vrijednost ± SD 2.16 ± 1.18 $\mu g\ L^{-1}$) dok za 31 osobu koja nije izložena, vrijednost je iznosila od 0.75 do 2.30 $\mu g\ L^{-1}$ (prosječna vrijednost ± SD 1.73±0.77 $\mu g\ L^{-1}$).

Ključne riječi: mangan, serum, urin, određivanje, elektrotermička atomska apsorpcijska spektrometrija

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