



Determination of aluminum in bovine liver SRM 1577c by Instrumental Cold Neutron Activation Analysis

Rolf Zeisler¹ · Danyal Turkoglu² · Nick Sharp¹ · Heather Chen-Mayer¹ 

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Abstract

Instrumental neutron activation analysis may need to correct for matrix interferences caused by fast neutron induced threshold reactions. The very low Al mass fraction in bovine liver Standard Reference Material (SRM) has eluded certification due to such complications. Cold neutron beam irradiation absent fast neutrons provides a possible alternative, which has been applied for the first time to determine Al in bovine liver SRM 1577c. The Al mass fraction was determined to be (0.80 ± 0.15) mg/kg using the traditional single peak-fitting method, and (0.78 ± 0.23) mg/kg using a new physics model-based spectral fitting method.

Keywords Activation analysis · Cold neutron · Gamma ray background · Low Al concentration measurement · VRF fitting

Introduction

Although aluminum has no known biological function, it is a well-established neurotoxin that has controversially been implicated as a factor in neurodegeneration diseases such as Alzheimer's [1]. Therefore, its determination is commonly considered in NIST biological standard reference materials (SRMs) that serve diagnostic, nutritional, and toxicological measurements in medical, veterinary, and environmental sciences. Al is assayed in instrumental neutron activation analysis (INAA) via detection of 1.7789 MeV gamma rays that are emitted following the ^{28}Al β^- decay with a 2.24 min half-life ($T_{1/2}$). Quantification of Al by INAA, although sensitive to Al at mg/kg mass fractions, is susceptible to erroneous results due to interferences in samples that contain Si and P, which produce other pathways to ^{28}Al (Fig. 1). For

example, assuming a 1.18% mass fraction of P in the 1577c material, INAA in one of our irradiation facilities would produce 0.14 mg/kg excess Al during irradiation. This value would not be able to be corrected without knowing the exact amount of P in the sample (not detectable by INAA) and would cause an obvious bias on our results of 0.80 mg/kg (effectively a +18% bias).

The Al mass fraction eluded certification in the most recent bovine liver tissue SRM 1577c [2] due to the lack of agreement between independent measurements as required. For Al, one of the independent measurements is normally INAA in order to provide a value assignment. A “pre-irradiation separation NAA” (PNAA) method [3] with modified digestion procedure and smaller sample sizes was necessary to reduce P in the sample for the determination of Al. However, the corrections for blank contributions from the pre-irradiation procedure add uncertainty to the result. The characterization of Al and V in SRM 1577c could not proceed without PNAA [3]. Therefore, at present, direct INAA for Al certification is not feasible, mainly due to the inability to quantitatively control the pre-separation INAA necessitated by the presence of interfering reactions created by in-reactor irradiation.

Irradiating bovine liver samples with cold neutron beams, termed Instrumental Cold Neutron Activation Analysis (ICNAA), avoids triggering the reactions that cause these interferences, and therefore is a good candidate method to solve the problem. However, various

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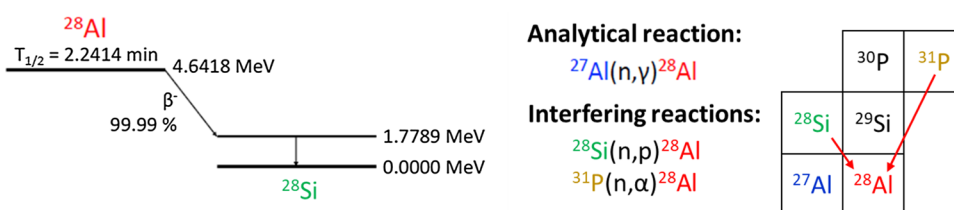
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✉ Heather Chen-Mayer
chen-mayer@nist.gov

¹ Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD, USA

² Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD, USA

Fig. 1 Al β^- decay scheme and interfering reactions from fast neutron irradiations



attempts of in-situ ICNAA at cold neutron prompt gamma activation analysis facilities, including the NIST Cold Neutron Prompt Gamma Activation Analysis (CNP-GAA) station at Neutron Guide D (NGD), suffered from high ^{28}Al background in the sample environment. With the recent implementation of a high-efficiency detector array in the vicinity of the CNP-GAA station, along with a newly installed neutron beam chopper for precise timing control, a semi-automatic ICNAA procedure was developed in this work.

Experimental

The CNP-GAA instrument at the NIST Center for Neutron Research (NCNR) is located at the end of NGD. CNP-GAA has been an important measurement technique for the certification of NIST SRMs and for characterizing elemental compositions in serving the neutron scattering community and industrial interests [4, 5]. The ICNAA measurement was carried out at the beam entrance port to the CNP-GAA sample chamber to obtain maximum neutron beam flux on the sample.

Establishing an ICNAA procedure at NIST was enabled by the following:

- The high thermal-equivalent neutron fluence rate ($6 \times 10^9 \text{ cm}^{-2}\text{s}^{-1}$) at the CNP-GAA instrument, with a nominal beam diameter of 2 cm with some divergence broadening;
- Wavelength spectrum peak at 0.4 nm, a clean cold neutron beam with insignificant fast components (which mainly comes from the ^6Li glass collimator, estimated to be about $10^5 \text{ cm}^{-2}\text{s}^{-1}$ at the current sample position based on an earlier study [6]);
- A high-efficiency detector array of high-purity germanium (HPGe) detectors, initially comprised of four detectors [7] and later two detectors at the minimum sample-to-detector distance for increased detector efficiency (see Fig. 2), located at the CNP-GAA station in a low-background environment;
- Software (qpx [8]) that facilitated data acquisition from multiple detectors;

- A fast beam shutter (in addition to local shutter) that provided precise timing for the irradiations [9].

The samples were prepared by loading 700 mg of bovine liver into a 20 mm diameter die and then pressed to create a 2 mm thick pellet. Multi-element standards for Al, Cu and Mn were prepared from high-purity metal standards dissolved into solution and deposited on filter papers and pressed into 20 mm pellets with 2 mm thicknesses. The sample and standard pellets were then contained within clean thin-film polyethylene bags. These were positioned on the upstream side of Ti-foil flux monitors in a 3-D printed plastic sample holder (Fig. 2) at the beam entrance port to the CNP-GAA sample chamber.

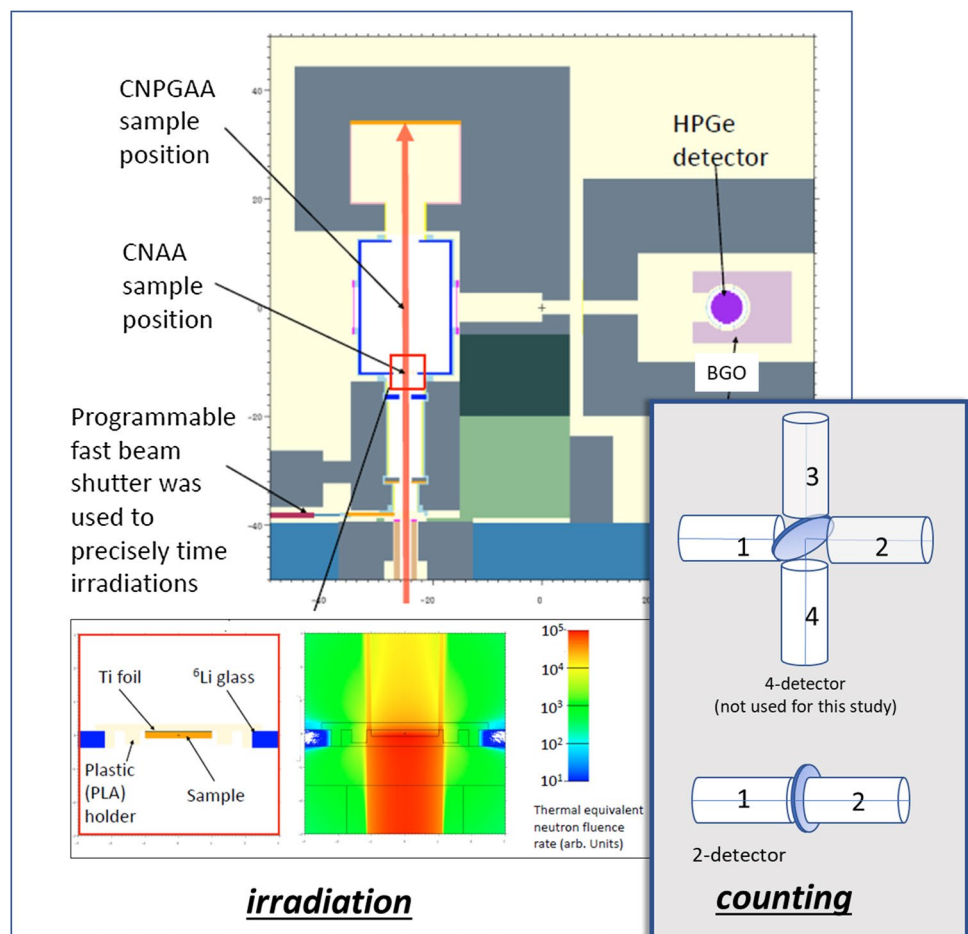
The following samples were measured:

- 7 samples of SRM 1577c, pressed pellet (2 mm thick \times 2 cm diameter) average mass about 700 mg, estimated density 1.15 g/cm^3 .
- 50.2 mg of multi-element standards (SRM 403a) containing Al (476.2 mg/kg), Mn (124.1 mg/kg) and Cu (2789 mg/kg) on filter paper, pressed into pellets with a resulting density closely matching the SRM 1577c samples.
- Blank cellulose pellet 2 mm thick \times 2 cm diameter.
- Background (empty sample holder).

Monte Carlo radiation transport simulations with Monte Carlo N-Particle 6 (MCNP6) software* [10] show that the neutron fluence rate has a buildup in the bovine liver pellet's upstream surface and is diminished on the downstream side with the Ti foil (Fig. 2). Although the samples and standards were approximately matched in mass and matrix, experimental results were normalized by activity of the Ti foil to account for different bulk fluence rates across the different samples.

For maximum signal-to-noise ratio for assaying Al, samples were irradiated for precisely 300 s, timed with the newly installed fast beam shutter. This allowed near saturation of the Al activity with minimal activation of Mn, Cu, Na, Cl, etc. The manual transfer and counting sequence are as follows:

Fig. 2 Schematic illustration of the CNPGAA instrument and the ICNAA irradiation setup, along with MCNP6 simulations of neutron beam attenuated by the bovine liver pellet before reaching the Ti foil, indicating the need for normalization to account for variation of bulk fluence rates from sample to sample. The off-beam counting geometry is shown in the inset, where a previously developed 4-detector array was rearranged into a 2-detector assembly for closer sample distance and, therefore, overall higher efficiency



- Retrieve sample following the 300 s cold neutron beam irradiation.
- Transfer sample to unirradiated PE or Teflon bags, heat seal, place bag in Petri slide.
- Insert Petri slide into the dual detector gamma ray spectrometer within 80–100 s and count for 300 s.
- Remove sample, insert the Ti foil and count for 120 s.

Since the two detectors were gain-matched prior to the experiment, the spectra from the two detectors were summed together. Data analysis was performed using commercially available software (Genie-VMS¹*[11]). Gamma ray attenuation is not calculated, and because there are two detectors on either surface of the sample disk (2 mm thick), it is assumed to be negligible.

Because of the very poor signal-to-noise ratio of the Al peak, subsequently the data were re-analyzed using the VRF software *[12] to assess possible dependence of the

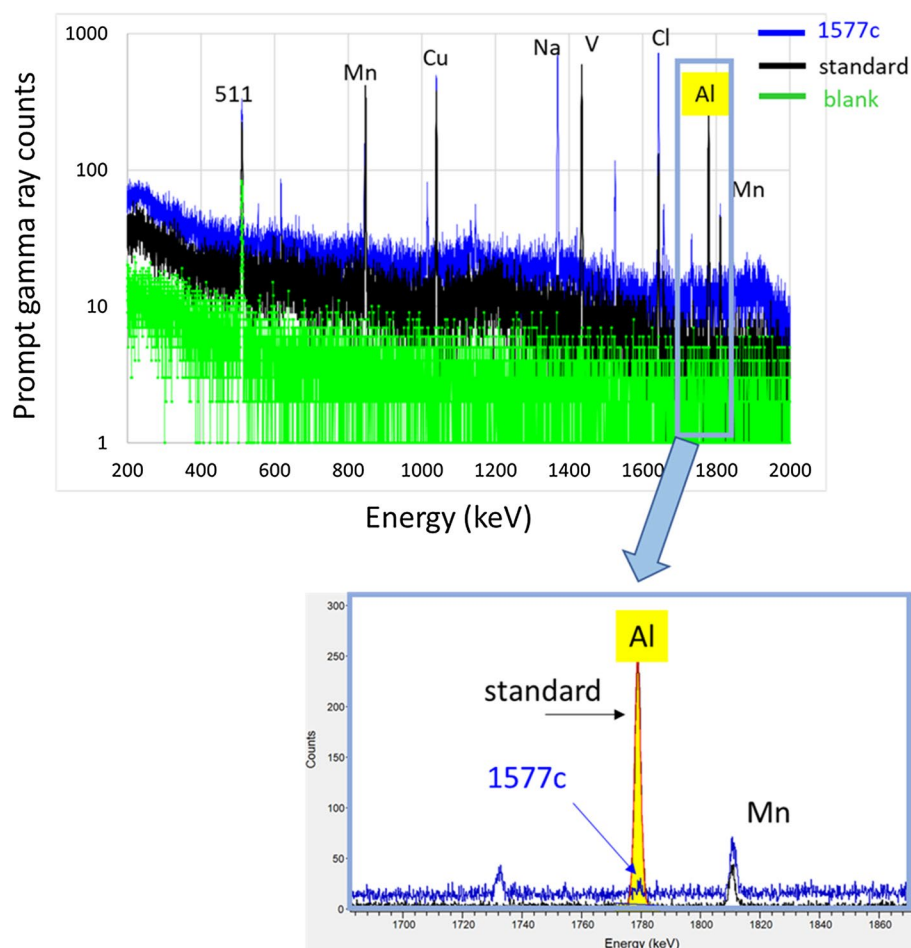
results on peak fitting algorithms. VRF is a new gamma spectral analysis package. Instead of fitting each peak individually, VRF fits all peaks as a single, spectrum-wide function which includes Compton continuum by non-linear least-squares fitting. It is suitable in this case with a limited number of contributing elements in the matrix. This serves as an independent verification using completely different approaches: a fit based on a physical model that includes all contributing peaks, as opposed to the conventional baseline subtraction peak fitting method which fits each peak manually. The global baseline obtained from the best-fit physical model should be more reliable than a local empirical construct.

Results and discussion

The three gamma spectra shown in Fig. 3 represent each of the following: sum of counts on the two opposing detectors in the 300 s count for an SRM 1577c sample, a multielement standard, and the cellulose blank (which is indistinguishable with the background spectrum, not shown). The background contribution is deemed negligible. Although the Al signals

¹ * Any mention of commercial products is for information only; it does not imply recommendation or endorsement by NIST.

Fig. 3 The gamma spectra (sum of counts on the two detectors) acquired for 300 s after 300 s irradiation in the cold neutron beam for a SRM 1577c sample, a multielement standard, and a blank. The inset shows the small Al peak in the sample under the much larger Al peak in the standard. The analysis was performed using Genie-VMS



from the SRM 1577c samples were weak, the peak search algorithm was able to find and fit peaks in the spectra.

The samples were analyzed individually using Genie-VMS; the peak area, after normalized to the Ti monitor counts and corrected for post-irradiation decay, was converted to mass fractions by ratio to the respective peak areas of the multi-element standard. The weighted means of mass fractions from the seven samples for Al, Cu and Mn are listed in Table 1, where mass fraction values of Cu and Mn can be used as quality control elements by comparing to the certified values of the Bovine Liver SRM series. The uncertainty for this measurement was dominated by the counting statistics with the average Poisson counting statistics uncertainty of approximately 30%. Other common sources of uncertainty for INAA analysis (such as standard purity, fluence corrections, gamma-ray attenuation) are significantly lower than the counting statistics [13]. Therefore, as this is not a value assignment measurement, the final expanded uncertainty was estimated to be dominated entirely by the Poisson counting statistics of the unknowns ($n=7$).

The same set of spectra were analyzed using VRF, with the main nuclide and the irradiation, decay and counting times as input parameters, a minimum chi-square fit to the

entire spectrum including the Compton continuum is carried out. When there are multiple peaks in the region, it adds to the constraint and increases the confidence of the outcome. A sample spectrum and fit are shown in Fig. 4.

The VRF software generates a report for the list of peaks fitted and the activity in Bq for each nuclide, decay corrected

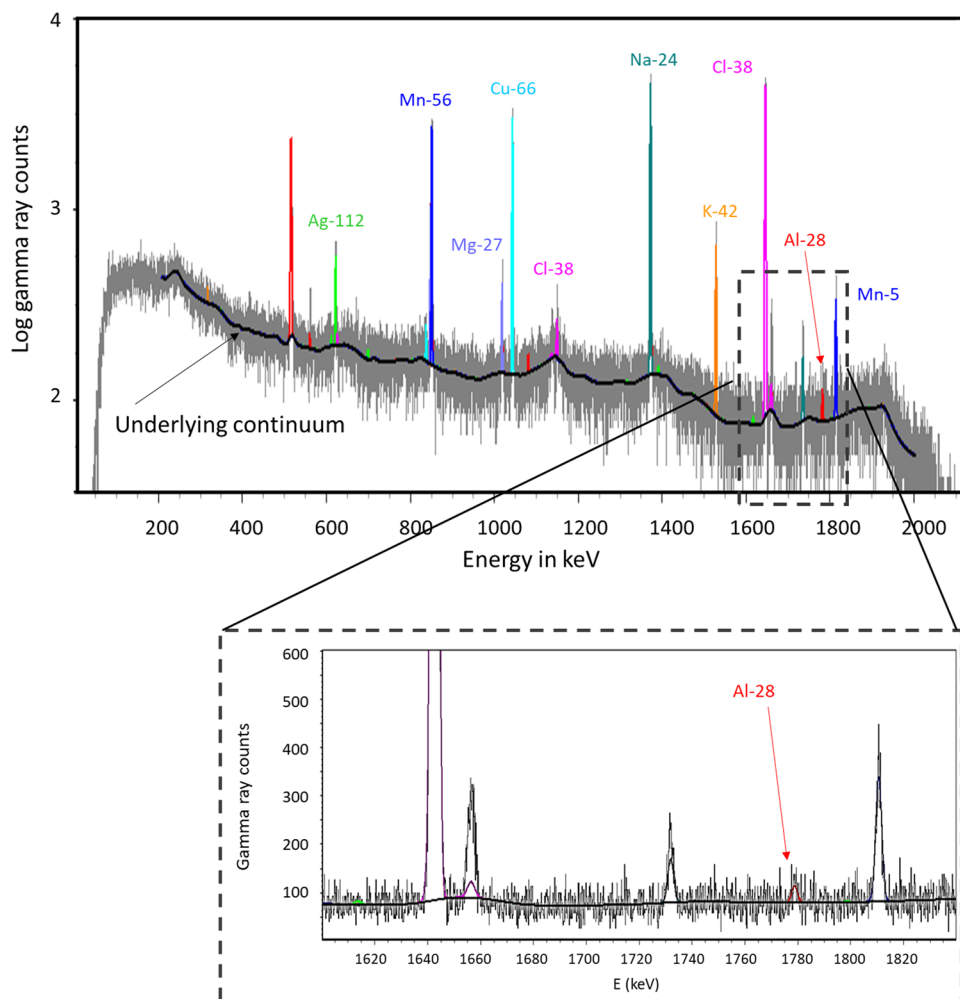
Table 1 Comparison of dry mass fractions from ICNAA with certified values for SRM 1577c, listed with their expanded uncertainty ($k=2$ for the certified values, and $k=2.45$ for the current analysis with 6 degrees of freedom)

Mass fraction (mg/kg) Element	SRM 1577c certificate	ICNAA results by method of analysis (uncertainty estimated based on counting statistics only)	
		Genie-VMS	VRF
Al	<i>Not certified</i>	0.80 ± 0.15	0.78 ± 0.23
Cu	275.2 ± 4.6	255 ± 16	285 ± 11
Mn	10.46 ± 0.47	10.76 ± 0.78	10.73 ± 0.46

The Al value was previously unavailable, reported here for the first time

The bold values represent certified values in SRM 1577c

Fig. 4 VRF fitting using physics-based model of decay spectrum from a 1577c sample, with the main contributing peaks identified and fitted along with the Compton continuum. The inset shows the portion of the spectrum enlarged and plotted in linear scale to highlight the small Al peak



to the time at the end of irradiation. The activity from the standard is obtained in the same manner, which is then divided by the known partial mass of each element to obtain a calibration of activity per unit monitor count per mg element. All Ti monitor counts were normalized to a nominal value such that counts from all samples were scaled to the same nominal incident neutron flux. In addition, the VRF reports a significance value, defined as the ratio of the activity to the uncertainty, as a statistical measure. A significance below 5, or loosely termed 5-sigma, is considered low and approaching the detection limit. The significance for Al in the 1577c data is on average 4.85, signifying the difficulty in the low concentration which is at or below the current detection limit of available measurement capabilities, even in the low background environment of the current detector configuration and under a favorable (300 s) irradiation condition to maximize ^{28}Al production.

The agreement between the two data analysis methods for ICNAA and the certified values for both Cu and Mn imply that there are no significant biases apparent in ICNAA analyses at the 200 mg/kg and 10 mg/kg level. However,

due to the much lower Al content (< 1 mg/kg) there are potentially unresolved biases present, though the two data analysis methods again show agreement. The Al value here is reported “as-is” for the purpose of illustrating the ability of ICNAA to measure elemental mass fractions at the sub mg/kg level and is not intended to satisfy the vetting necessary to be included in SRM 1577c’s Certificate of Analysis.

The limit of detection (LOD) for these measurements can be estimated following the Currie convention [14], using the Compton continuum baseline as the background under the peak. The area under the baseline, N_B , and by assuming Poisson statistics, its standard deviation, $\sigma_B = \sqrt{N_B}$, can be used to determine the null event limit $L_c = k_{1-\alpha, \nu} \sqrt{2\sigma_B}$, where $k_{1-\alpha, \nu}$ is the coverage factor. It can be shown that the minimum detection limit is $N_D = 2L_c + k_{1-\alpha, \nu}^2$. For $\alpha = 0.05$, corresponding to 95% confidence interval, and the degree of freedom ν is set to be N_B , then $k_{1-\alpha, \nu} \approx 1.645$. Applying this to the Al peak shown in Fig. 4, the value of N_D can be estimated as the net peak counts required for true event detection. This value is converted to mass fraction of Al in the 700 mg sample accordingly. The average LOD thus

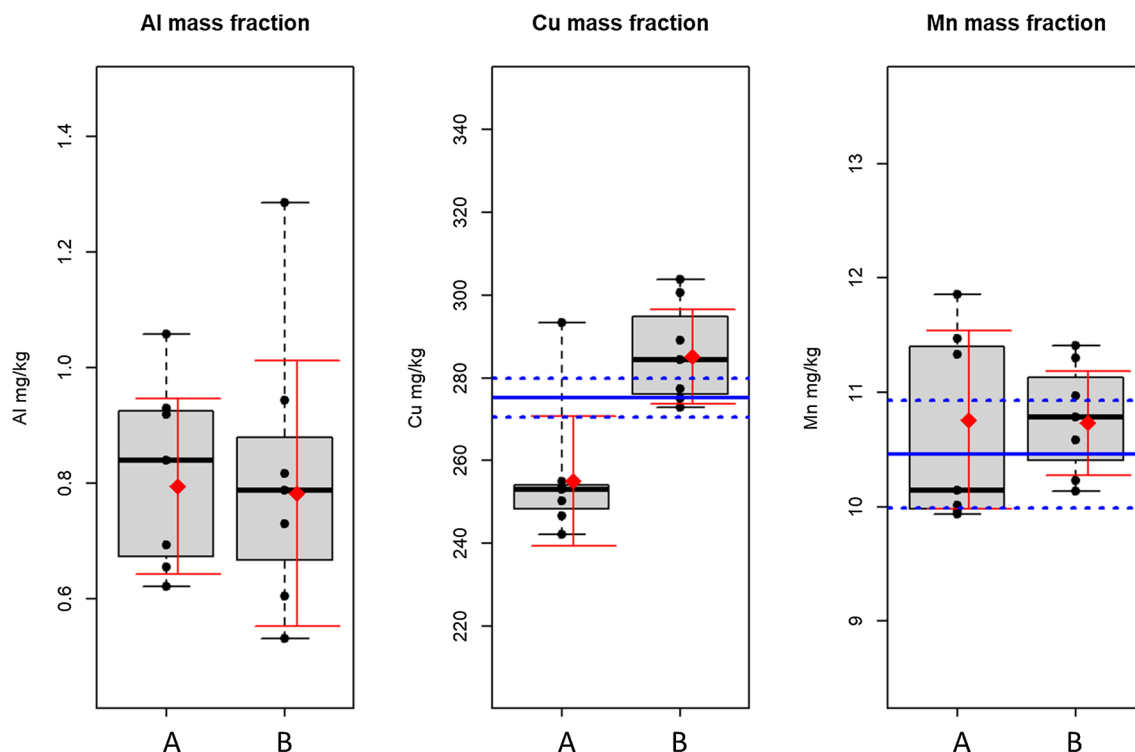


Fig. 5 “Box and whisker plot” of the mass fractions of Al, Cu, and Mn from the 7 bovine liver samples analyzed with Genie-VMS (a) and VRF (b). The solid box represents the middle two quartiles of the distribution, and solid black thick horizontal line is the median value. The top and bottom horizontal thin black lines span the entire data

range. The red dot is the weighted mean of the group, and the red lines are the corresponding expanded uncertainty reported in Table 1. For the Cu and Mn panels, the certified values of SRM 1577c are also shown in blue, along with the dashed lines as the expanded uncertainty of the certified value

estimated is 0.57 (0.08) mg/kg Al in SRM1577c, for the 300 s irradiation time and 60–90 s of time lapse between the end of irradiation and beginning of counting. This method of evaluation is a simplified version of the ISO 11,929 (see Supplemental Material for derivations).

The results of the weighted mean value and expanded uncertainty are shown in Table 1, together with the results from Genie-VMS. A statistical summary of the underlying data points is shown in Fig. 5 as a “box and whisker plot” of the 7 samples from Genie-VMS and VRF. There are some method dependent discrepancies, such as one data point in the Al value from the VRF method, and one in the Cu value from the Genie-VMS method, likely due to the different nature of the peak fitting method. The Cu values from the two methods are not overlapping within the expanded uncertainty which points to some systematic bias, although both results agree with the certified value within the expanded uncertainty. As a reference, previous results from two other independent techniques measuring Al mass fraction in SRM 1577c are shown in comparison to the ICNAA results from this work (Table 2). The disparity in the results suggests the difficulty in such low-level measurements with available techniques. We have shown that ICNAA can contribute

to this endeavor with the advantage of not requiring extra sample preprocessing.

Conclusions

This study was an attempt to independently quantify the Al concentration in bovine liver (SRM 1577c) at the sub-mg/kg level using the ICNAA method for the first time. The ICNAA experiment was carried out by irradiating the

Table 2 Comparison of the Al mass fraction with available data from two other techniques: ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) [15] and ICP-MS (inductively coupled plasma mass spectrometry) [16]

	Al mass fraction (mg/kg)	Expanded uncertainty (mg/kg)
ICP-OES	2.8	0.8
ICP-MS	0.569	0.262
ICNAA	0.76	0.22

These are values are based on “wet mass”, and the ICNAA (VRF) value has been converted to wet mass fraction accordingly

samples in a cold neutron beam to avoid activating possible interfering nuclides. The irradiation time was precisely (millisecond resolution) controlled at 300 s for both sample and standard to assure accurate decay correction. Because the cold-beam instrument is designed for prompt gamma activation analysis, it has an inherent environmental Al background that overwhelms the low Al signal from the sample. To overcome this, the ICNAA procedure was devised with sample transfer out of the irradiation chamber and into the a dual-detector assembly in high efficiency and low background configuration for decay counting. In addition, Ti foil was used as a local flux monitor to correct for neutron flux variation.

The data sets were analyzed using the traditional peak fitting methods which fits each peak locally with an estimated baseline and area. The aggregate of the seven samples produced Cu and Mn mass fractions that agreed with the certified values for bovine liver SRM 1577c within statistically uncertainties, supporting the feasibility of using this new technique and the associated data analysis. In addition, a new spectral analysis method based on a physical model that determines the baseline using the simulated Compton continuum was used to fit the data. The outcome of the two methods were compared to assess the possible bias and statistical significance of each. Based on these findings, we conclude that the measured Al mass fraction in SRM 1577c is likely at the level of 0.80 (0.15) mg/kg (Genie-VMS) and 0.78 (0.23) mg/kg (VRF) (value in the parentheses is the expanded uncertainty), although there could be unidentified biases that would preferentially affect low signal intensities from Al compared to Cu and Mn. Because VRF imposes constraints on the fit by a physical model, it should theoretically provide higher confidence in the outcome as shown by the improved agreement with Cu and Mn using the VRF software compared to the Genie-VMS. In addition, a globally fitted background taking into account the Compton continuum can be readily applied to the LOD estimate, hereby determined to be 0.57 (0.08) mg/kg for these irradiation and counting conditions. Future validation studies will need to be conducted to fully assess the accuracy and precision of the VRF software package.

Future experimental investigation includes automating the sample transfer mechanism for a more controlled study. Alternatively, implementing gamma-gamma coincidence measurement could eliminate extraneous Al background from the environment, thereby enabling in-situ measurements controlled by the neutron beam chopper without the need for sample transfer. The counting statistics is poor for the low-level Al in the current study, a major obstacle to making ICNAA useful for certification. In addition, systematic studies of Al concentrations using ICNAA are necessary to examine more definitively the possible bias in the low levels and the possible matrix effects.

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