Determination of urinary manganese by the direct chelation-extraction method and flameless atomic absorption spectrophotometry

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ABSTRACT The direct chelation-extraction method, originally developed by Hessel (1968) for blood lead analysis, has been successfully applied to urinalysis for manganese. The analyses of 35 urine samples containing up to 110 µg/l of manganese from manganese-exposed workers showed that the data obtained by this method agree well with those by wet digestion-flame atomic absorption spectrophotometry and also by flameless atomic absorption spectrophotometry.

Increasing attention has been focussed recently on the toxicity of manganese in the field of industrial and environmental health (Committee on Biological Effects of Atmospheric Pollutants, 1973). For biological monitoring of manganese exposure, urine is one of the materials of choice because it is apparently easy to sample even though faecal elimination constitutes the main route of excretion in human subjects (Horiuchi *et al.*, 1967; Mahoney and Small, 1968; Tolonen, 1972). A correlation on a group basis of manganese concentrations in the urine of workers with the manganese levels in their workroom air was reported by Tanaka and Lieben (1969), suggesting the validity of biological monitoring in manganese exposure. Digestion of biological materials, even of urine, is a troublesome step in analysis, however, and often forms a bottleneck in an entire procedure.

The direct chelation-extraction method, originally developed by Hessel (1968) for blood lead analysis, has been successfully applied in our laboratory to the analysis of manganese in urine. Another method of choice is flameless atomic absorption spectrophotometry. The results will be discussed here in comparison with those obtained by standard wet digestion-flame atomic absorption spectrophotometry.

Based on these findings, an automated system has been developed in our laboratory for the analysis of urinary manganese (Ikeda *et al.*, 1978). The results from the automated analysis will also be cited for comparison.

Materials and methods

Urine samples (35 in all) containing manganese up to 110 µg/l were collected from kiln operators and bagging personnel in a plant producing manganese dioxide from ore. Control urine samples (78 from men and 98 from women) were obtained from non-exposed workers and college students. These were kept in manganese-free containers and stored in a frozen condition until analysed.

All the reagents including standard manganese chloride (MnCl₂) solution, ammonium pyrrolidine dithiocarbamate (APDC), sodium diethyldithiocarbamate (SDDC), methyl isobutyl ketone (MIBK) and ethyl acetate (EA) were of trace metal analysis grade except Triton X-100 (TX) which was an extra-pure grade (all from Wako Pure Chemicals, Ltd., Osaka, Japan).

Wet digestion-flame atomic absorption spectrophotometry was carried out as follows: to 50 ml of urine sample in a Kjeldahl flask were added 20 ml of concentrated HNO₃, 0.5 ml of concentrated H₂SO₄ and 1.0 ml of concentrated HClO₄. After heating, the final digest was transferred to a 50 ml glass-stoppered tube together with a 30 ml wash. The pH was adjusted to about 6 by the dropwise addition of NH₄OH in the presence of methyl red

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as an indicator. An aliquot, 5 ml, of 20% ammonium citrate (pH 6.0) was added to the solution followed by 2 ml of 5% SDDC, and the mixture was shaken vigorously for seven minutes. The manganese chelate was extracted into 10 ml of EA by two minutes' vigorous shaking. The organic layer was analysed within 30 minutes by a Hitachi atomic absorption spectrophotometer (Model 207) equipped with a manganese hollow cathode lamp. A lamp current of 10 mA, a wavelength of 279.5 nm, an acetylene flow of 1.75 l/min, an air flow of 13 l/min, an atomiser aspiration rate of 3.5 ml/min and a recording chart speed of 20 mm/min were employed. A preliminary experiment showed that the use of EA for chelate extraction results in twice as high an absorption intensity as from application of MIBK, which had been used by Van Ormer and Purdy (1973). It was also found that the manganese chelate is stable in EA for at least 30 minutes after shaking for extraction had stopped (Table 1).

The procedure for the direct chelation-extraction method was as follows: the pH of 15 ml urine sample was adjusted to 6 in a 30 ml glass-stoppered bottle by the addition of 2 ml of 1 M HCl-sodium acetate buffer (pH 6). To this solution were added 2 ml of 5% SDDC and 1 ml of 5% TX, and the mixture was shaken vigorously for 10 minutes. Finally, 3 ml of water-saturated MIBK were added and the bottle was again shaken vigorously for five minutes followed by centrifugation for five minutes for the separation of the two layers. The organic layer was subjected to flame atomic absorption spectrophotometry as described in the preceding paragraph.

For flameless atomic absorption spectrophotometry, a 10 ml urine sample was acidified by the addition of 0.3 ml of concentrated HNO_3 ; 20 μl of the acidified urine were injected into a tube-type graphite furnace. The apparatus used was a Nippon-Jarrell Ash two-channel atomic absorption spectrophotometer (Model AA-8500) coupled with a Nippon-Jarrell Ash flameless atomiser (Model FLA-100) and a Rikadenki high speed recorder (Model B-281). The operational settings for the former were a wavelength of 279.5 nm with a manganese hollow cathode 8 mA lamp. The background extinction was automatically corrected with an arc-type deuterium 200 mA lamp, the display selector being set at the 'BKG' position. The furnace was operated at the drying stage for 35 s at a current of 19 A, at the ashing stage for 90 s at 85 A and at the atomising stage for 10 s at 220 A. The argon flow rate was 1.0 l/min with operation mode 'Auto-1'. The chart speed was 20 mm/min. Determinations were repeated three times for each sample, the mean value of which was taken to represent the manganese

concentration.

The automated system for urinary manganese determination is reported in a separate paper (Ikeda *et al.*, 1978).

Results and discussion

APPLICABILITY OF THE DIRECT CHELATION-EXTRACTION METHOD

When manganese chloride was dissolved in water at varying concentrations up to 80 $\mu\text{g/l}$ as Mn^{++} and subjected to analysis by the direct chelation-extraction method, it was confirmed that the extinction at 279.5 nm was linearly related to the concentration of manganese (Fig. 1). The analysis of manganese chloride added to control urine produced essentially the same result; the regression line was parallel to the former, the increment in the extinction being attributable to endogenous manganese in urine.

Each of several control urine samples was divided into three portions, manganese chloride added at 0-60 $\mu\text{g/l}$ urine as Mn^{++} , and subjected to analysis. The results, summarised in Table 2, indicated that the recovery rate of manganese was high and

Fig. 1 Linear relationship between amount of manganese added and extinction. Manganese chloride was added either to water (solid circles) or to control urine (open circles) at various concentrations up to 80 $\mu\text{g/l}$ as Mn^{++} .

Table 1 *Stability of manganese chelate in ethyl acetate*

Time after shaking had stopped (min)	Percentage recovery rate of Mn ⁺⁺ added at a level of	
	20 µg/l	60 µg/l
30	100.8 ± 1.0	100.3 ± 1.0
60	97.0 ± 1.8	98.6 ± 1.1
90	96.3 ± 1.0	96.2 ± 0.7
120	91.8 ± 1.6	93.0 ± 1.5

Five portions each of MnCl₂ solution at the concentration indicated were analysed by wet digestion-flame atomic absorption spectrophotometry (cf. Materials and methods section). Recovery rate is defined as the percentage of the observed value at the time indicated, taking the value measured within 5 minutes after shaking had stopped as 100. The percentage recovery rates shown are means ± SD (n = 5).

Table 2 *Recovery rates of manganese added to various urine samples, determined by the direct chelation-extraction method*

Sample number	Endogenous Mn (µg/l)	Percentage recovery rate of Mn ⁺⁺ added at a level of			
		5 µg/l	20 µg/l	30 µg/l	60 µg/l
1	0.8	106.8	98.7		
2	1.5	120.0	104.1		
3	1.2	109.6	100.9		
4	1.0	84.8	100.0		
5	1.0	105.0	98.5		
6	0.7	98.0	104.8	104.8	102.4
7	0.0			106.5	103.6
8	0.4			99.3	102.3
9	1.6			103.4	102.2
10	0.4			100.3	100.4
11	1.4			101.9	100.3
Mean recovery rates		104.0	101.1	102.7	101.8

Each of the control urine samples, 11 in all, was divided into several aliquots, to which manganese chloride was added at the various concentrations indicated.

The recovery rate (%) is defined as $\frac{\text{Mn observed}}{\text{Mn endogenous} + \text{Mn added}} \times 100$

essentially independent of manganese concentration or origin of urine samples. When three urine samples containing manganese at three different concentrations were each analysed five times and coefficients of variations were calculated, the variation was so small that the coefficient was less than 3% when manganese concentration was over 20 µg/l (Table 3).

The direct chelation-extraction method is applicable to various manganese compounds, both

Table 3 *Reproducibility of the results from various urine samples, determined by the direct chelation-extraction method*

Urine sample	Manganese concentration (µg/l)		CV(%)
	mean	SD	
I	14.3	1.01	7.07
II	24.2	0.57	2.35
III	38.3	2.65	2.58

Each urine sample was analysed five times.
CV = coefficient of variation.

organic and inorganic salts of different valencies including Mn⁺⁺ and Mn⁺⁺⁺ (Lekehal and Hanocq, 1976). When nine manganese compounds, listed in Table 4, were added to control urine and analysed by the direct chelation-extraction method, the results agreed well with those obtained by the wet digestion method, the ratio between the two results being in the range of 0.97-1.17 (Table 4). A popular inexpensive chelating agent, SDDC, is as suitable for the analysis as the specified reagent, 2,2,6,6-tetramethylheptan-3,5-dione, the latter being recommended by Lekehal and Hanocq (1976).

Table 4 *Applicability of the direct chelation-extraction method to various manganese compounds*

Manganese compounds		Values* obtained by		
		DCE	wet digestion	DCE/wet digestion
Acetate	Mn(CH ₃ COO) ₂ ·4H ₂ O	43.3	37.3	1.16
Ammonium sulphate	Mn(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O	41.7	43.0	0.97
Borate	MnB ₃ O ₆ ·8H ₂ O	28.0	25.8	1.09
Butyrate	Mn(C ₄ H ₇ COO) ₂ ·2H ₂ O	44.7	43.5	1.03
Carbonate	MnCO ₃	37.3	32.0	1.17
Chloride	MnCl ₂ ·4H ₂ O	43.2	37.7	1.15
Dioxide	MnO ₂	87.5	89.3	0.98
Phosphate (monobasic)	Mn(H ₂ PO ₄) ₂ ·2H ₂ O	34.5	30.0	1.15
Sulphate	MnSO ₄ ·4H ₂ O	42.7	41.5	1.03

Various manganese compounds were dissolved in control urine and analysed by the direct chelation-extraction method and also the wet digestion method coupled with flame atomic absorption spectrophotometry (cf. Materials and methods section).

*Each value is the mean of three determinations, using manganese chloride solution as a working standard.

DCE = direct chelation-extraction method.

MEASUREMENT WITH FLAMELESS ATOMIC ABSORPTION SPECTROPHOTOMETRY

For the confirmation of the linearity of peak heights in relation to manganese concentrations, one control urine sample was divided into several aliquots, to

Table 5 *Correlation between the results obtained by the four methods*

Comparison	n	Regression lines	r
I vs II	35	B = 0.943A + 1.452	0.971
I vs III	35	C = 0.932A + 2.385	0.974
I vs IV	35	D = 0.943A + 0.542	0.979
II vs III	35	C = 0.972B + 1.272	0.986
II vs IV	35	D = 0.967B + 0.273	0.975
III vs IV	35	D = 0.982C - 1.279	0.976

I = wet digestion-flame atomic absorption spectrophotometry; the value obtained is expressed as A (µg Mn/l urine).

II = direct chelation-extraction-flame atomic absorption spectrophotometry; the value obtained is expressed as B (µg Mn/l urine).

III = automated analysis (Ikeda *et al.*, 1978); the value obtained is expressed as C (µg Mn/l urine).

IV = flameless atomic absorption spectrophotometry; the value obtained is expressed as D (µg Mn/l urine).

n = number of samples: 35 urine samples collected from manganese-exposed workers were analysed by the four methods described.

r = correlation coefficient; P < 0.01 in every case.

which manganese chloride was added at various concentrations. The samples thus prepared were analysed by wet digestion-flame atomic absorption spectrophotometry or flameless atomic absorption spectrophotometry as described in the Materials and methods section. The results clearly showed that the peak height obtained by the latter method was linearly related to the manganese concentration as was the case in the former procedure. The endogenous manganese concentration in the urine sample was about $3 \mu\text{g/l}$. The present method using automatic deuterium lamp correction allows direct injection of the urine sample into the furnace. Buchet *et al.* (1976) previously reported flameless atomic

absorption spectrometry of manganese-cupferron chelate in MIBK. Quantitative injection of volatile MIBK in the furnace at a high temperature is apparently difficult to achieve.

CORRELATION BETWEEN THE VALUES OBTAINED BY THE FOUR METHODS

In order to determine whether the value obtained by any of the methods studied is in agreement with that by any of the other methods, 35 urine samples containing manganese up to $110 \mu\text{g/l}$ were collected from manganese-exposed workers and analysed by (1) the standard method of wet digestion coupled with flame atomic absorption spectrophotometry; (2) the direct chelation-extraction method coupled with the same; (3) the automated method (Ikeda *et al.*, 1977), the principle of which is based on the preceding method; and (4) flameless atomic absorption spectrophotometry. The results are summarised in Table 5 in terms of regression lines and correlation

Fig. 2 Scatter diagrams of the results obtained by different methods. Urine samples obtained from 35 manganese-exposed workers were analysed (see also Table 5).

Fig. 3 Distribution of manganese in control urine samples.

coefficients, and comparative scatter diagrams are shown in Fig. 2. It is quite clear that all four analytical methods achieve essentially the same results, and that the three time- and labour-saving methods are as reliable as the standard method of flame atomic absorption spectrophotometry after lengthy wet digestion of urine.

MANGANESE LEVELS IN THE URINE OF NON-EXPOSED SUBJECTS

Urine samples were collected from 78 men and 48 women who were either factory workers without occupational manganese exposure or who were college students, and these samples were analysed by the direct chelation-extraction method. As shown in Fig. 3, the distribution of manganese levels is skewed. With the assumption of log-normal distribution as suggested by Horiuchi *et al.* (1967), the geometric mean of values from men was 0.53 $\mu\text{g/l}$ and the 95% confidence range was 0.11-2.67 $\mu\text{g/l}$ whereas for women the values were 0.54 $\mu\text{g/l}$ and 0.08-2.37 $\mu\text{g/l}$ respectively. There is no statistically significant difference between the two means by the *t* test ($P > 0.10$). The present values are very close to those reported by Buchet *et al.* (1976) (mean 0.65 $\mu\text{g/l}$, standard deviation 0.53 $\mu\text{g/l}$) although these authors did not discuss the distribution pattern, and somewhat lower than those reported by Cholak and Hubbard (1960) (1.8 $\mu\text{g/l}$). The discrepancy between the values found in the present study and those reported by Horiuchi *et al.* (1967) is apparently attributable to the higher specificity of the method used here.

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