EXTENDED AUTOMATED SEPARATION TECHNIQUES IN DESTRUCTIVE NEUTRON ACTIVATION ANALYSIS; APPLICATION TO VARIOUS BIOLOGICAL MATERIALS, INCLUDING HUMAN TISSUES AND BLOOD

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1. INTRODUCTION

The development of sensitive trace-element analysis techniques and their proven value for analyzing various biological materials have gradually stimulated the interest of biological, medical, and environmental scientists in the role and behaviour of trace elements. Multi-element techniques are to be preferred since they provide simultaneously information on a number of trace elements in only one sample aliquot, thus enabling an accurate study of correlations between trace-element levels. Correlations between trace elements mutually and also between trace elements and biological parameters (e.g. concentration of specific metabolites, enzymatic activities, levels of vitamines) may give more insight into the origin, uptake, metabolism, function and/or effects (including synergetic and antagonistic effects) of trace elements.

Neutron activation analysis may be performed as a multi-element and low-level technique for many important trace elements in biological materials, provided that post-irradiation chemical separations are applied. This paper describes a chemical separation, consisting of automated procedures for destruction, distillation, and anion-chromatography. The system developed enables the determination of 14 trace elements in biological materials, viz. antimony, arsenic, bromine, cadmium, chromium, cobalt, copper, gold, iron, mercury, molybdenum, nickel, selenium, and zinc. The method is an extended and improved version of that described earlier /1/. In this paper the aspects of sample preparation, neutron irradiation, gamma-spectrum evaluation, and blank-value contribution are also discussed.

2. EXPERIMENTAL

2.1. Sample preparation

Although neutron activation analysis is often presented as a blank-free technique, special attention should be given to sample handling prior to activation in order to avoid contamination. Furthermore, contamination of the samples may also occur due to trace-element contributions from the walls of the quartz irradiation vials; this latter aspect will be discussed in section 2.6.

In order to minimize contamination, a clean room at a certain distance from the laboratory was choosen as sample preparation room. Inside this room a glove box was installed. Cleaning of the cabinet is performed by spray-rinsing of the interior with demineralized water. Metallic objects are avoided as much as possible. For sample handling utensils made of plastic did not always prove to be adequate. Therefore we developed knives, scissors, tweezers, and homogenator blades made of titanium metal.

The quartz irradiation vials are thoroughly cleaned before use. First, the quartz tubes are refluxed in hot concentrated nitric acid for 24 hours, rinsed with demineralized water and dried. Then the quartz tubes are sealed at one end and steamed out with the sealed end up for 5 minutes.

2.2. Irradiation procedure

Irradiations are carried out in the high flux facility of the Hoger Onderwijs Reactor of the IRI at Delft; the thermal neutron flux amounts to 1.0×10^{13} n/cm^2s. The irradiation time is limited due to the pressure build-up in the

quartz vials caused by radiolysis of biological materials. To reduce radiolysis, the samples are lyophilized prior to irradiation. Although quartz capsules may withstand internal pressures of over 100 atmospheres, depending on diameter and wall thickness, the pressure build-up should be limited to about 10 atmospheres at room temperature. This is necessary in order to minimize loss of irradiated material when the vials are broken after cooling in liquid nitrogen. Before irradiating new types of biological material, the pressure build-up as a function of irradiation time is checked in a special system. For most lyophilized biological samples of 200 to 300 mg in quartz vials of 3 ml an irradiation time of 12 to 24 hours can be maintained.

Each irradiation batch consists of eight items, i.e. four samples, one liquid multi-element standard, one aliquot of (standard) reference material; two 4 mg pieces of zinc metal serve as a flux monitor. The insertion of a (standard) reference material - e.g. Bowen's kale /2/ or NBS bovine liver /3/ - is used for analytical quality control purposes.

2.3. Radiochemical separations

The irradiated samples are allowed to cool for three days, resulting in a substantial reduction of the radiation level, mainly due to sodium-24. Before opening the vials, the outside surface contamination is removed by keeping the vials in boiling aqua regia for 20 minutes and by rinsing twice with water. The vials are then cooled in liquid nitrogen, wrapped in paper tissue, and crushed in a pneumatic press. The sample and quartz splinters are processed together.

The wet destruction with concentrated sulfuric acid and 30 percent hydrogen peroxide and subsequent separation of the volatile elements by distillation with hydrobromic acid are carried out according to the automated procedure described in the earlier paper /1/. In the automated apparatus six samples are processed simultaneously. In the subsequent chemical separation procedure, using Dowex 2x8 anion exchangers, a determination of the elements antimony, arsenic, cadmium, copper, mercury, selenium and zinc was reported. Since then we have added to this list the elements bromine, cobalt, gold, iron, molybdenum, and nickel (via an (n,p) reaction on nickel-58). The element chromium was included recently.

Chromium is obtained as a separate fraction by volatilization as chromyl-chloride from a concentrated perchloric acid solution with dry hydrogen chloride. This manipulation comes as a second step in the separation scheme after the distillation of the volatile bromides (see figure 1). The sulfuric acid residue is evaporated to dryness, the rest is taken up in15 ml of 70 percent perchloric acid solution and 30 mg of trivalent chromium as a carrier is added. At a temperature of 200 + 5°C, dry hydrogen chloride is passed through the perchloric acid at a rate of 100 ml/minute, volatilizing over 90 percent of the chromium in about 10 minutes. The chromium is collected in a receptacle with 5 ml of a 10 percent hydroxylamine hydrochloride solution. An excellent decontamination factor from interfering activities (mainly sodium-24, potassium-42 and phosphorus-32) is obtained. The shape of the distillation vessel (figure 2), and the constancy of the temperature and the hydrogen-chloride gas flow proved to be crucial in this separation.

The further chemical separation of the hydrobromic distillate and the perchloric evaporation rest via Dowex anion chromatography has been improved. The separation scheme now yields 14 elements in 10 separate fractions (figure 1). For a better separation of the elements, Dowex 2x8 200-400 mesh columns of 0.9 cm diameter are enlarged to 9 cm height (4 grams of Dowex per column). Reproducibility of the results is assured by using aliquots of a 232 lbs batch of Dowex, which was divided into 1 lb portions in well-stoppered bottles upon arrival at the laboratory five years ago.

The element cobalt can now be separated from iron. Copper and cobalt are eluted with 20 ml 3M hydrochloric acid with 0.2 percent hydrogen peroxide (fraction 3 in figure 1). Experience shows that about 6 percent of the total

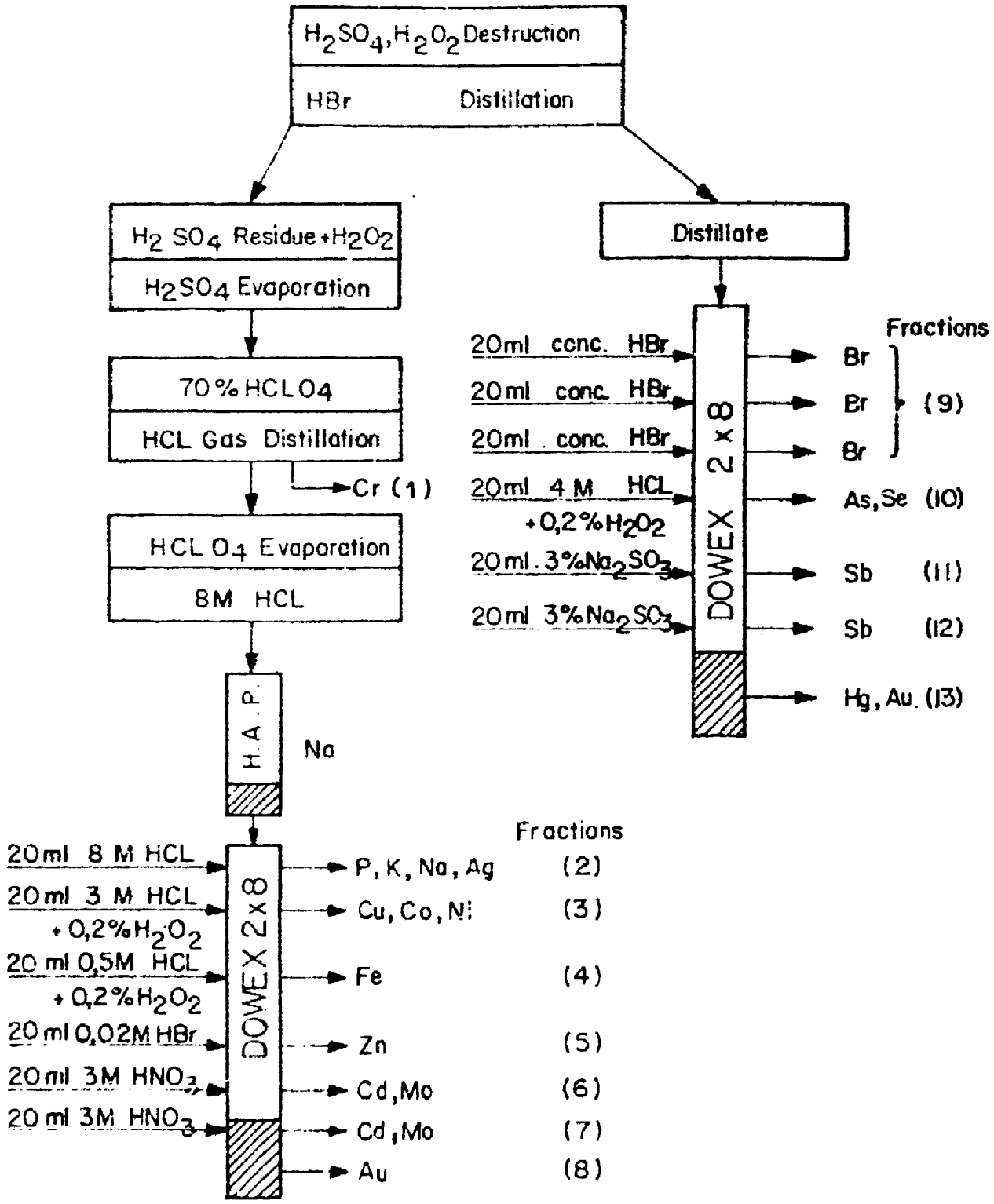

Figure 1 Separation scheme for the analysis of trace amounts of antimony, arsenic, bromine, cadmium, chromium, cobalt, copper, gold, iron, mercury, molybdenum, nickel, selenium, and zinc in biological materials

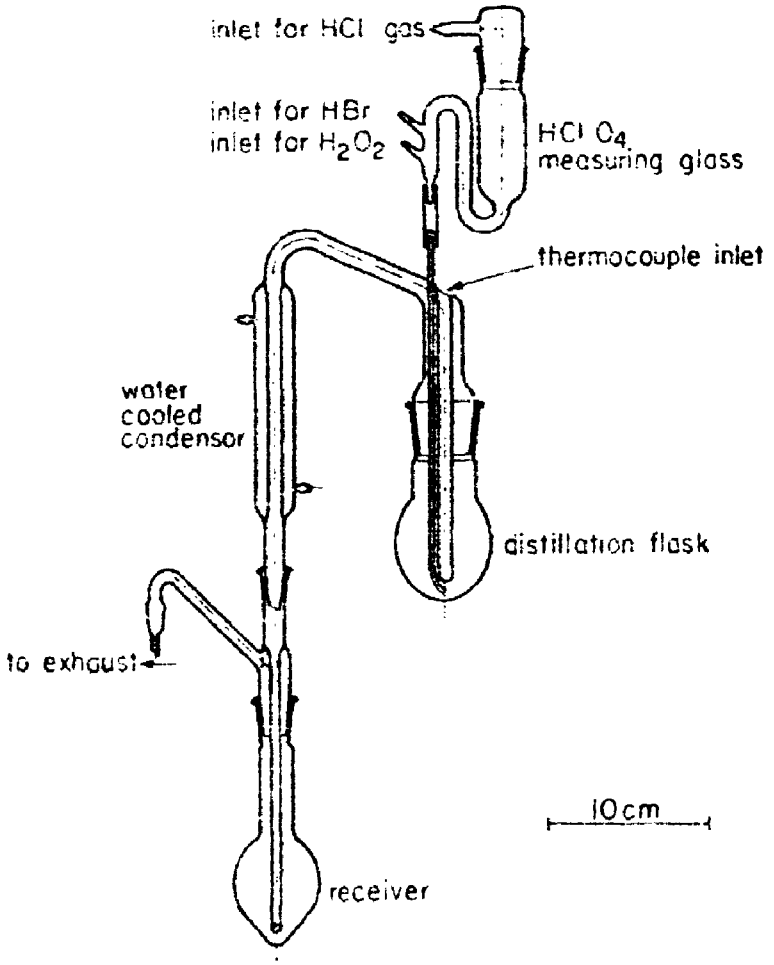

Figure 2 Distillation unit

iron-59 activity is present in fraction 3, which may apparently enhance the cobalt levels when much iron is present in the samples. This problem may be solved by adjusting fraction 3 to 8 M hydrochloric acid again - after counting of the copper-64 activity -, passing the solution over a new Dowex 1x8 column and by eluting the cobalt again with 3 M hydrochloric acid.

The element molybdenum, if present in the oxidized form, stays tightly bound on the column in the hydrochloric acid medium. Molybdenum and cadmium are eluted together by 3 M nitric acid into two 20 ml fractions (fraction 6 and 7 in figure 1).

The element gold is only partially volatilized in the hydrobromic acid distillation. The amounts of gold left in the residue remain well absorbed onto the Dowex after the successive elutions (fraction 8 in figure 1). The same is true for the gold in the distillate. Thus, both Dowex columns contain the total gold-198 activity, and from them the gold levels in the sample may be calculated.

Bromine may be determined by collecting all hydrobromic acid passings through the distillate Dowex column (fraction 9 in figure 1). After making up to 200 ml, a 20 ml aliquot is used for counting.

For a proper absorption and elution of the elements selenium and arsenic, the hydrobromic acid distillate should be of a light orange colour due to free elementary bromine. If necessary, the colour may be adjusted by oxidizing with hydrogen peroxide or by reducing with hydroxylamine or sulfur dioxide. The elements arsenic and selenium are quantitatively eluted with 4 M hydrochloric acid which contains 0.2 percent hydrogen peroxide in order to keep selenium in soluble form (fraction 10 in figure 1).

Antimony is separated from mercury by eluting antimony with two aliquots of 20 ml of a 3 percent sodium sulfite solution (fraction 11 and 12 in figure 1). This procedure ensures an interference-free spectrum of mercury, except for gold-198 which stays on the column.

2.4. Gamma-spectrum evaluation

The fractions resulting from the separation scheme are counted in an 1¼" well of a 3" x 3" NaI(Tl) scintillation detector; counting times range from 200 to 40,000 s. Since the gamma spectra are not complex, a simple computerized

... linear calculation is applied. Difficulties arise, however, in assessing the cobalt-59 peak that stands on the Compton edge of an usually much larger cobalt-60 peak. Linear regression analysis, as proposed by de Bruin e.a. /4/ proved to be succesful in resolving the cobalt spectra. Linear regression analysis also proved to be valuable for accurate determinations of the cobalt-60 activity in the presence of iron-59.

2.5. Calibration of the activities

From the standard in each irradiation run an ETZR-value is calculated. The ETZR (element-to-zinc-ratio) value is the ratio of the activity of the standard (expressed as the decay-corrected activity per microgram of element) to the activity of the zinc flux monitor (expressed as the decay-corrected zinc-65 activity per mg of zinc). A discussion of the advantages of calibration via ETZR-values is given elsewhere /5/. The ETZR-values for an irradiation run are checked with the corresponding averages of ETZR-values calculated from a number of preceding irradiations and should not deviate by more than 10 percent. For routine analysis the calibration is carried out with average ETZR-values.

2.6. Blank value

For most biological materials studied, the contribution of trace elements from the container walls is negligible. However, for matrices such as blood or serum, a correction for the blank contribution should be applied. The main problem in this correction is the high variability of the blank value. In order to minimize this, vials are made from quartz tubes purchased as one large batch from the supplier. However, since the process of crushing of irradiated vials is not reproducible, fractures of variable areas may be leached out during the chemical processing, enhancing the blank variability. Table 1 presents typical

Table 1: Blank values for two types of quartz irradiation vials

	average blank value and standard deviation in nanogram	
	Vitrosil*)	Spectrosil*)
antimony	0.6 ± 0.5	1.4 ± 0.6
arsenic	0.14 ± 0.14	0.17 ± 0.10
bromine		70 ± 55
cadmium	2.7 ± 4.0	0.38 ± 0.20
chromium	9.3 ± 7.8	1.6 ± 0.3
cobalt	0.61 ± 0.36	0.13 ± 0.07
copper	2.1 ± 1.0	2.0 ± 1.3
gold		0.002 ± 0.002
iron	430 ± 380	69 ± 46
mercury	0.05 ± 0.05	0.03 ± 0.01
molybdenum	1.0 ± 0.6	0.4 ± 0.2
nickel	< 50	< 50
selenium	0.29 ± 0.15	0.16 ± 0.09
zinc	30 ± 21	3.3 ± 2.5

*) Supplied by Thermal Syndicate Ltd, Wallsend, Tyne and Wear, NE28 6DG, England

blank values for Vitrosil and Spectrosil quartz. The Vitrosil quartz is normally used. If low blank values are required - especially for the elements cobalt and chromium - the more expensive (synthetic) Spectrosil quartz is applied.

2.7. Further improvements and extensions

When ultimate sensitivity is required, increase of the irradiation time and additional radiochemical purification steps may be used, e.g. for the determination of cobalt, molybdenum, and nickel in whole human blood, for which the levels are close to the detection limit of the system. In the case of blood, the fraction with cobalt-58 and cobalt-60 may be purified by passing over a Dowex 2x8 column a third time (c.f. section 2.3); cobalt-57 may hereby be used as an indicator for the chemical yield. Molybdenum may be further purified by an extraction. For this the two 20 ml nitric acid fractions are evaporated after addition of 1 ml concentrated sulfuric acid and 1.5 mg ammonium molybdate. The evaporation rest is taken up in 50 ml of water and extracted twice with 10 ml of a 3 percent solution of trioctylamine in carbon tetrachloride.

The possibility of elaborating the separation scheme to more elements is still open. The first fraction of the residue Dowex column (fraction 2 in figure 1) is presently discarded, but with additional separations more elements could be measured, e.g. rubidium.

3. RESULTS AND DISCUSSION

In the past 4 years the automated separation techniques have been successfully applied to a wide variety of biological materials, e.g. plant material, fish and shell fish /6, 7/, bird tissues /7/, various organs of aquatic and terrestrial mammals /7, 8/, hair, whole blood /9/ and serum, soil and sediments /14/.

The value of a multi-element technique - as indicated in the introductory section - is well illustrated in our previous study of mercury levels in aquatic mammals /7/. When applying the automated separation technique, we found a strong correlation, over 4 decades, between mercury and selenium levels. This finding led us to the assumption of a natural detoxification process for methylmercury on the basis of selenium available in food. The occurence of high mercury levels in the liver of (apparently) healthy aquatic mammals could not have been understood without knowledge of the selenium levels.

Another illustration may be found in a trace-element study of human carcinoma, where we were able to benefit from the fact that for most samples analyzed for trace elements the activity of 14 enzymes had been measured earlier. Although no large differences in average trace-element levels were found between the various tissues, correlation studies between trace-element levels and enzymatic activities yielded a number of significant and interesting correlations /8/. The nature of these correlations is not yet understood, but they may be a good starting point for a closer biochemical investigation.

The accuracy (degree of absence of systematic errors) and precision (reproducibility) of the method developed is regularly tested with the help of (standard) reference materials distributed among the samples. Table 2 presents typical results for Bowen's kale and NBS Bovine liver. Comparison of our results with the "grand means" for Bowen's kale /2/ and the certified values for NBS bovine liver /3/, yields no significant systematic errors, except for the element gold. The precision is in general 15 percent or better, except in cases where the trace-element levels reach the detection limit, e.g. for gold and nickel in bovine liver. The low precision of chromium in the bovine liver cannot be explained on the basis of counting statistics and variation in the analytical method alone and may indicate a certain inhomogeneity of this reference material with respect to chromium.

We have analyzed the four IAEA biological materials: Dried animal whole

Table 2: Trace-element levels in ppm for Bowen's kale and NBS bovine liver

element	kale of Bowen			NBS bovine liver		
	this study N = 9		Bowen's "grand mean" ± standard deviation /2/	this study N = 9		certified values or indicator values in parenthesis /3, 10/
	average ± standard deviation	relative standard deviation		average ± standard deviation	relative standard deviation	
antimony	0.070 ± 0.008	12%	0.072 ± 0.017	0.015 ± 0.004	30%	
arsenic	0.111 ± 0.015	13%	0.14 ± 0.03	0.08 ± 0.03	38%	(0.055)
bromine	21.0 ± 2.3	11%	24.8 ± 2.4	8.0 ± 1.0	13%	
cadmium	0.87 ± 0.17	19%	0.76 ± 0.19	0.30 ± 0.02	8%	0.27 ± 0.04
chromium	0.45 ± 0.06	13%	0.36 ± 0.13	0.13 ± 0.05	36%	
cobalt	0.077 ± 0.008	10%	0.059 ± 0.010	0.246 ± 0.014	6%	(0.18)
copper	4.49 ± 0.27	6%	4.68 ± 0.64	177 ± 7	4%	193 ± 10
gold	0.0010 ± 0.0003	33%	0.0022 ± 0.0003	0.00023 ± 0.00016	70% *	
iron	117 ± 18	15%	117 ± 16	240 ± 12	5%	270 ± 20
mercury	0.184 ± 0.022	12%	0.174 ± 0.030	0.016 ± 0.002	10%	0.016 ± 0.002
molybdenum	2.59 ± 0.35	13%	2.33 ± 0.51	3.71 ± 0.25	7%	(3.2)
nickel	0.87 ± 0.09	10%		0.27 ± 0.12	43% *	
selenium	0.112 ± 0.014	12%	0.138 ± 0.017	1.06 ± 0.10	9%	1.1 ± 0.1
zinc	34.7 ± 1.8	5%	31.9 ± 2.1	134 ± 5	4%	130 ± 10

* near detection limit of the analysis

blood, wheat flour, dried potatoes, and powdered milk. The results for these four reference materials are listed in table 3. The agreement of our results with the "overall means of accepted laboratory averages" /11/ is satisfactory to good, except for cobalt and iron in wheat flour. Therefore, we have repeated the measurements for both latter elements in wheat flour. Three separate series with in total 11 determinations yielded a mean value and standard deviation for cobalt and iron of respectively 0.014 ± 0.003 ppm and 31 ± 4 ppm instead of 0.037 ppm and 84 ppm as indicated by the IAEA. The (standard) reference materials Bowen's kale and NBS bovine liver analyzed simultaneously in the three runs yielded their correct values for cobalt and iron.

Table 3: Trace-element levels for various IAEA reference materials; average values and standard deviation expressed in ppm

element	dried animal whole blood (A-2) N=4	wheat flour (V-2/1) N=4	dried potatoes (-4) N=4	powdered milk (A-8) N=4	oyster homogenate (MA-M-1) N=6
antimony	0.045±0.016	0.008±0.002	0.005±0.001	0.006±0.003	0.11±0.05
arsenic	0.212±0.065	0.013±0.002	0.026±0.001	0.013±0.004	13±2
bromine					390±40*
cadmium	0.049±0.014	0.057±0.013	0.20±0.01	0.019±0.011	1.9±0.2*
chromium					0.65±0.45
cobalt	0.52±0.08	0.011±0.002**	0.020±0.001	0.022±0.011	0.41±0.04
copper	46±1	3.8±0.5	4.0±0.2	1.6±0.1	400±50
iron	3,200±60	32±2***	18±2	39±12	310±30
mercury	0.013±0.002	0.0022± 0.0003	0.0012± 0.0001	0.0021± 0.0003	0.18±0.04
molybdenum	0.076±0.061	0.29±0.02	0.24±0.03	0.12±0.02	0.51±0.05*
selenium	0.54±0.04	0.23±0.03	0.015±0.003	0.028±0.004	2.2±0.2
zinc	95±15	28±3	13.0±0.5	40±2	2,700±300

* N=4
** Experiments carried out later yielded 0.014±0.003 ppm (N=11)
*** Experiments carried out later yielded 31±4 ppm (N=11)

By request of the IAEA at Vienna we participated in an intercomparison of trace elements in the marine/environmental reference material oyster homogenate. The results /6/ are also presented in table 3, and agree well with the preliminary "best values" as issued by the International Laboratory on Marine Radioactivity at Monaco /12/.

In an intercomparison of trace elements in pooled human whole blood we were able to determine 13 trace elements in 1 ml aliquots /9/. For this particular material the irradiation duration was increased to 48 hours, and for the elements cobalt, nickel and molybdenum additional purification steps were inserted (c.f. section 2.7). As may be seen from table 4 the values obtained for bromine, copper, iron, mercury, selenium, and zinc agree well with Bowen's "best mean values" for trace-element levels in whole human blood /13/. However, the results for antimony, arsenic, cadmium, cobalt, molybdenum, and nickel are (considerably) lower than Bowen's "best mean values". We feel that this may be due to minimizing contamination of the 1 ml aliquots prior to irradiation on the one hand, and to a good radiochemical purity of the counting fractions on the other hand.

Table 4: Trace-element levels in a sample of pooled whole human blood

element	this study, irradiation in Spectrosil vials	ranges reported by Bowen /13/ from literature	"best mean values" as suggested by Bowen /13/
antimony	1.6 ppb	1 - 8 ppb	4.7 ppb
arsenic	1.7 ppb	2 - 800 ppb	7.7 ppb
bromine	3.7 ppm	1.3 - 10 ppm	3.3 ppm
cadmium	2.0 ppb	5 - 7 ppb	6.5 ppb
cobalt	0.4 ppb	0.2 - 200 ppb	587 ppb
copper	0.85 ppm	0.52 - 2.0 ppm	1.1 ppm
gold	0.01 ppb	0.35 ppb	
iron	460 ppm	350 - 525 ppm	464 ppm
mercury	3.8 ppb	1 - 13 ppb	8.3 ppb
molybdenum	0.3 ppb	1 - 160 ppb	1 ppb
nickel	11 ppb	20 - 400 ppb	47 ppb
rubidium*	3.1 ppm	1.2 - 6 ppm	2.3 ppm
selenium	0.11 ppm	0.07 - 0.32 ppm	0.19 ppm
zinc	5.7 ppm	3.4 - 13.7 ppm	6.5 ppm

* About 20 percent of the rubidium is present in de HAP column (see figure 1) and 80 percent in fraction 2. After decay of the sodium-24 activity in the HAP column and after absorption of fraction 2 onto a Dowex 50x8 column in 0.1 M hydrochloric acid medium, the rubidium-86 activity was measured with a Ge(Li) semiconductor detector.

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