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by

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Research Establishment Risø
Isotope Division

Abstract

A method for the simultaneous determination of arsenic, copper, manganese, selenium, and zinc in biological material was developed by the incorporation of separation procedures for copper and zinc into an existing procedure.

Investigation of the performance characteristics of the method was carried out with reference to copper and zinc. For certain materials characterized by a high Cu/Zn ratio, or a high zinc content, or both, such as liver, copper interferes in the determination of zinc thus requiring a small correction by an iterative procedure. Blank values for copper depend on the rinsing of the irradiation container, and a single rinsing with redistilled water was found superior to other rinsing procedures. Nuclear interference was negligible.

The accuracy of the method was checked by analysis of Standard Reference Materials and the precision verified by analysis of Intercomparison Samples.

Results are presented for 5 male foetuses of 3-5 months' gestational age. The distribution of arsenic, manganese and selenium is similar to that previously reported for adults. With the exception of liver, concentrations of copper in foetal organs were lower than values in the literature indicate.

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CONTENTS

INTRODUCTION

The discovery by Danks et al.[1] of a connection between low copper concentrations in plasma and the genetic defect characterized as Menkes' syndrome, led to a sudden interest in reliable determinations of copper in biological samples.

Zinc is known to interact with copper[2], resulting in symptoms of copper deficiency, and it was therefore decided to extend our current method for the determination of arsenic, manganese and selenium by neutron activation analysis[3] to include the determination of copper and zinc, so that all 5 trace elements could be determined in the same sample.

In order to minimize the effort connected with a thorough documentation and investigation of the performance characteristics of an analytical method, the previous procedure for arsenic, manganese and selenium was to remain virtually unchanged and thus its characteristics would be directly applicable to the revised method.

The starting point of the extension was therefore the copper-containing precipitate of iodides and cupferrates, from which the other 4 elements were separated. According to the separation scheme[3], this precipitate contains a dozen elements, but only a few have high effective values, in particular tungsten and antimony.

Separation of copper from these elements is achieved by precipitation with thioacetamide in ammoniacal solution[4].

Zinc is present in the separated manganese sample, but improvement of the separation procedure is required to determine this element with satisfactory precision, and it was desirable to determine the two elements in the same sample.

Simultaneous separation of manganese and zinc can be achieved by ion exchange[5] or by extraction[6-11]. In this study the procedure previously described for the determination of manganese in serum[12] by extraction with diethylammonium diethyldithiocarbamate was modified to include zinc.

Incorporation of these procedures into our previous method did not increase the time needed for separation.

ANALYTICAL METHOD

Our previous method for the determination of arsenic, manganese and selenium in biological materials calls for a one-hour irradiation of a one-gram sample followed by radiochemical separation and measurement of the

activities of ^{76}As, ^{56}Mn and ^{81m}Se using scintillation detectors. The chemical yield is determined by added ^{54}Mn, respectively re-irradiation, of the separated arsenic and selenium samples.

In addition, ^{64}Cu and ^{69m}Zn are used as indicators and the chemical yield is determined by re-irradiation of the separated copper sample and by added ^{65}Zn.

Determination of Arsenic and Selenium

No changes are required in the analytical procedure for these two elements. However, simultaneous irradiation of a bromine reference together with the 5 comparator standards was found useful when correcting for ^{82}Br at very low arsenic concentrations[12].

Determination of Copper

Copper is removed from the other elements as cuprous iodide in the precipitate resulting from the addition of potassium iodide and cupferron.

Separation of copper from antimony, as well as from traces of ^{24}Na, is achieved by dissolution of the precipitate in ammonium hydroxide and precipitation of the sulphide with thioacetamide.

Counting of ^{64}Cu is based on the 511 keV annihilation peak in spite of its poor specificity; the only γ-ray of 1345 keV is subject to strong interference from traces of ^{24}Na, unless counted with a Ge(Li) detector[13].

Determination of Manganese

Simultaneous separation of manganese and zinc can be obtained by extraction with diethylammonium diethyldithiocarbamate in chloroform from ammoniacal solution provided that the remaining hydrogen sulphide is removed before the addition of ammonium hydroxide. Satisfactory removal was achieved by passing sulphur dioxide through the solution.

The amount of added ^{54}Mn was reduced to one fifth to allow counting of the separated sample for zinc in a well-type NaI(Tl) scintillation detector.

The chemical yield was increased from 4% in the existing method to about 80%, permitting a reduction in counting time.

The 835 keV peak of ^{54}Mn is positioned on the Compton edge of ^{65}Zn requiring stripping of the 1115 keV peak of ^{65}Zn from the ^{54}Mn spectrum of the separated sample before calculation of the chemical yield.

Determination of Zinc

Only one counting of the separated sample is required to permit determination of the ^{69m}Zn indicator and the ^{65}Zn tracer.

Counting takes place in a well-type scintillation detector to keep counting time to a minimum.

The determination of zinc is not entirely interference-free. The 511 keV annihilation peak of ^{64}Cu interferes with the ^{69m}Zn indicator and the sum peak of 1022 keV interferes with the ^{65}Zn tracer.

Correction for interference is required for materials with high copper and/or zinc concentrations, such as liver. The correction is applied by an iterative procedure.

In the calculation of zinc, the same fixed boundaries are used as those in the determination of interference, instead of boundaries selected by the sign change of the first derivative.

Procedure

The present procedure is to be superimposed on our previous procedure so common features are omitted; a schematic presentation is shown in fig. 1.

Reagents

Ammonium hydroxide 25%
Ammonium hydroxide, 4M
Nitric acid 65%
Sulphur dioxide, Matheson
Chloroform
Diethylammonium diethyldithiocarbamate (DDDC)

Carriers

Mn-carrier, 10 mg/ml as Mn(II) in 4 M nitric acid, containing 0.4 µCi/ml of ^{54}Mn
Zn-carrier, 10 mg/ml as Zn(II) in ammoniacal solution, containing 0.2 µCi/ml of ^{65}Zn

Comparator Standards

Cu-comparator, 5 µg Cu/ml in 0.1 M nitric acid
Zn-comparator, 200 µg Zn/ml in 0.5 M ammonium hydroxide

The addition of nitric acid to the Cu-comparator improves the stability, so that the change of concentration is reduced from several per cent to well below 1% per month.

Also the Zn-comparator changes significantly less than 1% per month.

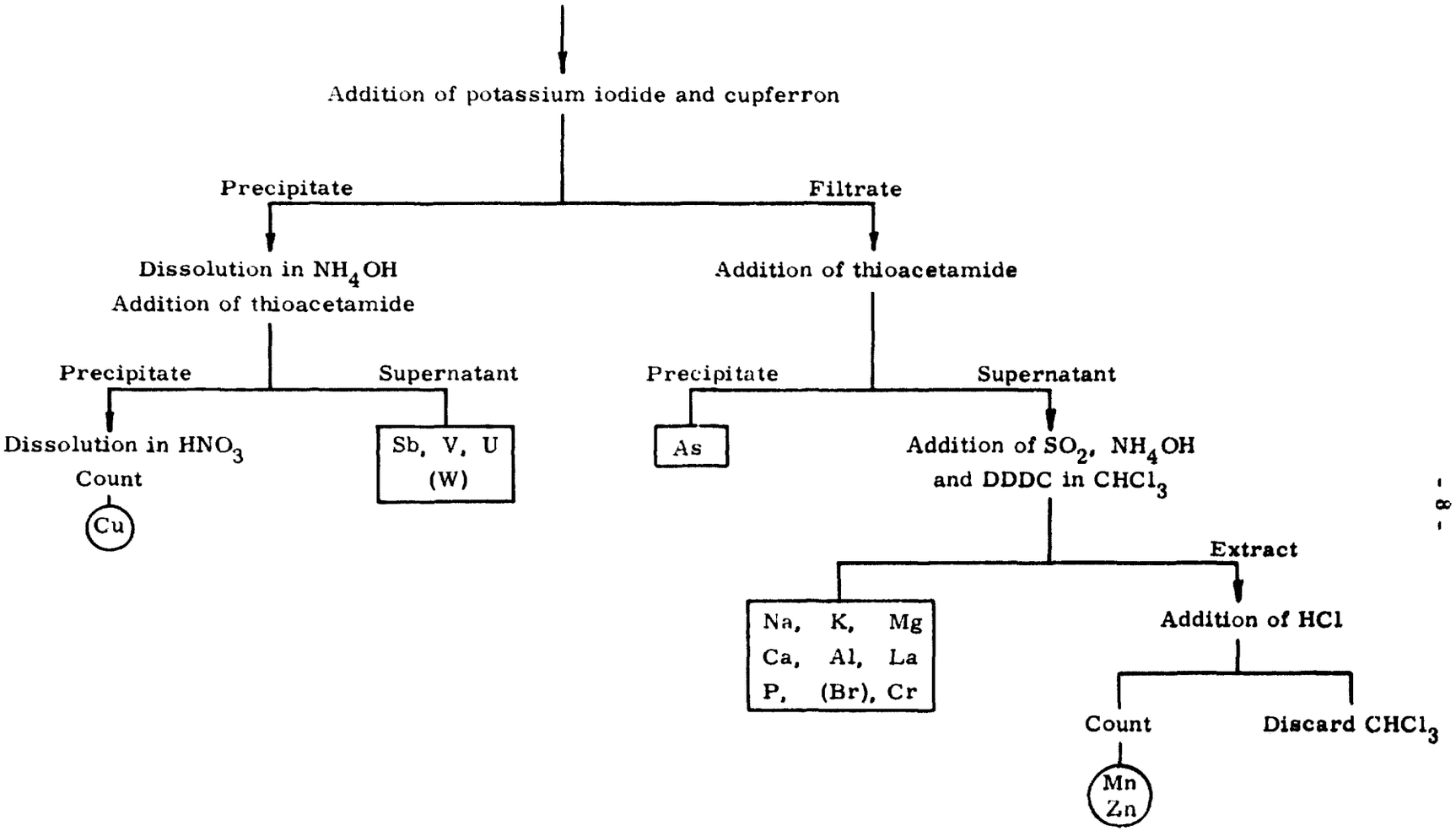

Fig. 1. Radiochemical separation scheme for copper, manganese and zinc.

Irradiation

The sample is irradiated together with the comparator standards of all 5 elements in heat-sealed, half-dram polyvials for 1 h, as shown in fig. 2. Irradiation takes place in the Danish reactor DR 2 in a thermal neutron flux density of 7×10^{12} neutrons/cm^2/s.

A ^{82}Br reference is produced by simultaneous irradiation of about 1 µg of Br as NH_4Br in a 1 ml aqueous solution.

Decomposition

The 50 ml beaker contains 0.1 ml Sb-carrier, 100 µl As-carrier, 2000 µl Cu-carrier, 100 µl Mn-carrier, 500 µl Se-carrier, 100 µl Zn-carrier and sulphuric acid. The sample is decomposed as previously described.

For yield determination, a Cu-carrier sample is prepared by transferring 1000 µl of Cu-carrier to a half-dram polyvial; separate Mn- and Zn-carrier samples are made in polyvials from 500 µl of Mn-carrier and 500 µl of Zn-carrier, respectively, both diluted to 1.0 ml.

Separation of Copper

The precipitate from the addition of potassium iodide and cupferron is washed twice with water and dissolved on the filter* in 20 ml of 4M ammonium hydroxide.

About 100 mg of thioacetamide are added to the solution, and copper is precipitated as the sulphide by gentle heating in a water bath.

After centrifugation the supernatant is discarded, and the precipitate is dissolved in 1.0 ml of nitric acid. Finally, the solution is transferred to a half-dram polyvial, which is then heat-sealed and ready for counting.

Counting of Copper-64

A comparator standard is made by transferring 1000 µl of the irradiated Cu-comparator to a half-dram polyvial.

The copper sample is counted for at least 4 min live time about 24 h after pile-out with a 3"× 3" scintillation detector at a gain of 6.7 keV/channel. The comparator standard is counted for 4 min under the same conditions.

Yield of Copper

The separated copper sample and the Cu-carrier sample set aside for yield determination are irradiated together in the reactor for 10 s.

About 24 h after pile-out each sample is counted for 4 min under the same conditions as above.

The chemical yields of copper averaged 70%.

Calculation of Result

Copper is determined from the 511 keV peak areas of sample and comparator corrected for differences in decay and counting times.

The chemical yield is calculated in the same way from the spectra of the re-irradiated sample and carrier.

The copper content, corrected for chemical yield, is converted into nanograms.

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* Munktell 20 H (recommended by Berzelius)

Separation of Manganese and Zinc

Sulphur dioxide is passed through the supernatant from the arsenic sulphide precipitation for about 5 min until a clear solution is obtained. Ammonium hydroxide is added to a pH of 7-8, and manganese and zinc are extracted simultaneously by stirring for 1 min with 5 ml of freshly prepared 3% w/v of DDDC in chloroform. The organic phase is transferred to a centrifuge tube, and the extraction is repeated with an additional 5 ml of DDDC solution.

The combined chloroform phases are washed 3 times with water and, after the addition of 1.1 ml of hydrochloric acid, manganese and zinc are back-extracted by stirring for 1 min. The acid layer is transferred to a half-dram polyvial that is then closed ready for counting.

Counting of Manganese-56

The manganese sample is counted for 4 min live time not later than 5 h after pile-out with a 3"×3" scintillation detector at a gain of 6.7 keV/channel.

Counting of Manganese-54

The separated manganese sample is counted for 20 min live time not earlier than 40 h after pile-out under the same conditions as above.

Calculation of Result

Before calculation of the chemical yield, the 1115 keV peak of ^{65}Zn is stripped from the sample spectrum of ^{54}Mn by means of a ^{65}Zn reference spectrum.

The chemical yield averaged 80%.

Counting of Zinc

A comparator standard is made by transferring 1000 µl of the irradiated Zn-comparator to a half-dram polyvial.

The zinc sample is counted for 20 min live time about 24 h after pile-out in a 3"×3" well-type scintillation detector at a gain of 6.7 keV/channel. The comparator standard is counted for 4 min under the same conditions.

Yield of Zinc

The Zn-carrier sample set aside for yield determination is counted for 4 min immediately after the comparator standard and under the same conditions as above.

The chemical yield averaged 70%.

Calculation of Result

Zinc is determined from the 438 keV peak areas of sample and comparator corrected for differences in decay and counting time. The sample area is corrected for the contribution of ^{65}Zn, and this correction is made by stripping of the 1115 keV peak of ^{65}Zn in the sample by means of the spectrum of the Zn-carrier sample. The areas are calculated over 15 channels, 7 channels on each side of the peak.

The chemical yield is calculated from the 1115 keV peak areas of sample and carrier, the sample area being corrected for ^{65}Zn formed during the irradiation. This correction is calculated from the comparator

standard. The areas are calculated over 31 channels, 15 channels on each side of the peak.

The zinc content, corrected for chemical yield, is converted into nanograms.

For materials for which correction for interference is required (p. 12), the above procedure is modified to an iteration with the following steps:

1. Calculate the error on the indicator and the error on the tracer from the copper concentration and the experimentally determined values for interference.
2. Calculate the yield assuming that the 1115 keV peak area is due to ^{65}Zn tracer.
3. Correct the ^{69m}Zn peak area for interference from the annihilation peak of ^{65}Zn.
4. Calculate the amount of zinc in the separated sample and add the error on the indicator multiplied by the chemical yield.
5. Calculate the ^{65}Zn formed during irradiation.
6. Subtract the ^{65}Zn formed from the 1115 keV peak area and correct the difference for interference on the yield.
7. Calculate the ^{65}Zn in the sample.
8. Calculate the yield and the concentration.
9. Repeat steps 3 to 8 with the new values for ^{65}Zn and yield until the change in concentration is well below the detection limit.

ANALYTICAL EVALUATION

The errors originating from systematic differences in neutron flux density between sample and comparator can be cancelled by re-irradiation yield determination in the same positions. Random errors from the uncertainty of positioning of the irradiation container during activation are unavoidable, but their magnitude should be reduced the closer the sample and comparator are to each other.

In the present case copper was chosen as the most favoured element, its comparators occupying the position closest to the sample.

Interference

For arsenic, selenium and copper, interference from other elements is only possible on the indicator of the element to be determined, while, for manganese and zinc, interference may be on the indicator as well as on the tracer used for the chemical yield determination.

The interference on the tracer is the product of the separation factor and an effective value of the interfering element that is irradiated and counted as a sample and calculated by reference to a carrier sample.

This interference can be expressed as % error of the yield per µg of interfering element, or its reciprocal value expressing the concentration of the interfering element corresponding to an absolute error of 1% of the chemical yield.

The analytical procedure for arsenic and selenium is unchanged, and consequently interference from other elements in the determination of these two elements is the same as previously reported[3].

The measurement of ^{64}Cu by the 511 keV annihilation peak is particularly susceptible to interference, not only from radionuclides emitting γ-rays with neighbouring energies, but also from all nuclides with γ-transitions greater than 1022 keV producing electron-positron pairs.

Experimentally determined interferences are therefore particularly important in this case, and effective values for 7 elements are presented in table 1.

For manganese, the effective values are slightly changed because of the reduction of the added ^{54}Mn tracer and the longer time between the first and the second counting of the separated manganese sample. Separation factors are identical to those reported for the DDDC extraction method[12] Revised interferences are listed in table 2.

Experimentally determined interferences for zinc are given in table 3.

The contribution from other elements to the analytical results is well below the a priori detection limits according to Currie[14] for all 5 elements, except that from copper to the zinc result.

Copper interference in the determination of zinc has been treated in detail elsewhere[15], and is only summarized in the following.

Correction for copper interference is required when the sum of the two errors, interference on the indicator and interference on the tracer, exceeds the detection limit for zinc. For copper and zinc concentrations in ppm, the total error (table 3) of the zinc result is

$$\text{Error, ppm} = 0.023 \cdot Cu + 3.6 \cdot 10^{-4} \cdot Cu \cdot Zn$$

This equation is used to determine whether a zinc result should be corrected for copper interference by the iterative procedure.

The copper interference is below the detection limit for zinc for all analyzed materials but liver. For human liver samples the correction is 1-3%.

Table 1

Experimentally determined interferences in copper analysis

Interfering element	Radioactive tracer	Activity μCi	Mass μg	Separation factor S	Effective value f	ppm of element ~ ± 1 ppb of Cu
Na	Na-24	•		1.4×10^{-5}	0.033	2,000
Zn	Zn-65	12	1000	1.4×10^{-3}	-0.016	40
Ga	Ga-72	200	6	3.7×10^{-1}	-0.13	0.02
As	As-76	25	••	1.3×10^{-3}	0.93	0.8
Br	Br-80m	16	1.2	5.0×10^{-5}	0.32	60
Sb	Sb-122	3	••	2.6×10^{-2}	0.27	0.15
W	W-187	10	0.1	1.5×10^{-3}	0.96	0.7

• Irradiated sample

•• Irradiated carrier

Table 2

Interferences in the determination of manganese

Interfering element	Separation factor S	Effective value indicator	Effective value tracer	ppm of element ~ ± 1 ppb of Mn	ppm of element ~ ± 1% of yield
Ga	2.7×10^{-5}	7.2×10^{-4}	0.52	50,000	70,000
Br	7.1×10^{-4}	2.8×10^{-3}	11	500	100

Table 3

Experimentally determined interferences in zinc analysis

Interfering element	Radioactive tracer	Activity μCi	Mass μg	Separation factor S	Effective value indicator	Effective value tracer	ppm of element ~ ± 1 ppb of Zn	ppm of element ~ ± 1% of yield
Na	Na-24	•		7.8×10^{-6}	-0.27	-13	500	100,000
Cu	Cu-64	5	••	1.9×10^{-3}	-12	19	0.04	30
Br	Br-80m	16	1.2	2.3×10^{-4}	-0.76	-18	6	200

• Irradiated sample

•• Irradiated carrier

Blank

The half-dram polyvials release up to a few nanograms of manganese to a water sample during irradiation, which is important in the analysis of serum[12]. Cleaning with a 3% hydrogen-peroxide solution produced the lowest and most consistent results, but the use of an alternative polyethylene ampoule with a volume of 5 ml reduced the problem to insignificance.

A similar investigation was carried out for copper and zinc. The results for copper are summarized in table 4 and show that a single rinsing with redistilled water is superior to the classic nitric acid procedure[16] as well as to the hydrogen-peroxide treatment. No zinc could be detected in the water.

The copper content in the redistilled water used in this investigation was determined by irradiation of a frozen water sample surrounded by solid carbon dioxide. After irradiation the sample was removed from the container, the surface layers were allowed to melt away, and the solid core of ice, uncontaminated by the container, was used for the analysis. The copper content was found to be 3 ng/ml. The copper blank found for polyvials rinsed with water therefore represents the actual copper present in the redistilled water and not the release of copper from the polyvial.

Nuclear Interference

For arsenic, manganese and selenium, the interference from nuclear transmutation caused by fast neutrons has been reported previously[12]. More recent cross sections for (n, p) and (n, α) reactions[17] in selenium, bromine and cobalt reduce the estimated interference to even less importance.

Nuclear interference is independent of irradiation time, and the previous experimentally determined values for iron and bromine are directly applicable to the present method.

The magnitude of interferences in the determination of copper and zinc was estimated from data given by Calamand[17], and the highest value was found for the reaction $^{64}Zn(n, p)^{64}Cu$. This interference was therefore verified experimentally.

Samples of ZnO (Analar) were irradiated together with dried copper comparator standards within and without a 0.5 mm thick cadmium box. Copper was determined instrumentally with a semiconductor detector; all ^{64}Cu was produced by the (n, p) reaction on zinc.

Table 4

Copper blank values for different irradiation containers

Irradiation container		Blank value			
Type	Supplier	Total Cu ng	Volume ml	Number of analyses	Concentration of Cu ng/ml
Polyvial		91 ± 41	1.1	10	3.4 ± 0.4
H_2O_2 cleaned	Olympic Plastic Company	105 ± 49		10	10 ± 4
HNO_3 cleaned		101 ± 15		10	33 ± 9
Polyethylene ampoule	Atomic Industrial Co.		4.5	6	7.2 ± 0.9

Table 5 shows the experimentally determined interference together with other estimated interferences based on a thermal to fast neutron ratio of 44.

On the basis of these values it was concluded that nuclear interference is negligible for the analysis of biological material.

The fast neutron cross section for $^{64}Zn(n, p)^{64}Cu$, calculated on the basis of a thermal cross section for copper of 4.4 ± 0.2 barn, was 36 ± 2 millibarn which is in satisfactory agreement with the reported value of 31 ± 3 millibarn[18].

Table 5

Interference from nuclear transmutation

Element determined	Interfering reaction	Error of 1 ppb produced by
Cu	$^{64}Zn(n, p)^{64}Cu$	7.79 ± 0.07 ppm Zn
	$^{69}Ga(n, p)^{69m}Zn$	~0.7 ppm Ga
Zn	$^{72}Ge(n, \alpha)^{69m}Zn$	~ 20 ppm Ge

PRECISION AND ACCURACY

Estimation of Precision

Random variations in neutron fluence between sample and comparators placed side by side during irradiation give rise to a standard deviation of 3 %.

While it is possible to achieve an improved a priori precision for a single element by careful positioning of sample and comparator on top of

each other, this does not apply to the simultaneous determination of several elements, where the positions of the individual standards become equivalent.

Thus, with the arrangement of sample and comparators shown in fig. 2, all 5 elements are assigned an a priori precision of $3\frac{1}{2}$ %.

Counting statistics are calculated as usual and include contributions from yield determinations by counting of ^{54}Mn and ^{65}Zn. Positrons from ^{64}Cu were found to be annihilated within the liquid samples, and no special absorber was needed. The correction of the values for zinc by the iterative procedure described did not influence the precision of the results, only their accuracy.

The overall precision of the individual results was calculated as the combined effect of counting statistics and a priori precision.

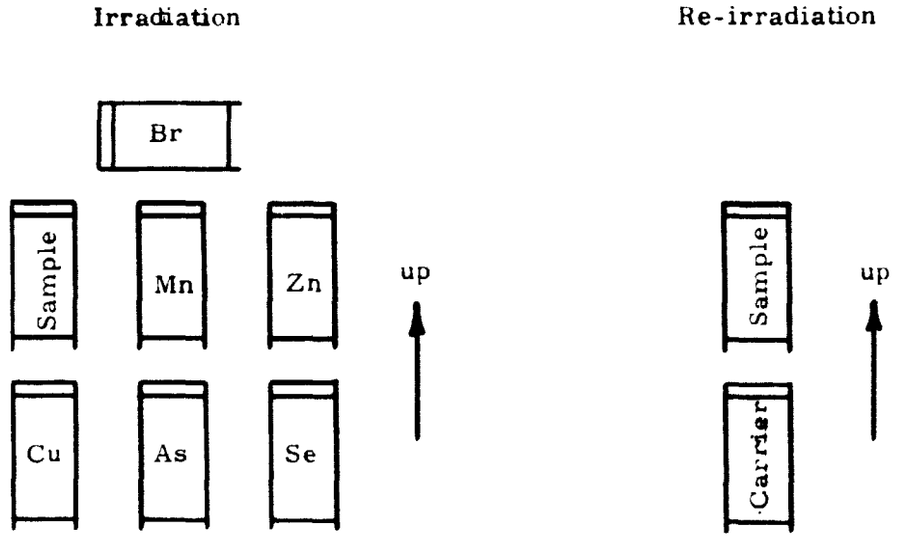

Fig. 2. Positions of sample and comparators during activation.

Analysis of Precision and Accuracy

The analysis of Standard Reference Materials permits in principle the verification of precision as well as of accuracy. However, both SRM 1571 Orchard Leaves and SRM 1577 Bovine Liver have been analyzed many times for all 5 elements, although not simultaneously, using previous versions of the present analytical method. Its consistently good accuracy was reconfirmed by one additional analysis of SRM 1577 for all 5 elements in the

same sample, which gave results in full agreement with previously reported values.

Verification of precision was performed by the analysis of Intercomparison Samples distributed by the International Laboratory of Marine Radioactivity in 1975 and by the International Atomic Energy Agency in 1976.

Results are given for Mediterranean Oyster Homogenate, Code Ma-M-1, 1975, in table 6 and for Dried Animal Muscle, Code H-4, 1976, in table 7.

Table 6

Analysis of Mediterranean Oyster Homogenate, Code MA-M-1

Element determined	Number of samples	T	d.f.	Mean value ppm
As	3	0.66	1	12.3 ± 0.3
Cu	4	2.75	2	331 ± 6
Mn	3	0.08	1	68.2 ± 1.5
Se	4	2.11	2	2.26 ± 0.08
Zn	3	0.76	1	2950 ± 75
	Analysis of Precision	6.36	7	$P(\chi^2 > T) = 0.50$

Table 7

Analysis of Animal Muscle, Code H-4

Element determined	Number of samples	T	d.f.	Mean value
As	4	2.24	3	6.7 ± 0.5 ppb
Cu	6	9.90	5	3.40 ± 0.05 ppm
Mn	4	3.25	3	447 ± 9 ppb
Se	4	2.81	3	310 ± 11 ppb
Zn	4	1.92	3	78.5 ± 1.5 ppm
	Analysis of Precision	20.12	17	$P(\chi^2 > T) = 0.27$

Table 8

Replicate analysis of beef heart for copper and zinc in µg/g wet tissue

Cu	Zn
-	20.5 ± 0.8
4.09 ± 0.15	19.1 ± 0.8
4.17 ± 0.15	21.0 ± 0.8
3.83 ± 0.14	18.6 ± 0.7
4.14 ± 0.15	21.3 ± 0.8
4.08 ± 0.15	22.3 ± 0.8
3.96 ± 0.14	22.3 ± 0.8
4.38 ± 0.16	20.2 ± 0.8
3.95 ± 0.15	21.5 ± 0.8
4.18 ± 0.15	20.3 ± 0.8
4.29 ± 0.16	-
4.11 ± 0.16	22.4 ± 0.9
4.37 ± 0.16	21.5 ± 0.9
-	19.4 ± 0.7
-	22.0 ± 0.8
4.19 ± 0.15	21.4 ± 0.8
-	21.0 ± 0.8
4.12 ± 0.15	21.9 ± 0.8
-	22.1 ± 0.8
4.09 ± 0.15	22.4 ± 0.8

Replicate Analysis of Beef Heart

No existing reference material represents actual samples, as such samples are taken by autopsy. It is therefore necessary to verify the precision of the analytical method by analyzing actual samples; in addition these are usually of considerably lower concentration than reference materials, and therefore more sensitive to absolute errors.

Such verification has already been carried out for arsenic, manganese and selenium[19], and therefore only copper and zinc are included in the present investigation.

A total of 20 samples was taken from the same beef heart and analyzed for copper and zinc by the present method. Results are presented in table 8; the missing copper results are due to leaks in the counting vial used for re-irradiation yield determination.

The Analysis of Precision (table 9) shows excellent agreement between estimated and actual variation between samples for copper thus no lack of homogeneity is observed in the material. For zinc, however, additional sources of variation were detected, and an unidentified error must be present. Similar problems were observed in the analysis of IAEA Bovine Blood, but the good agreement achieved with the certified value of 25 ppm in the analysis of SRM 1571 Orchard Leaves indicates that the absolute magnitude of the error is small; the relative value is estimated to be about 5%, which was found acceptable for the present use.

Table 9

Analysis of precision of beef heart

Element determined	Number of samples	T	d.f.	$P(\chi^2 > T)$
Cu	15	13.75	14	0.47
Zn	19	41.3	18	< 0.005

Table 10

Case No. 16 1974	Weight 247 g	CR length 155 mm CH length 225 mm	Abortion by prostaglandine		
Organ	Zinc µg/g	Copper µg/g	Selenium µg/g	Manganese ng/g	Arsenic ng/g
Liver	144 ± 5	29.5 ± 1.0	0.31 ± 0.02	437 ± 16	1.72 ± 0.19
Brain	4.6 ± 0.3	0.39 ± 0.01	0.08 ± 0.01	111 ± 4	1.35 ± 0.12
Lung	9.0 ± 0.4	0.52 ± 0.02	0.10 ± 0.01	86 ± 3	1.8 ± 0.3
Muscle and skin	-	-	-	-	-
Spleen	9.5 ± 1.0	0.62 ± 0.02	0.20 ± 0.02	137 ± 6	2.1 ± 0.8
Kidney	11.1 ± 0.5	0.74 ± 0.03	0.18 ± 0.02	215 ± 8	1.6 ± 0.3
Pancreas	23.3 ± 1.6	1.33 ± 0.05	0.18 ± 0.08	308 ± 12	1.4 ± 0.5
Intestine	11.2 ± 0.5	5.50 ± 0.20	0.14 ± 0.01	540 ± 20	1.25 ± 0.14

Table 11

Case No. 18 1974	Weight 448 g	CR length 200 mm CH length 280 mm	Abortion on mental indications		
Organ	Zinc µg/g	Copper µg/g	Selenium µg/g	Manganese ng/g	Arsenic ng/g
Liver	-	34.1 ± 1.2	0.33 ± 0.10	560 ± 20	2.1 ± 0.2
Brain	-	0.52 ± 0.02	0.09 ± 0.01	123 ± 5	0.64 ± 0.12
Lung	-	0.57 ± 0.02	0.13 ± 0.01	77 ± 3	1.5 ± 0.2
Muscle and skin	-	1.02 ± 0.04	0.09 ± 0.01	21.0 ± 1.1	1.67 ± 0.17
Spleen	-	1.15 ± 0.04	0.08 ± 0.05	119 ± 12	0.9 ± 1.0
Kidney	-	1.14 ± 0.04	0.19 ± 0.01	195 ± 7	1.6 ± 0.2
Pancreas	7.6 ± 0.8	1.60 ± 0.06	0.22 ± 0.04	165 ± 7	1.3 ± 0.4
Intestine	14.1 ± 0.6	4.18 ± 0.15	0.12 ± 0.01	950 ± 30	1.29 ± 0.19

Table 12

Case No. 98 1974	Weight 112 g	CR length 130 mm CH length 185 mm	Spontaneous abortion		
Organ	Zinc µg/g	Copper µg/g	Selenium µg/g	Manganese ng/g	Arsenic ng/g
Liver	72 ± 3	17.5 ± 0.6	0.20 ± 0.01	428 ± 17	3.44 ± 0.19
Brain	4.6 ± 0.3	0.29 ± 0.01	0.07 ± 0.01	108 ± 4	2.00 ± 0.17
Lung	10.0 ± 0.4	0.49 ± 0.02	0.11 ± 0.01	117 ± 4	2.37 ± 0.19
Muscle and skin	9.5 ± 0.4	0.45 ± 0.02	0.04 ± 0.01	78 ± 3	3.09 ± 0.19
Spleen	13.1 ± 1.9	0.90 ± 0.03	0.24 ± 0.05	87 ± 4	5.7 ± 1.1
Kidney	9.0 ± 0.4	0.80 ± 0.03	0.19 ± 0.01	260 ± 10	2.9 ± 0.2
Pancreas	12 ± 3	1.12 ± 0.04	0.05 ± 0.02	219 ± 12	10 ± 2
Intestine	10.8 ± 0.4	6.6 ± 0.2	0.14 ± 0.01	1110 ± 40	7.0 ± 0.4

Table 13

Case No. 126 1974	Weight 74 g	CR length 98 mm CH length 143 mm	Abortion by sectio parva on mental indications		
Organ	Zinc µg/g	Copper µg/g	Selenium µg/g	Manganese ng/g	Arsenic ng/g
Liver	87 ± 3	37.0 ± 1.3	0.22 ± 0.01	635 ± 25	0.49 ± 0.17
Brain	5.7 ± 0.3	0.35 ± 0.01	0.08 ± 0.01	142 ± 5	0.50 ± 0.15
Lung	-	0.35 ± 0.01	0.09 ± 0.01	85 ± 3	0.3 ± 0.2
Muscle and skin	6.9 ± 0.3	0.43 ± 0.02	0.06 ± 0.01	55 ± 2	0.41 ± 0.14
Spleen	21 ± 7	0.89 ± 0.04	0.30 ± 0.13	155 ± 10	1 ± 3
Kidney	10.9 ± 0.8	0.52 ± 0.02	0.17 ± 0.02	211 ± 8	1.3 ± 0.4
Pancreas	13 ± 9	-	0.09 ± 0.06	510 ± 25	1 ± 4
Intestine	12.2 ± 0.5	7.5 ± 0.3	0.11 ± 0.01	1180 ± 50	0.69 ± 0.15
Placenta	9.6 ± 0.5	0.76 ± 0.03	0.15 ± 0.01	78 ± 3	1.1 ± 0.2

Table 14

Case No. 127 1974	Weight 128 g	CR length 130 mm CH length 185 mm	Abortion by sectio parva on social indications		
Organ	Zinc µg/g	Copper µg/g	Selenium µg/g	Manganese ng/g	Arsenic ng/g
Liver	286 ± 13	33.2 ± 1.2	0.09 ± 0.01	880 ± 30	5.8 ± 0.4
Brain	5.1 ± 0.3	0.27 ± 0.01	0.07 ± 0.01	121 ± 5	1.9 ± 0.2
Lung	9.9 ± 0.4	0.41 ± 0.01	0.10 ± 0.01	80 ± 3	2.0 ± 0.2
Muscle and skin	16.9 ± 0.7	0.99 ± 0.04	0.07 ± 0.01	173 ± 7	3.6 ± 0.2
Spleen	17.9 ± 1.4	1.27 ± 0.05	0.16 ± 0.02	151 ± 7	3.6 ± 1.0
Kidney	11.3 ± 0.4	0.84 ± 0.03	0.12 ± 0.01	179 ± 7	2.6 ± 0.2
Pancreas	28.3 ± 1.9	1.27 ± 0.05	0.13 ± 0.03	330 ± 14	-
Intestine	19.4 ± 0.7	4.06 ± 0.15	0.18 ± 0.01	950 ± 40	3.3 ± 0.2

RESULTS AND DISCUSSION

The purpose of the present investigation was to determine the concentrations of arsenic, copper, manganese, selenium, and zinc in foetuses of 3-5 months' gestational age.

With the exception of the liver, foetal organs are not well investigated for their trace element content, because of the limited sample size and the lack of sensitivity of most classical methods of analysis. There were no really reliable control values for copper, and values for arsenic were practically non-existent.

It was therefore decided to analyze the foetal organs for which previous results were available for normal, adult individuals, but only for male foetuses that can be used as controls in cases suspected of Menkes' disease.

Samples weighed approximately 1 gram wet weight, whereever possible, and results are also given on a wet weight basis. Precision is stated as the standard deviation of each individual result, calculated as the overall effect of an a priori error of $3\frac{1}{2}$% and counting statistics. It should be noted that additional sources of variability are present for the zinc values.

The scarcity of abortions performed in normal pregnancies after 3 months' gestation made it reasonable to include samples from foetuses that were not strictly normal.

Results for 5 foetuses are presented in tables 10-14 together with other pertinent information for judging the value of the data.

With the exception of liver, results for copper are significantly lower than those reported by Fazekas et al. [20]. Other elements do not exhibit any striking differences from the results obtained for normal adults by Larsen et al. [21].

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