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AN ACCURATE PROCEDURE FOR MULTIELEMENT NEUTRON ACTIVATION ANALYSIS OF TRACE ELEMENTS IN BIOLOGICAL MATERIALS

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A procedure for radiochemical neutron activation analysis of biological materials for As, Cd, Co, Cu, Ga, Hf, Hg, Mn, Na, Sb, Sc, Se, Zn, and the rare-earth elements (REE) has been developed. Maximum control over accuracy has been emphasized. Samples are digested under reflux in the presence of carrier for every element determined, and a chemical yield is measured for every element assayed. The procedure has been tested by replicate analysis of National Bureau of Standards bovine liver sample (SRM-1577). Values agree to within their uncertainties with those given by NBS for the 9 elements of this group that they have analyzed. Accuracies at the 90% confidence level for elements in the liver sample are estimated as better than ±10% for Cu, Na, and Zn, between ±10 and ±25% for As, Co, Hg, La, Mn, and Se, and between ±25 and ±50% for Cd, Sb, Sc, and Sm.

Introduction

Concentrations of many trace elements are very low in natural materials of concern to environmental studies. Thus, extra special care must be taken to assure that such materials do not become contaminated with the elements of interest in any way that would interfere with accurate analysis. High sensitivity is required of the analytical technique to be used. In order to learn the most from inter-element correlations (which may indicate the presence and nature of a contaminant, among other things) it is essential to analyze the exact same sample for several trace elements. The level of control over the accuracy of the analysis at each step should be high so that the greatest attainable accuracy in sampling will not later be compromised by the analysis, in cases for which accuracy is important.

In an attempt to meet these criteria, we have developed procedures for the radiochemical neutron activation analysis of As, Cd, Co, Cu, Ga, Hf, Hg, REE, Sb, Sc, Se, and Zn. Problems of contamination are limited to those associated with sample acquisition and initial preparation, i.e., only those steps that must be done prior to neutron irradiation. Sample digestion is done under reflux in order to preserve rel-

atively volatile compounds. Carrier is added for every element determined before the digestion and a chemical yield is measured for each element so that the need to depend on quantitative separations is eliminated. These procedures offer tighter control over accuracy than appears possible by the procedures of Samsahl and coworkers[1,2] and provide greater accuracy and sensitivity for some elements than can be obtained by strictly instrumental methods.[3-7]

In addition, Na and Mn are determined on the same samples by instrumental neutron activation analysis.

These procedures have now been used on a variety of materials in our laboratory. They have been evaluated by repeated analyses of bovine liver, NBS standard SRM-1577. The procedures and their evaluation are given below.

Experimental

Stock solutions, carriers, standards

A stock solution containing approximately 10 mg of the element per ml is made up for each element determined. Methods used to determine the exact concentrations of the stock solutions are indicated in Table 1. The stock solutions as prepared have remained stable in concentration for a period of one year. Analytical reagent grade chemicals and reagent grade nitric, hydrochloric, and acetic acids and sodium hydroxide are used to prepare the stock solutions.

Separate solutions with approximate concentrations as shown in Table 1 are made up for carrier. That for As is made from $(NH_4)_3AsO_4$, which is acid soluble. Carriers for each chemical group (4 of which are single elements) are prepared and stored separately until they are mixed together on the day the analysis is made. Quantities of carrier for each element have been chosen to optimize the ease of chemical separation and to provide appropriate levels of radioactivity upon irradiation to provide chemical yields.

Except for the carriers for Hg and Se, the mixture of carriers used for each sample is brought to dryness in the flask to be used for the digestion of the sample. This minimizes dilution of the acid used during the digestion. Carrier in an equivalent amount for each element is added separately to the irradiated standard for each chemical group.

Standards are prepared for each chemical group by appropriate dilution of stock solutions with deionized water and enough reagent grade nitric acid to lower the pH to unity. The concentrations of the standard solutions used are given in Table 1 along with the approximate quantities of each that are weighed out exactly and packaged for irradiation. The freshly prepared standard solutions are pipetted into 9 mm o. d. fused silica tubes for irradiation, and the water is evaporated off in a clean oven at 95 °C, except for those for Hg and Se, which are lyophilized. As soon as the water has evaporated, each tube is sealed in a gas-oxygen flame, with proper care taken not to heat that portion of the tube containing the standard to a high enough temperature to volatilize any of the standard or fuse it into the wall of

the tube. A series of standards in tubes is made up when standards are freshly prepared by dilution of the stock solutions in order to avoid difficulties of storage of very dilute solutions. Note that subsequent to their irradiation, the standards for Se and Hg are removed from their tubes by washing with 6M nitric acid, then are diluted with water to 500 ml, and only 1.00 ml of that solution is used for radioassay. Quantitative removal of Se and Hg from the irradiation tubes is difficult if smaller quantities of those elements are used.

Sample preparation

A detailed discussion of sampling and sample handling for biological materials of environmental interest is well outside the scope of this work and tends to be specific to the research problem. The NBS liver sample used to test the precision of the procedures was received in a glass jar with a screw cap. This jar was placed in a clean polyethylene bag and stored in a freezer. Whenever a portion of the sample is to be used, the jar and its contents are allowed to warm to room temperature before the plastic bag is opened. The jar is opened only in a relatively clean room in which neither radioactive materials nor chemical reagents are allowed. Various portions of the sample come into contact with carefully cleaned stainless steel (a spatula), pyrex (freeze-drying apparatus and funnel), and fused silica (tube for neutron irradiation). The first samples were freeze dried in accordance with the instructions of the National Bureau of Standards, but when the weight loss was found to be negligible for samples that were removed quickly from the container and packaged for irradiation, the practice was abandoned.

Samples weighing approximately 0.125 g are placed in 9 mm o.d. fused silica (Amersil) tubes which have been closed on one end, are weighed on a clean balance, and are sealed into the tubes with a torch, care being taken to avoid any pyrolysis during the sealing operation.

Small powdered samples of USGS standard rock BCR-1 (analyzed many times in our laboratory) were used as the irradiation standards for Na and Mn. Samples and standards were assayed for those elements by counting them in the fused quartz tubes used for the irradiation. Blank levels for Na and Mn in the tubes were found to be negligible.

Irradiations

For analysis of Na and Mn, samples and standards are irradiated in the hydraulic (whale) facility of the University of Wisconsin nuclear reactor for 5 min. The reactor flux is $\sim 1 \cdot 10^{13} n \cdot cm^{-2} \cdot sec^{-1}$. For analysis of the other elements, samples are irradiated for 7 hrs in that facility. Individual lengths of Fe wire are attached to the exterior of each tube over the portion containing sample or standard in order to determine correction factors for gradients in neutron flux to the samples. Groups of samples and standards are packaged together in a single polyethylene container for each irradiation.

Table 1
Information on stock solutions, carriers, and standards

Chem. group	Element	Carrier, mg	Concentration of standard, μg/g	Standard solution used, mg	Stock solutions
I	Se	10	10,370	25	Se^{o} weigh, diss. in HNO_3
II	As	10	9.08	50	As_2O_3 weigh, diss. in NaOH
	Sb	10	0.964	50	$SbCl_3$ weigh, diss. in HCl
III	Hg	10	9,980	50	Hg^{o} weigh, diss. in HNO_3
IV	Cu	10	10.09	50	$Cu(NO_3)_2$ EDTA, PAN
V	Cd	10	9.74	50	$Cd(OAc)_2$ EDTA, PAN
VI	Zn	10	101.5	250	$Zn(OAc)_2$ EDTA, Erio-T
	Ga	1	0.571	250	$Ga(NO_3)_3$ EDTA, Pyrocatechol violet

VII	Co	10	1.055	250	$Co(OAc)_2$ EDTA, Murexide	
	Sc	1	1.57	250	$Sc(NO_3)_3$ EDTA, PAN	
VII REE	La	2	0.3000	250	REE see Ref.[10]	
	Ce	1.5	0.7237	250		
	Nd	1	0.7993	250		
	Sm	1	0.0300	250		
	Eu	1	0.01245	250		
	Gd	1	0.1981	250		
	Tb	1	0.0100	250		
	Ho	1	0.0150	250		
	Yb	2	0.02505	250		
	Lu	2	0.00500	250		
IX	Na	INAA	-	-		
	Mn	INAA	-	-		

Chemical separations

Opening of the sample and standard. Prior to the end of the irradiation, a 100 ml, round-bottom, digestion flask is prepared for each sample by pipetting into it appropriate quantities of carrier solution for each element to be analyzed. The mixed carriers, except for Hg and Se, are evaporated to dryness in a clean oven at 110 °C. Solutions of the slightly volatile Hg and Se carriers are then placed in the flask after it has cooled to room temperature. Amounts of carrier for each chemical group equal to the amounts used for the sample are pipetted into separate 40 ml centrifuge cones to receive the standards.

The wire monitor for flux gradient for each tube irradiated is removed and labelled as soon as the samples and standards are brought into the laboratory. Then each tube is carefully rinsed with 6N nitric acid, distilled water, and methanol before it is opened.

For opening, the end of each tube that contains the standard or sample is chilled in liquid nitrogen to reduce internal pressure. Each tube is wrapped in a clean paper towel and its tip is carefully broken off with a pliers. The tube is then carefully unwrapped to make sure that none of the contents of the tube (if it contained sample) have blown out onto the towel and to make sure that no large pieces of fused silica have fallen into the sample or standard.

Digestion of the samples. Each sample is slowly and carefully poured from its irradiation tube into the 100 ml digestion flask via a 30 mm glass funnel. The last traces of sample are transferred by rinsing the irradiation tube with 1 to 2 ml quantities of the acid used for the digestion, a mixture of 1 : 9 (vol) concentrated sulfuric acid to concentrated nitric acid. A disposable pipet is used in transferring the suspension to the digestion flask and a second pipet is used in washing the glass funnel. When the transfer is complete and 15 ml of acid have been added to the digestion flask, the flask is attached to the collection trap which is topped by a condenser and a bubble trap. The collection trap is supplied with a 3-way stopcock at the bottom that can be closed to collect up to 25 ml of distillate or opened either to return the distillate to the digestion flask or route it into a separate container. This apparatus is similar to that described by Gorsuch.[8] The flask is warmed to initiate and maintain the digestion reaction (gently at first to prevent foaming). The system is heated under reflux for about 5 min, then the stopcock to the trap is closed and 10 to 12 ml of distillate are collected. The system is cooled and the distillate returned to the flask. The stopcock is closed and the collection cycle repeated until the solution is clear except for a fine, white suspended solid. This usually requires four to six cycles. After the last distillation, the distillate is tapped off and an equal amount of distilled water is added to the flask through the condenser. Then, one more distillation is done to break the nitric - sulfuric acid complex. This distillate is also tapped off, leaving only 3–5 ml of liquid in the flask along with some finely divided, white solid. The mixture remaining in the flask, which contains the dissolved radioactive sample thoroughly mixed with the

carriers, all of the sulfuric acid, and some of the nitric acid, is then transferred to a 40 ml centrifuge tube with a disposable pipet. This transfer of the sample does not have to be quantitative because the thorough mixing of sample and carriers make it possible to determine an overall chemical yield for each element for this and all subsequent operations.

Group I. Se. The acid-digested sample is diluted to 10 ml, centrifuged, and decanted. The precipitate is saved, and the decantate is treated with solid hydroxylamine hydrochloride to destroy the remaining nitric acid. After the nitric acid has been destroyed, excess hydroxylamine hydrochloride reduces Se to the elemental form. This serves both to separate the Se in a pure form from the rest of the elements and as an endpoint indicator for the destruction of nitric acid. The pH during this operation is about −0.5 because of the sulfuric acid from the digestion mixture. After this solution is heated in a boiling water bath for about 10 min to convert the nearly colloidal, red, amorphous Se to the black, crystalline form (which centrifuges and filters better), the mixture is centrifuged, and the supernatant decanted. The Se is washed, filtered, and mounted for radioassay.

Group II. As, Sb. The decantate from the centrifugation of Se is combined with the precipitate obtained prior to treatment with hydroxylamine hydrochloride. Solid sodium acetate trihydrate is added to the mixture in small amounts to raise the pH to approximately 0.4, as determined by pH paper. The resulting suspension is treated with H_2S for 25 sec while being heated in a boiling water bath. More sodium acetate is added to raise the pH to unity and the H_2S treatment is repeated. This time the insoluble sulfides of Cu, Hg, Cd, As, and Sb are separated by centrifuging. The decantate now contains Zn, Ga, Co, Sc, Hf and REE. The sulfide precipitate is washed twice with water. The first wash is added to the decantate, the second is discarded.

The washed sulfide precipitate is slurried twice with hot ammonium sulfide to dissolve As and Sb. The insoluble sulfides are centrifuged away and As and Sb are reprecipitated as sulfides by addition of 12M hydrochloric acid to a pH of 4 to 5, washed with water, filtered, and mounted for radioassay.

Group III. Hg. The sulfides of Cu, Cd, and Hg are washed with 0.5M ammonium acetate and treated with 3M nitric acid to dissolve Cu and Cd. The remaining HgS is washed with very dilute nitric acid and dissolved in a small amount of aqua regia. The solution is centrifuged to remove any insoluble solid. Such solid, if present, represents a residue of material left after the digestion. The solution containing Hg is diluted to 10 ml, adjusted to pH 1 with HCl, and treated with H_2S. The mercury sulfide is washed with water, filtered, and mounted for radioassay.

Group IV. Cu. The nitric acid solution of Cu and Cd is made strongly basic with 16M NaOH, centrifuged, and the precipitated hydroxides are dissolved in the minimum amount of sulfuric acid and diluted to 10 ml. Solid sodium dithionite is

added slowly to reduce Cu to the metallic form, which is digested in a boiling water bath, centrifuged, washed with water, filtered, and mounted for radioassay.

Group V. Cd. Cadmium is removed from the remaining solution by precipitation with 16M NaOH, then dissolved in the minimum required amount of 36N sulfuric acid. The solution is diluted to 10 ml (pH ~1), treated with H_2S, and the pH raised to about 3 with NaOH to complete the precipitation. The CdS is digested by heating it in a boiling water bath, washed with water, filtered, and mounted for radioassay.

Group VI. Ga, Zn. The solution containing the carriers that were not precipitated by H_2S at pH~1 is treated as follows to remove acetate. The pH is raised to 8 with 16M NaOH, five drops of ammonium sulfide are added, and the precipitate is digested by heating in a boiling water bath and centrifuged. The decantate is adjusted to pH 12 with 16M NaOH, treated again with ammonium sulfide, digested, and centrifuged. The two precipitates thus obtained are combined and contain the sulfides or hydroxides of Co, Ga, Hf, REE, Sc, and Zn. The precipitate is dissolved in 12N HCl with addition of a few drops of 16M HNO_3. Sulfur is removed with a stirring rod and the solution is diluted to 10 ml and boiled to remove any H_2S.

To the sulfide-free solution are added about 2 ml of 16M NaOH (to a pH of about 14) to precipitate the hydroxides of Co, Sc, Hf, and the REE, but hold in solution the amphoteric Zn and Ga. After centrifuging, the solution pH is carefully lowered to 3 with 12M HCl, the solution is treated with H_2S, the pH is raised to 7 with ammonium hydroxide, and the solution is treated with H_2S again. The ZnS and $Ga(OH)_3$ mixed precipitate is centrifuged, washed with water, filtered, and mounted for radioassay.

Group VII. Co, Sc, Hf. The hydroxide precipitates of Co, Hf, REE, and Sc are dissolved in the minimum required amount of 12M HCl and diluted to 25 ml with 6M HCl. This solution is extracted twice with diethyl ether to remove Fe, which otherwise lowers the chemical yields of the REE. (The removal of Fe is deemed necessary only if that element is suspected of being present in concentrations greater than one percent.) The pH of the aqueous phase is then raised to 12 with 16M NaOH to precipitate the hydroxides of Co, Hf, REE, and Sc. The hydroxides are again dissolved in the minimum required amount of 12M HCl. To this solution are added an additional 1 ml of 12M HCl plus 15 g of ammonium thiocyanate, then the solution is diluted to about 30 ml (~0.5M in HCl, ~8M in NH_4SCN). This solution is extracted twice with diethyl ether. The thiocyanate complexes of Co, Hf, and Sc enter the ether phase. 25 ml of water are then added to the separated ether phase and the ether is evaporated away on a hot plate. The resulting aqueous solution is treated with 16M NaOH to precipitate the hydroxides, which are redissolved in 12M HCl. That solution is adjusted to pH 7–9 with aqueous ammonia and treated with ammonium sulfide. Then the resulting precipitate of CoS and the hydroxides of Sc and Hf is centrifuged, washed with water, filtered, and mounted for radioassay.

Group VIII. REE. The REE are precipitated from the aqueous phase left from the ether extraction by 16M NaOH and redissolved in the minimum necessary amount of 12M HCl. Additional HCl and water are added to adjust the pH to 1 and the volume to 3 ml. The solution is warmed, then 1.5 ml of saturated oxalic acid are added with vigorous stirring and the solution is cooled in an ice bath. The precipitated REE oxalates are washed with water, filtered, and mounted for radioassay.

Standards

Standards are rinsed from their irradiation tubes into the centrifuge cones containing the carriers for their respective groups with 4 portions of 6M nitric acid, except those for Hg and Se. Hg and Se standards are separately diluted to 500 ml with distilled water as described previously, then 1.00 ml of each is transferred to the centrifuge cone for mixing with carrier. Following mixing with carrier, the pH in each cone is adjusted so the standard for each chemical group is precipitated in the same way as was done for the sample. No special precautions are taken at this step to insure isotopic mixing, as this appears to occur readily under the conditions of acid transfer and precipitation. No effort is made to insure quantitative precipitation of the carrier and standard for any group, because a relative chemical yield for the sample and standard are obtained at the end of the analysis.

Radioassay

Preparation of precipitates. Precipitates are filtered through demountable chimneys onto circles of Whatman No. 50 filter paper supported by glass frits of medium or coarse porosity. Chimneys of 1.60 cm i.d. and 2.2 cm diameter filter disks are used for most precipitates. Larger chimneys (2.6 cm dia) and filter discs (3.3 cm dia) must be used for groups II and VII because of their more voluminous precipitates and for group VIII in order to approximate more closely a "thin" sample during reirradiation for yield determination. Precipitates are washed with water while in the filter chimney and are partially dried by drawing air through them. The filter circles with the precipitates (still slightly damp) are then sealed between two sheets of 2 mil polyethylene by use of a heat-sealing wheel. (A sheet of 5 mil teflon is inserted between the polyethylene and the wheel to prevent sticking.) Sealing the precipitates while slightly damp helps prevent crumbling during handling, which results in serious changes in counting geometry. Each precipitate, sealed in polyethylene, is placed on a cardboard counting card and covered with a small, close-fitting square of polyethylene which is taped to the card holding the precipitate package in position. Thus, after radioassay the sealed precipitate can easily be removed for neutron irradiation (yield determination) by lifting the tape and polyethylene square off the card with no risk of breaking open the sealed package.

Flux monitors

The wire flux monitors from the irradiation tubes are cleaned of the last bits of the low-Na content tape (3M-549) used to hold them in place during the irradiation, rinsed with water to remove any traces of sodium, rinsed in methanol, and air dried. Each wire is formed into a coil about 1 cm in diameter and mounted on a cardboard card with cellophane tape. The flux monitors are counted with a NaI(Tl) detector whose output is fed through a single channel analyzer for times adjusted to give well over 10,000 counts to ensure counting errors of less than 1% for this step of the analysis.

Gamma-ray spectrometry

Spectra for the element groups were taken with 20 or 30 cm^3 Ge(Li) detectors. The Ge(Li) spectrometer is described more fully elsewhere.[9, 10] Information on times and durations for counting, energy settings, nuclides and their half-lives, and gamma-ray peaks is summarized in Table 2.

Chemical yields

With the exception of chemical group V (Cd), whose precipitate is essentially opaque to thermal neutrons, chemical yields were determined by activation of the precipitates used for radioassay. Details of this procedure have been published previously.[9, 10] The Cd precipitates are dissolved in 6M HCl and diluted to 100 ml. A 2.0 ml aliquot is taken of that solution and further diluted to 100 ml. The resulting solution is analyzed by atomic absorption spectrophotometry. Table 3 contains irradiation and counting information pertinent to determination of chemical yields.

Calculations

Calculations are done as indicated in previous work.[9, 10] The method for whole peak integration (rather than for half-peak integration) gives superior performance with the Ge(Li) detectors now in use. Integration of well-isolated gamma-ray peaks is now done by a computer, whose program makes essentially the same judgements described earlier.[9, 10]

Results and discussion

Table 4 contains results of four separate determinations of NBS bovine liver sample SRM-1577. The first four columns of Table 4 contain the data from the individual analyses, which were obtained in four separate irradiations in order to give the best

Table 2
Information for radioassay by gamma-ray spectrometry

Counting group	Day of count	Duration, sec	Energy range observed, MeV	Nuclide observed	Half-life	γ-Peak used, MeV
I Se	14	20,000	1	^{75}Se	120 d	0.136, 0.265
II As	3	10,000	1	^{76}As	26.4 h	0.559, 0.657
Sb	10	20,000	1	^{122}Sb	64.3 h	0.564
			1	^{124}Sb	60.3 d	0.603
III Hg	3	20,000	1	^{197}Hg	65.0 h	0.0776, 0.0688
IV Cu	2	2,000	2	^{64}Cu	12.8 h	0.511, 1.346
V Cd	2	20,000	1	^{115}Cd	53.5 h	0.528
				^{115m}In	4.5 h	0.336
VI Zn	2	20,000	1	^{69m}Zn	14.1 h	0.439
Ga	2	–	1	^{72}Ga	14.1 h	0.630, 0.834
VII Co	14	20,000	2	^{60}Co	5.26 y	1.173, 1.333
Sc	14	–	2	^{46}Sc	83.9 d	0.889, 1.120
Hf	14	–	2	^{181}Hf	42.5 d	0.133, 0.482
VIII La	3	40,000	1	^{140}La	40.2 h	0.3288, 0.4872
Sm	3		1	^{153}Sm	47.0 h	0.1032
IX Na	1	2,000	5	^{24}Na	15.0 h	1.368, 1.732, 2.754
Mn	1		5	^{56}Mn	155 m	0.847, 1.811, 2.113

Table 3
Information about determination of chemical yields

Chem. group	Element	Duration of irradiation, min	Day of count	Duration of count, sec
I	Se	10	6	1,000
II	As	2	1	2,000
	Sb	2	1	2,000
III	Hg	2	1	1,000
IV	Cu	2	1	1,000
V	Cd	A.A.S.		
VI	Zn	10	2	2,000
	Ga	10	2	2,000
VII	Co	10	6	1,000
	Sc	10	6	1,000
	Hf	10	6	1,000
VIII	La	10	7	2,000
	Sm	10	7	2,000

possible estimate of our precision. The uncertainties accompanying the first set of numbers correspond to one standard deviation based solely on counting statistics. The average for the four analyses and one standard deviation from that average are given in column 5. Column 6 gives one standard deviation for the mean value for each element. From other work in our laboratory, we know that there is an additional uncertainty of $\sim 0.6\%$ in preparing irradiation standards that affects the accuracy of our analysis.[11] This is combined with the results from column 6 to give an estimated percent standard deviation for the accuracy of our results (column 7). The values from column 7 are then multiplied by the appropriate Student t factor to give (slightly overestimated) uncertainties in our results that correspond to a 90% confidence level (column 8). Finally, column 9 gives the ratio of our results for the various elements to those supplied by the National Bureau of Standards. A significant discrepancy is seen only for Co, for which NBS has so far provided only a

Table 4

Concentrations in ppm and uncertainties in concentration for trace elements in bovine liver NBS-SRM 1577*

Element	A (±s)	B	C	D	Average (±s)	s_m, %	s_{acc}, %	90%	Ratio to NBS
Se	1.34±0.06	1.03	1.08	1.25	1.18±0.14	6.1	6.1	14	1.07
As	0.057±0.001	0.057	0.048	–	0.054±0.005	5.3	5.3	15	0.96
Sb	0.0084±0.0004	–	0.0069	0.0118	0.009±0.003	17	17	50	–
Hg	0.0166±0.008	–	0.0161	–	0.0164±0.0004	1.6	1.7	11	1.02
Cu	187±2	185	191	189	188±3	0.7	0.9	2.1	0.97
Cd	0.27±0.02	–	0.31	–	0.29±0.03	7.0	7.0	44	1.07
Zn	127±3	146	130	141	136±9	3.3	3.4	8.0	1.05
Ga	<0.004	0.005	0.004	<0.009	~0.004				–
Co	0.233±0.009	0.22	–	0.26	0.24±0.02	4.8	4.8	14	~1.3
Sc	0.0004±0.0002	0.0006	0.0007	0.0007	0.0006±0.0001	12	12	28	–
La	0.009±0.001	0.011	–	0.009	0.010±0.001	7.1	7.1	21	–
Sm	0.00091±0.00005	0.0012	–	0.0010	0.0010±0.0002	9.1	9.1	27	–
Na	2,400±30	2,400	2,420	2,420	2,410±10	0.23	0.64	1.5	1.01
Mn	9.3±0.2	9.8	–	–	9.6±0.4	3.2	3.3	21	1.06

*Columns A, B, C, and D are results from 4 separate analyses; (±s) accompanying A is one standard deviation uncertainty in A obtained solely from counting statistics; Average is the average for the 4 results and accompanying (±s); is one standard deviation from the average; s_m is the standard deviation of the mean value; the next column is s_m multiplied by the Student t factor to give a 90% confidence interval; final column is ratio of our result to that reported by NBS.

tentative value. Otherwise, all values agree to well within our stated 90% confidence level.

No values for Hf are reported because the level of concentration for that element was below the sensitivity of this analysis. Levels of concentration for all REE except La and Sm were too low for observation, also. The value for Cu is 3% lower than that given by NBS and the 90% confidence limit allows only $\pm$2.1%, but the NBS stated uncertainty is $\pm$5.2%, which easily covers the difference between their value and ours.

For As, Sb, Se, Co, and Zn, the standard deviation from the average exceeds that expected solely from counting statistics by more than a factor of 2. In the case of As and Sb this is probably a problem of counting geometry for one of the samples (column C, Table 4) because the precipitate broke up somewhat. For the rest, combinations of small factors, including counting geometry, probably contribute to the somewhat larger errors than expected. (An analysis of the uncertainties in similar measurements for REE in powdered rock samples has been made by Kosiewicz et al.[11]). The rather high values for uncertainties for several of the elements (e.g., Ga, La, Sc, and Sm) may be associated with the very small quantities of those elements in the sample (0.0006 to 0.01 ppm). None of those elements is regarded as biologically essential, so their presence in the sample could be the result of small pieces of insoluble inorganic matter which may be inhomogeneously distributed through the sample. Such low concentrations also make the material supersensitive to contamination. Significant extents of contamination or inhomogeneity, however, would cause more drastic variations than were observed.

One reason for determining non-essential, apparently low-toxicity elements such as Ga, La, Sc, and Sm is to discover the presence of contamination by common mineral matter in the biological samples. The abundance of Ga, for example, is typically about 20 ppm in common materials at the Earth's surface. Thus, the observed concentration of $\sim$0.004 ppm limits the amount of contamination by such material to about 0.2 mg/g of the dried liver. Similar limits may be derived from concentrations of La, Sc, and Sm.

In a similar manner, limits on contamination from the spatula used to handle the samples can be obtained from the values for Co, Cu, and Mn, elements which are present (but usually only Mn intentionally) in the stainless steel spatulas used in sample transfer. Approximate concentrations of these elements in a sample of one of the stainless steel spatulas were determined to be Co, 970 ppm, Cu, 1,400 ppm; and Mn, 15,500 ppm. This gives an upper limit (from the Co value) of 0.3 mg/g dried liver for contamination by the spatula. However, Co is an essential element whose concentration in the liver is not expected to be unobservably low. Furthermore, the ratio of Co : Mn in the sample is a factor of two lower than that of the spatula, indicating that even if all the Co determined in the sample came from the spatula, not all of the Mn did (assuming no preferential loss of Mn over

Co from the spatula). The concentration of Cu in the sample is far too high for that element to have come from contamination by the spatula.

There is, of course, no reason to presume that any of the elements present in the liver sample is there as a result of contamination. The above examples merely illustrate the use of trace element data in determining upper limits for amounts of potential contaminants whose chemical characteristics are approximately known.

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