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Neutron activation analysis with pre- and post-irradiation chemical separation for the value assignments of Al, V, and Ni in the new bovine liver SRM 1577C

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Abstract Instrumental neutron activation analysis as carried out at the National Institute of Standards and Technology (NIST) is inadequate for determining Al, Ni, and V at the levels found in the newly prepared Standard Reference Material® (SRM) 1577c Bovine Liver. To overcome shortcomings in the value assignment, the authors initiated a cooperative approach using NAA with previously established chemical separation procedures and with significantly different neutron energy spectra to determine Al and V with pre-irradiation separation of the elements at NIST, and V and Ni with post-irradiation separation at the Nuclear Physics Institute Řež. The determinations were confirmed with the analyses of several SRMs. The work supported the certification of mass fraction values for V and Ni in SRM 1577c.

Keywords Blank contributions · Bovine liver · Low-level trace elements · Standard addition · Standard Reference Material · Value assignment

Introduction

The National Institute of Standards and Technology (NIST) has prepared a freeze-dried powdered bovine liver tissue material to renew the supply of the well-known Bovine Liver Standard Reference Material® (SRM). The collection and preparation of this material was performed under strict contamination control resulting in an SRM with very low levels of some trace elements typical for uncontaminated biological tissues [1]. The analytical approach to the value assignment in the certification process had to address the challenges of low level trace element determinations, the changes in available analytical techniques compared with those available for use in the certification of the original bovine liver SRM, and the addition of new elements of interest. The Centers for Disease Control and Prevention (CDC) have identified ten elements of interest in their efforts to monitor population health and nutritional status (National Health and Nutrition Examination Survey) which together with a selection of elements with known toxicological effects formed the target for the analytical characterization.

Three of the elements from CDC's priority list, Al, Ni, and V, are present at levels well below those found in previously investigated tissue samples and constitute a challenge for most analytical techniques. In the process of value assignment for the Certificate of Analysis (COA) of SRM 1577c it became apparent that analytical values for these elements did not meet all NIST criteria for certification. The initial value assignment for Al, Ni, and V could only be based on the combination of results from inductively coupled plasma mass spectrometry (ICP-MS) and graphite furnace atomic absorption spectrometry (GFAAS) determinations for Al (600 μg/kg), and only ICP-MS for Ni (40 μg/kg) and V (10 μg/kg). These results did not allow a full estimate of uncertainties since GFAAS was operating near the procedural detection limit and there are no suitable control materials available.

The conventional comparator instrumental neutron activation analysis (INAA) method as carried out at NIST

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is inadequate for determining Al, Ni, and V at the levels found in the SRM 1577c. The insensitivity for Al and V results from the interference by the $^{31}P(n,\alpha)^{28}Al$ nuclear reaction during irradiation and from the high matrix activity, while the low fast-neutron flux at the NIST Center for Neutron Research (NCNR) prevents the determination of Ni via the reaction $^{58}Ni(n,p)^{58}Co$ even if radiochemistry is considered. To overcome these shortcomings in the value assignment, the authors initiated a cooperative approach to determine Al and V with pre-irradiation separation neutron activation analysis (PNAA) at NIST, and Ni and V with post-irradiation radiochemical separation (RNAA) at the Nuclear Physics Institute Řež (NPI).

Experimental

PNAA determination of aluminum and vanadium

PNAA was carried out with bovine liver samples and control samples by Chelex column separation of the metal elements from the matrix elements followed by INAA of the columns [2]. This procedure was recently modified, including the use of microwave sample digestion and smaller sample sizes, which resulted in a corresponding decrease in the amount of resin and reagents used [3]. Higher sensitivity obtained through high-rate counting techniques [4] in the INAA portion of the determinations permitted use of these smaller sample sizes.

Prior to processing the certification and control samples, test samples for the total procedural blanks for each of the designated digestion vessels were processed in a class 10 clean room. Twelve clean microwave vessels were processed to assess potential residual blanks in each vessel as well as the contamination risk for the entire process. The samples were taken from three bottles of SRM 1577c. The control materials were samples of SRM 1643e Trace Elements in Water and SRM 1598a Animal Serum. In addition a dilute solution of Al (2.080 mg/kg) and V (105.6 μg/kg) was prepared from SRM 3101 and SRM 3165 to be used in a standard addition experiment. This solution was added at two increasing mass levels to SRM 1577c samples, and 0.1 g samples of the solution were processed as well. The digestions were carried out in batches of six digestion vessels; a total of seven batches were processed.

Samples, controls and procedure blanks were separated with Chelex 100 (Bio-Rad, Richmond, CA)[1] 200–400 mesh resin prepared from suspension in water as 25 mm high and 3 mm diameter columns. Metals are retained by the Chelex resin and the matrix elements are eluted with 2 mol/L ammonium acetate, followed with high purity water. The columns were allowed to sit uncovered on a Class 10 clean bench for 3 weeks until the resin bed dried completely. When dry, the resin shrank into a solid 15 × 2 mm plug that could be easily removed and packaged for INAA.

The dry Chelex columns were loaded without any additional implements under Class 10 airflow into acid-cleaned micro-centrifuge vials that had been cut in length to the diameter of the irradiation rabbits. The vials were closed with their snap cap top and on the bottom with Parafilm. The standards were taken from previously prepared multi-element solutions and pipetted (10 mg) on disks of 8 mm diameter Whatman Grade 41 filter paper. After air-drying, the standards were sealed in envelopes made from linear polyethylene (LPE). Titanium flux monitor foils were 3 mm (1/8″) diameter, 0.1 mm thick, and aluminum foils added as standards were 3 mm (1/8″) diameter 0.025 mm thick; they were loaded into LPE envelopes.

For the irradiations, each resin column tube was placed into the center of the rabbit perpendicular to its axis via a holder made from polyethylene foam. Slots on the anterior and posterior side of the bore for the tube held the standards on one side and the flux monitor on the opposite side, alternating the positions of standards and flux monitors from posterior to anterior and vice versa for each individual irradiation to exactly evaluate and correct the flux gradient [5]. Forty-four irradiations were carried out in this configuration in the RT-2 pneumatic facility of the NCNR at a thermal neutron fluence rate of 3.2×10^{13} cm^{-2} s^{-1} for 300 s. For the quantitative assay one 300 s count was carried out for each of the samples starting after 120 s decay, followed by a 300 s count for the standards on a coaxial high purity germanium detector (HPGe) (43% relative efficiency, 1.75 keV FWHM resolution for 1332.5 keV) at 5 cm distance from the detector cap. The flux monitors were counted for 120 s on a second comparable detector.

RNAA determination of V

The reaction $^{51}V(n,\gamma)^{52}V$ with thermal neutrons followed by radiochemical separation of vanadium by solvent extraction as described earlier [6] was used for V determination in the new SRM 1577c, and SRM 1515 Apple Leaves and RM 8433 Corn Bran quality control samples. Samples weighing 200–250 mg were irradiated in sealed pre-cleaned polyethylene vials in an experimental reactor (denoted LWR-15) of the NPI at a thermal neutron fluence

[1] Certain commercial equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

rate of 4×10^{13} cm^{-2} s^{-1} for 120 s. The samples and a V standard were irradiated individually with fluence rate monitors (10 μg of Au deposited on a disk of chromatographic paper Whatman Grade 1).

The samples were wet-ashed in the presence of weighed aliquots of ^{48}V tracer solution (50 μL) for chemical yield determination and 50 μg of V carrier. Subsequently, V^{5+} was extracted with a 0.2% solution of *N*-benzoyl-*N*-phenyl hydroxylamine in toluene for 30 s. Finally, the organic fraction was scrubbed with 5 mol/L HCl prior to counting. The whole separation procedure was completed within 6–8 min and its chemical yield was in the range of 95–98% as found by measurement of the ^{48}V tracer activity.

Counting of the 5 mL separated fractions, V standard, and ^{48}V tracer standard in 30 mL PE vials was carried out with a coaxial HPGe detector (23% relative efficiency, 1.8 keV FWHM resolution for 1332.5 keV) for 420 s. The separated fractions and V standards were measured on the detector cap. The 1434.1 keV gamma-line of ^{52}V was used for quantification of V, whereas the 983.5 keV and 1312.0 keV gamma-lines of ^{48}V were employed for determination of the chemical separation yield.

RNAA determination of nickel

The reaction ^{58}Ni(n,p)^{58}Co with fast neutrons followed by ion exchange chromatography and precipitation procedure of the product nuclide as described earlier [7] was used for SRM 1577c and the control materials SRM 1548a Typical Diet and RM 8433 Corn Bran. Irradiations of 250 mg samples in sealed pre-cleaned synthetic silica ampoules (Suprasil AN, Heraeus) were carried out in the LWR-15 reactor at a fast neutron fluence rate of 3×10^{13} cm^{-2} s^{-1} for 72 h. The samples were irradiated together with blank vials and a vial with a Ni standard. Each vial carried an iron wire for neutron flux monitoring.

The irradiated samples were cooled for 7–10 days, then the vials were cleaned externally by boiling in aqua regia and rinsed with water. The vials were cooled in liquid nitrogen, wrapped in tissue paper, inserted into a polyethylene bag with flap and crushed. The samples and silica fragments (plus tissue paper) were processed together. The blank ampoules were treated in the same way as the vials with the samples.

Wet ashing was carried out in the presence of weighed aliquots of ^{57}Co tracer solution (50 μL) for chemical yield determination and 100 μg of Co carrier solution. The residue was taken up in about 5 mL of 9 mol/L HCl and quantitatively transferred to an anion exchange column of Dowex 1 × 8, Cl^- form, 100–200 mesh resin. The cobalt fraction was eluted with 40 mL of 4.5 mol/L HCl and evaporated to a small volume. To achieve the highest counting efficiency on a coaxial HPGe detector, a thin flat source of separated cobalt fraction was prepared by precipitation of $Co(OH)_2$. The precipitate was filtered off forming a thin disc with a 22 mm diameter, which was sealed into a PE envelope for counting. The chemical yield of separation was in the range of 87–95% as ascertained by measurement of the ^{57}Co tracer activity.

Counting of the separated fractions and Ni standard (^{57}Co + ^{58}Co) was carried out with a coaxial HPGe detector (53% relative efficiency, 1.8 keV FWHM resolution for 1332.5 keV) for 10 h. The separated fractions and Ni standard were measured on the detector cap. The 810.8 keV gamma-line of ^{58}Co was used for quantification of Ni, whereas the 122.1 keV gamma-line of ^{57}Co was employed for determination of the chemical separation yield.

Results and discussion

Results from the PNAA procedure are presented in Tables 1, 2, 3, 4. All values are corrected for the irradiation and counting geometry differences between samples and standards. Tables 5 and 6 present the results from the RNAA determination of V and Ni. Where available, results determined in SRM control materials are compared with the values in the Certificate of Analysis (COA) [8].

Aluminum

A high potential for contamination with aluminum exists in all chemical procedures. In the PNAA procedure an approximately 1 in 20 chance for contamination was observed. The results in Table 1 reveal a blank of <16–70 μg/kg. However, several elevated Al results were

Table 1 PNAA determinations of process blanks (5 or 7 mL HNO_3) in each vessel carried through digestion and separation (uncertainties are 1 sigma counting statistics)

Material	TFA vessel #	INAA ID	ng Al on column
Process blank + H_2O	1	PNAA01	<7.8
Process blank	3	PNAA30	<13
Process blank	5	PNAA11	15.4 (4.3)
Process blank	7	PNAA20	<15
Process blank	8	PNAA25	<7.9
Process blank	18	PNAA16	17.8 (2.9)
Process blank	20	PNAA05	<8.4
Process blank	25	PNAA18	22.9 (3.8)
Process blank	27	PNAA28	32.9 (3.6)
Process blank	28	PNAA27	30.2 (4.2)
Process blank	45	PNAA02	24.7 (7.1)
Process blank + H_2O	47	PNAA17	14.6 (3.1)

Table 2 PNAA determination of Al, V, and Cu in SRM 1577c, values based on dry mass, Al values corrected for average blank

INAA-ID	Vessel #	Al (μg/kg)		V (μg/kg)		Cu (mg/kg)	
		Value	1 sigma	Value	1 sigma	Value	1 sigma
BL1444A	7	97.5	9.9	6.95[a]	0.92	238.37[a]	0.71
BL1444B	20	106.7	16.1	7.00	0.95	282.97	0.82
BL1444C	47	183.6	14.8	7.18	0.96	291.06	0.84
BL1444D	8	123.6	9.3	5.92	0.59	264.64	0.89
BL556A	8	1246.8[a]	24.5	6.84	1.03	253.31	0.75
BL556B	28	49.4	22.2	8.44[a]	1.12	227.21[a]	0.74
BL556C	5	55.2	13.0	9.42	1.04	288.21	0.84
BL556D	18	276.1	11.6	6.30	0.56	264.59	0.89
BL940A	3	297.8	17.2	10.15	0.97	258.12	0.76
BL940B	18	115.9	14.5	6.74	0.93	269.29	0.79
BL940C	45	89.8	16.1	11.69	1.33	283.89	0.84
BL940D	3	349.1	16.8	6.93	0.78	240.09	0.83
BL940E	28	278.2	11.4	7.05	0.66	265.42	0.90
Mean ± S.D.		164 ± 104		7.75 ± 1.8		269 ± 16	
Recommended value		<200		7.8 ± 2.2		269 ± 25	

Uncertainties are 1 sigma counting statistics and expanded uncertainties with a coverage factor of 2 for the recommended values. The assigned SRM 1577c COA value for Cu is (272.5 ± 4.6) mg/kg

[a] Not included in average, see discussion

Table 3 PNAA of standard addition samples, the difference between measured value and spike reflects the base amount contained in the sample

Sample	Al			V		
	Spike (μg/kg)	Measured value (μg/kg)	Base value (μg/kg)	Spike (μg/kg)	Measured value (μg/kg)	Base value (μg/kg)
Spike Soltn. A	2080	2366	286	105.6	99.5	−6.1
Spike Soltn. B	2080	2066	−14	105.6	96.7	−8.9
BL1444E	521	491	−30	26.84	32.6	5.7
BL556E	609	706	97	30.65	36.7	6.1
BL1444F	1033	1133	100	52.38	64.7	12.3
BL556F[a]	1200	544	−656	60.92	60.9	0.0

Values for Al are corrected with the average process blank (15 ng per processed sample)

[a] Incomplete digestion

found later in the samples and controls. These erratic high values are due to occasional contamination from airborne particles and are omitted from all considerations. No measurable blanks were found for V or Cu, the latter element is determined concurrently with the PNAA procedure. The correction of Al with an average value of 30 μg/kg may have resulted in over- as well as under-correction of the measured values, therefore only a probable value of <200 μg/kg is estimated. This value is also supported by the standard addition experiment. Table 3 lists these results, showing the spike amounts in the processed solutions and samples; the resulting sample values for Al fall in the range of the probable value. One sample was omitted because it was not completely digested. These observations contradict previously reported values of about 600 μg/kg, and consequently a certified value could not be assigned with the currently available results.

Vanadium

The PNAA values for V appear to be consistent throughout the samples. The values for V are confirmed with the standard addition experiment and found in good agreement with the RNAA determinations.

Nickel

The RNAA results for Ni were obtained after subtraction of a Ni blank value of 3.9 ± 0.1 ng per vial ($n = 3$), which resulted from wet ashing of the irradiated samples together with the vial splinters. Although the blank value formed almost a half of the Ni signal in the SRM 1577c, its subtraction did not significantly increase the combined relative uncertainty of Ni values in this and other materials analyzed, because its variation was reasonably low.

Table 4 PNAA determination of Al, V, and Cu in control samples, blank corrections based on average are applied to Al values

SRM 1598a		Al (μg/L)		V (μg/L)		Cu (μg/L)	
INAA-ID	Vessel #	Value	1 sigma	Value	1 sigma	Value	1 sigma
1598a_SA	47	N.D.		1.85	0.17	1549	22
1598a_SB	25	13.79[a]	1.67	2.13	0.16	1563	19
1598a_SC	1	2.08	0.58	1.84	0.07	1520	11
1598a_SD	25	4.37	0.56	1.91	0.05	1652	11
Mean ± S.D.		3.2 ± 1.6		1.93 ± 0.14		1571 ± 57	
COA value		2.3 ± 0.6		1.88 ± 0.11		1580 ± 90	
SRM 1643E		Al (μg/kg)		V (μg/kg)		Cu (μg/kg)	
INAA-ID	Vessel #	Value	1 sigma	Value	1 sigma	Value	1 sigma
1643E_SA	1	565.2[a]	7.2	36.22	0.50	N.D.	
1643E_SB	27	125.6	2.5	39.30	0.30	N.D.	
1643E_SC	25	143.3	8.4	34.48	0.77	N.D.	
1643E_SD	47	825.6[a]	15.0	36.42	0.93	N.D.	
Mean ± S.D.		134 ± 12		36.6 ± 2.0			
COA value		138.33 ± 8.4		36.93 ± 0.57		22.20 ± 0.31	

Uncertainties are 1 sigma counting statistics and expanded uncertainties with a coverage factor of 2 for the certified values

[a] Not included in average, see discussion

Table 5 RNAA determination of Ni content in biological reference materials in μg/kg, dry mass

Sample	SRM 1577c		SRM 1548a		RM 8433	
	Value	1 sigma	Value	1 sigma	Value	1 sigma
1	42.3	3.4	382	12	220	6
2	46.0	3.2	352	11	147	4
3	46.5	3.8	344	11	170	5
4	46.5	3.7	NA		NA	
5	38.8	3.7	NA		NA	
Mean ± S.D.	44.0 ± 3.4		359 ± 20		179 ± 38	
COA value			369 ± 23		158 ± 54	

Combined 1 sigma uncertainties due to counting statistics of the analytical radionuclide, radiotracer, and blank subtraction are given for the results of this work and expanded uncertainties with a coverage factor of 2 are given for the certified values

NA not analyzed

Table 6 RNAA determination of V contents in NIST biological reference materials in μg/kg, dry mass

Sample	SRM 1577c		SRM 1515		RM 8433	
	Value	1 sigma	Value	1 sigma	Value	1 sigma
1	9.2	0.6	249	4	4.5	0.4
2	7.6	0.5	235	4	4.6	0.4
3	7.9	0.5	246	4	5.5	0.5
4	9.5	0.6	NA		NA	
5	7.8	0.5	NA		NA	
Mean ± S.D.	8.4 ± 0.9		243 ± 8		4.8 ± 0.6	
COA value			260 ± 30		5 ± 2	

Combined 1 sigma uncertainties due to counting statistics of the analytical radionuclide and radiotracer are given for the results of this work and expanded uncertainties with a coverage factor of 2 are given for the certified values

NA not analyzed

Quality assessment

The PNAA procedure carried out in the clean room could not use radiotracers for yield determinations. However, the concurrent determination of Cu, which was previously determined by INAA, gives indication on the quantitative nature of the process with the exception of two processed samples where residues were observed in the irradiation container. Excluding these in the average, agreement is found with the COA value. The accuracy of results is further supported with the agreement of the measured values in the various SRMs with their respective COA values. The RNAA procedures deliver similar accuracy based on the results obtained for the SRM control samples.

Conclusions

The inclusion of PNAA and RNAA procedures for the determination of Al, V, and Ni was essential to meet NIST criteria [9] for value assignment. This work showed the importance of control and determination of blanks in all

chemical procedures when investigating these low natural levels. However, the reported values for Al could not be confirmed. Further investigations are required to establish a presumed value of 200 μg/kg or less for Al. Combining the values [10] found in this work with ICP-MS values obtained previously resulted in a NIST certified value of (8.17 ± 0.66) μg/kg for V and (44.5 ± 9.2) μg/kg for Ni. Although labor-intensive, the combination of chemical procedures with neutron activation analysis can elucidate problems and provide critical information for benchmark values.

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