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Determination of toxic metals in blood by X-ray fluorescence spectrometry

by RIITTA VAASJOKI, M.Sc., and JORMA RANTANEN, M.D.¹

VAASJOKI, R. and RANTANEN, J. Determination of toxic metals in blood by X-ray fluorescence spectrometry. *Scand. j. work environ. & health* 1 (1975) 184—192. An application of X-ray fluorescence spectrometry for the analysis of various toxic metals (lead, cadmium, manganese, chromium, nickel, vanadium) in whole blood was studied, and two techniques for sample preparation (vacuum drying and nitric acid combustion) were compared. The acid combustion method appeared to be superior on the basis of the accuracy and precision of the determinations. The tungsten-target X-ray tube was suitable for determining manganese, nickel, and vanadium, the lowest limits of detection being 0.021 $\mu\text{g/ml}$, 0.035 $\mu\text{g/ml}$ and 0.017 $\mu\text{g/ml}$, respectively, and the precisions being between 4.25 and 6.92 %. The sensitivity and reproducibility for lead, cadmium, and chromium were unsatisfactory. A comparison between the recoveries of X-ray fluorescence and atomic absorption analyses demonstrated that the X-ray method is suitable for determining manganese, vanadium, nickel, cadmium, and lead. The poor sensitivity of the X-ray method, however, restricts its practical use. For vanadium the results obtained by X-ray fluorescence were superior to those measured by a colorimetric method. No significant differences were observed between the results of single component and multicomponent analyses at the metal concentrations usual in physiological and toxicological cases.

Key words: lead, cadmium, manganese, chromium, nickel, vanadium, whole blood, acid combustion, vacuum drying, atomic absorption, X-ray fluorescence.

Most of the present methods for determining heavy metals in blood require complicated preparation of samples and allow an analysis of only one component per sample. Preparation of the material to be analyzed with X-ray fluorescence spectrometry is rapid. Moreover, the X-ray determination is specific and simple and leaves the sample intact for further study. Another advantage of the method is the possibility to analyze several metal elements from one and the same specimen. Due to the low atomic number of carbon, nitrogen, oxygen, and

hydrogen the organic matter does not disturb the analysis of the heavier elements in blood. On the other hand, absorption-excitation interactions, bremsstrahlung, background, and overlapping fluorescence lines (in blood especially those of iron) limit the applicability of the method in analyzing biological samples with low metal concentrations.

Up to now only a few X-ray fluorescence analyses of human samples have been reported. Mostly the previous papers involve the determinations of ions of clinical interest (Na, K, Ca, Sr, Fe, Cu, Cl, J, S and P) in serum (20, 22, 23, 24), in whole blood (10, 15, 16, 21), or in certain tissues (1). Concentrations of these relatively nontoxic elements in normal blood are higher than the concentrations of toxic metals in toxicological patients.

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X-ray fluorescence has been used for the single component analysis of blood lead (11), zinc (4, 26), arsenic, and bromine (10). A two component analysis of bismuth and mercury, as well as of mercury and gold, in blood has been successfully carried out (19). The multicomponent determination of trace metals (chromium, arsenic, strontium, silver, cesium, tungsten, mercury, lead) in whole blood has given the lowest limit of detection of about 2–150 ppm when an energy-dispersive system has been used (14).

In the present investigation the applicability of X-ray fluorescence spectrometry for the analysis of the most common toxic metals in blood was studied, two methods of sample preparation were compared, and the results of single component and multicomponent analyses were evaluated.

MATERIALS AND METHODS

X-ray fluorescence spectrometry

The X-ray fluorescence measurements were carried out with a Philips PW 1410 vacuum X-ray fluorescence spectrometer equipped with a pulse height analyzer and a flow proportional counter, as well as with a scintillation counter (Philips Co., Eindhoven, Holland). A tungsten-target X-ray tube and a lithium fluoride analyzing crystal were used. Adjustment of the pulse height analyzer was made with pure standard reagents. The conditions for the

analysis of various components are presented in table 1.

Reagents

The reagents used for standards were spectrum clean metal oxides or salts (Johnson Matthey Chemicals Ltd., London, England) except in the case of chromium, which was of p.a. grade (Merck Ag, Darmstadt, GFR). Deconex® (Borer Chemie, Soloturn, Switzerland) was used for washing the glassware employed in the analyses. The cellulose-resin base for wet combustion samples was prepared by mixing Polyamid 11-resin (Merck) with Whatman CF 11 cellulose (Balston Ltd., Maidstone, Kent, England) in a weight ratio of 1:10.

Procedure

The stock solutions of the standards were prepared by dissolving in redistilled, deionized water (PbCO_3 , NiCO_3 , CrO_3 , V_2O_5) or in 1 ml of concentrated nitric acid (p.a.) (CdO , MnO_2), and water was added to get a final concentration of 1 to 100 μg of metal per milliliter. Two microliters of $\text{H}_2\text{O}_2/\text{ml}$ (Merck p.a.) was used as an oxidizing agent in the preparation of the manganese standard.

The blood samples for the single component analysis were prepared by pipetting aliquots containing 0–20, 30, 40, 60, 70, and 100 μg of the metal standards into 10-ml volumes of heparinized pig

Table 1. X-ray spectrometer settings for lead, nickel, manganese, chromium, cadmium, and vanadium.^a (SC = scintillation counter; FLC = flow proportional counter^b)

Component	Pb	Ni	Mn	Cr	Cd	V
Counter	SC	SC	FLC	FLC	FLC	FLC
Counter voltage (V)	1080	1080	1670	1670	1670	1670
Collimator	Fine	Fine	Fine	Fine	Coarse	Fine
Pulse height base-line voltage (V)	4.15	4.00	4.44	4.44	4.50	4.44
Pulse height "window" (V)	3.5	4.0	1.7	1.7	2.2	1.7
Goniometer, 2θ settings						
Peak	33.88°	48.60°	62.95°	69.31°	53.85°	76.90°
Background	34.50°	48.00°	62.00°	68.00°	55.20°	75.00°
Spectral line ($n = 1$)	L	K	K	K	L	K
Gas in flow proportional counter,						
Flow rate (l/h) ^b	—	—	3	3	3	3

^a Tungsten-target X-ray tube (60 kV, 40 mA, LiF crystal).

^b Gas mixture: 90 % argon and 10 % methane.

blood (Medica Co., Helsinki, Finland) supplemented with 1 million IU/liter of Penicillin G (Leiras Co., Turku, Finland). The samples for the multicomponent analysis were obtained by pipetting corresponding amounts of each metal standard into the same 10-ml volume of blood.

Two methods of sample preparation were utilized: (a) the conventional combustion by nitric acid (15 ml of HNO_3 per sample, main phase for 2 h at 370–390°C) and (b) vacuum drying (11). The combusted samples were ground, weighed, and collected on a cellulose-resin base. The vacuum-dried samples were ground, 200 mg of Polyamid 11 resin was added, and the mixture was homogenized by

subsequent thorough grinding. Fifteen samples for each metal component for either method were pressed into pills with a hydraulic press. The pills were stored in a vacuum before the X-ray analysis.

Just before the analysis the pills were covered with a Mylar film (6 μm thick, Philips) to prevent contamination of the spectrometer. The maximum intensity of the characteristic line of each metal component, as well as the background values, were measured during three periods of 40 s. As a control of the homogeneity of the pills, the vacuum-dried samples were measured on both sides of the pills. No matrix corrections were made.

Fig. 1. Calibration curves for cadmium, chromium, manganese, nickel, lead, and vanadium at the microgram level from wet combusted single component and multicomponent standards. The range of practical toxicological significance is presented at an enlarged scale in the upper left-hand corner of the figure. (Open symbols: single component analysis; closed symbols: multicomponent analysis)

Fig. 2. Calibration curves for microgram amounts of cadmium, chromium, manganese, nickel, lead, and vanadium from vacuum-dried single component and multicomponent standards. The range of practical toxicological significance is presented at an enlarged scale in the upper left-hand corner of the figure. For manganese and vanadium only data from the single component analysis are presented. (Open symbols: single component analysis; closed symbols: multicomponent analysis).

Table 2. Sample homogeneity, lowest limit of detection, accuracy, and precision of the vacuum drying and acid combustion methods in X-ray analysis.

Element	Atomic number	Preparation method	Pellet homogeneity (%)	Lowest limit of detection ($\mu\text{g/ml}$)	Accuracy ($\mu\text{g/ml}$)	Precision (%)
Lead	82	vacuum	13.90	0.354	0.327	6.08
		combustion		0.205	0.123	7.21
Cadmium	48	vacuum	11.54	0.571	0.400	— ^a
		combustion		0.269	0.185	3.37
Chromium	24	vacuum	18.90	0.179	0.073	15.03
		combustion		0.087	0.905	17.29
Manganese	25	vacuum	11.30	0.049	0.043	6.32
		combustion		0.021	0.014	5.51
Nickel	28	vacuum	6.39	0.094	0.066	6.40
		combustion		0.035	0.055	6.92
Vanadium	23	vacuum	9.15	0.062	0.054	10.19
		combustion		0.017	0.010	4.25

^a Not calculated because of the small number of duplicate determinations.

Evaluation of data

The lowest limit of detection (LLoD) was calculated on the basis of the variation of the background (8). The within-run precision was determined as the percentage standard deviation of the measurement error for duplicate determinations, and the accuracy of the spectrometry, as the standard error (SE) of the estimate (3). The total recoveries of the wet combusted samples were calculated and compared with those obtained from the same samples by atomic absorption spectrometry for cadmium, lead (5, 25), manganese, nickel (7), and chromium (17). In the case of vanadium values for comparison were obtained by a colorimetric method (9).

RESULTS

Linearity and sample homogeneity

A linear correlation between the net counts of the apparatus and the metal concentration of blood was found in the single component and multicomponent analyses for every metal studied. The sample preparation method had no effect on the linearity (figs. 1 and 2). However, the regression line for the vacuum-dried samples of chro-

mium intersects the net count axis markedly above the origin. For the acid-combusted samples of chromium the relationship becomes clear only at very high concentrations. The difference between the net counts measured on both sides of the vacuum pills varied between 6.4 and 18.9 % (table 2).

Accuracy and sensitivity

The vacuum-dried samples gave only 21 to 44 % of the net counts obtained for the same metal concentrations after the acid combustion method (figs. 1 and 2). The lowest limit of detection was 1.7 to 3.8 times higher for vacuum-dried preparations than for acid combusted samples (table 2). A comparison of the X-ray and atomic absorption analyses demonstrated that the X-ray method above the LLoDs is applicable for the determination of lead, cadmium, manganese, nickel, and vanadium (table 3).

Precision

The within-run precision for standard samples (table 2) varied greatly and depended on the element (range 3.4—17.3 %). The sample preparation technique had an effect on the reproducibility only in the case of vanadium.

Table 3. Comparison of the accuracy of the vacuum method in X-ray fluorescence and atomic absorption spectrophotometry (AAS) or colorimetric analyses.

Component	Amount added ($\mu\text{g/ml}$)	Measured		Recovered	
		AAS ($\mu\text{g/ml}$)	X-ray ($\mu\text{g/ml}$)	AAS (%)	X-ray (%)
Lead	0.2	0.21	0.14	105	70
	0.3	0.43	0.39	143	130
	0.4	0.53	0.41	133	103
	0.5	0.68	0.74	136	148
	0.8	0.86	0.81	108	101
	1.0	1.20	1.04	120	104
mean				124	109
Cadmium	0.03	0.03	0.07	100	233
	0.2	0.17	0.22	85	115
	0.4	0.28	0.39	70	98
	1.0	0.70	1.12	70	112
mean				81	140
Manganese	0.04	0.04	0.03	100	75
	0.1	0.15	0.07	150	70
	0.2	0.22	0.18	110	90
	0.6	0.76	0.56	127	93
	0.8	1.12	0.77	140	96
	1.5	1.77	1.52	118	101
mean				124	88
Nickel	0.1	0.09	0.07	90	70
	0.3	0.33	0.28	110	93
	0.8	1.06	0.79	133	99
	1.0	1.12	0.98	112	98
	1.5	1.58	1.51	105	101
mean				110	92
Vanadium	0.02	0.03 ^a	0.04	150 ^a	200
	0.10	0.29	0.07	290	70
	0.50	0.31	0.50	62	100
	0.80	0.46	0.83	58	104
mean				140	119
Chromium	0.03	0.02	0.06	67	200
	0.05	0.07	0.07	140	140
	0.15	0.11	0.11	73	73
	0.20	0.14	0.16	70	80
mean				88	123

^a Colorimetric determination.

Comparison of the single component and multicomponent analyses

No differences were observed between the results of the single component and multicomponent analyses for lead, cadmium, chromium, and nickel independently of the method of preparation (figs. 1 and 2). For manganese and vanadium the acid-combusted samples behaved in a similar

manner (fig. 1). The vacuum-dried samples of manganese and vanadium, however, differed markedly. At higher concentrations (5–10 $\mu\text{g/ml}$) the net counts of the single component samples reached only about 90 % of the net counts of the multicomponent samples (fig. 3). At physiological and toxicological concentrations (less than 2 $\mu\text{g/ml}$) practically no differences between the single component and multicomponent samples were observed.

DISCUSSION

For both methods of sample preparation and all the metals investigated a linear relationship between the metal concentrations and net counts of the spectrometer was found (figs. 1 and 2). The observed pellet inhomogeneity of the vacuum-dried pills would be decreased by prolonged mechanized grinding during preparation. If homogeneity still remains poor, measurement on both sides of the pill is necessary. The net counts were higher and the lowest limits of detection were better for the acid-combusted samples than for the vacuum-dried specimens (figs. 1 and 2). Therefore, use of the acid combustion method is advisable in the preparation of toxicological samples having low metal concentrations.

Results of the determinations of vacuum-dried X-ray fluorescence samples (table 3) showed that the method is applicable for the analysis of lead, cadmium, manganese, nickel, and vanadium at concentrations exceeding the LLoDs (table 2). The atomic absorption (AAS) analyses of lead, manganese, and nickel systematically gave values too high, whereas those measured for cadmium and chromium were lower than expected. It may be argued that the poor results of the AAS analyses resulted from the relatively high concentrations measured. This is supported by the tendency for the lowest concentrations to be best in quality in the analyses.

The precision of the X-ray method varied greatly. It was good for cadmium and vanadium and poor for chromium. The poor quality of the chromium determinations depends largely on the chromium impurities of the tungsten-target of the X-ray tube, which causes some characteristic chromium radiation. This is illustrated by the regression line for the vacuum-dried chromium samples, which showed a "background" value at the concentration of zero. This interference could be overcome either by inserting a titanium filter (18) or by employing, e.g., a molybdenum-target tube.

No significant difference between determinations from single component and multicomponent samples of lead, cadmium, manganese, nickel, chromium, and vana-

Fig. 3. Comparison of single component (solid line, open symbols) and multicomponent (broken line, closed symbols) standard curves for vacuum-dried samples of manganese and vanadium. The range of practical toxicological significance is shown at an enlarged scale in the upper left-hand corner of the figure.

dium could be detected either in the combustion or the vacuum preparations from normal up to the highest metal concentration met in intoxication cases. At extremely high concentrations, net counts for manganese and vanadium multicomponent analyses from vacuum-dried samples exceeded those obtained for the corresponding single component specimens. On the basis of their electron structure a similar behavior could be expected for nickel and chromium. The concentration of chromium in the samples was, however, too low, and for nickel a corresponding observation was not made.

The determination of lead and cadmium was disturbed by an abnormally high background. Both of these elements

Table 4. Human whole blood normal values found in the literature for lead, cadmium, chromium, manganese, nickel, and vanadium. (Elemental concentration: $\mu\text{g/ml}$ of sample)

Toxic metal	Mean $\pm$ SE	Range, mean 95th percentile	Range, mean	Mean	Upper limit	Maximum normal value
Pb	0.177 ± 0.008	—	0.010—1.093	0.300 (males) 0.237 (females)	—	0.300
Cd	—	0.003—0.054 0.007 0.017	0.132 0.005—0.142 0.018	—	—	0.010
Cr	0.107 ± 0.014	0.013—0.055 0.027 0.045	—	—	—	0.100
Mn	0.040 ± 0.001	—	—	—	—	0.040
Ni	0.327 ± 0.010	0.009—0.455 0.030 0.098	—	—	—	0.120
V	0.126 ± 0.003	—	—	—	< 0.010	0.020
Determination method	emission spectro-chemistry	Cd and Ni IR-spectrography; Cr, spectrophoto-metry	atomic absorption spectro-photometry	titrimetric dithizon method	colorimetry	atomic absorption spectro-photometry
Population size	50	154	243	138	89	unknown
Sex	female and male	male	male	female 51 male 87	male	female and male
Reference	Butt et al. (6)	Imbus et al. (12)	Kubota et al. (13)	Zurlo et al. (27)	Allways et al. (2)	Institute of Occupational, Health, Helsinki, Finland

were determined from their L-lines with a much lower intensity than that of the K-lines. The tungsten-target X-ray tube yields several lines in the area of the L-lines of lead, and the measurement of cadmium is disturbed by the K-line of iron. Furthermore, the sensitivity of the scintillation counter increases at the shorter wavelengths of the L-lines, which also produces a higher background.

Only little information is available on normal values of toxic metal concentrations in blood. The values given in the literature are quite contradictory and depend on the method of determination and preparation and on the reagents, population, and standardization (table 4). Evidently the limit of detection of the metal components in either of the present preparation methods is not low enough for reliable analysis of mean normal values. The LLoDs of the vacuum method are higher for lead, cadmium, manganese, chromium, and vanadium than the mean normal values of blood. For cadmium the acid combustion method also appeared to be unsatisfactory. This is at least partly due to the low intensity of the L-lines used

for identification. The combustion method must be considered applicable for the determination of manganese, nickel, and vanadium, since the normal values (table 4) exceed the lowest limits of detection (table 2). For lead and chromium the data presented is contradictory. The mean normal values of lead reported by Kubota et al. (13) are lower than the lowest limits of detection of the present study; for chromium the values found by Imbus et al. (12) are also below the LLoD of our method. Normal values reported by other authors would speak for the applicability of the acid combustion method for lead and chromium determinations.

The present findings are consistent with the fact that the X-ray fluorescence instrument is more sensitive for the determination of lighter elements (around the atomic number 30) than for heavier elements when it is used for the analysis of microgram quantities. The difference in sensitivity is due to the inability of the instrument to excite the K-lines of heavier elements and also to the fact that the background of the lighter elements can almost completely be cut off by a pulse height analyzer.

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