Certified Reference Material

National Research Council Canada | Conseil national de recherches Canada

FEBS-1

Otolith Certified Reference Material for Trace Metals

FEBS-1 is a saggital otolith reference material procured from red snapper (*Lutjanus campechanus*).

Certified values are based on unweighted mean results from data submitted by collaborating laboratories. The expanded uncertainty (U_{CRM}) in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the ISO Guide [1] and k is the coverage factor. The value of u_c is determined from the combined uncertainties of the various analytical methods (u_{char}) as well as uncertainties associated with homogeneity (u_{hom}).

It is intended that U_{CRM} encompasses every aspect that reasonably contributes to the uncertainty of the measurand [2]. A coverage factor of 2 was applied for all elements. The table below lists measurands certified in FEBS-1.

CERTIFIED VALUES
(milligram/kilogram)

Barium (d,i,p)	5.09	±	0.23
Lithium (d,i,n,p)	0.305	±	0.044
Magnesium (d,i,n,p)	23.6	±	1.3
Manganese (d,n)	0.686	±	0.016
Sodium (d,i,n)	2594	±	161
Strontium (d,i,p)	2055	±	79

(percent)

Calcium (d,i,n,x)	38.3	±	1.4

Coding

The coding refers only to the instrumental method of analyte determination.

d - Inductively coupled plasma mass spectrometry (ICP-MS).
i - Inductively coupled plasma atomic emission spectrometry (ICP-OES).
n - Neutron activation (INAA).
p - Isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS).
x - X-ray fluorescence spectrometry (XRF).

NRC · CNRC

Intended Use

This reference material is primarily intended for use in the calibration of procedures and the development of methods used for the analysis of bulk elemental composition of otoliths and other marine aragonites.

Storage and Sampling

This material should be kept tightly closed in the original bottle and should be stored in a cool location. The contents should be well mixed by rotation and shaking of the bottle prior to use. The bottle should be tightly closed immediately after use.

Instructions for Drying

FEBS-1 can be dried to constant weight in an oven for 3 hours at 105 °C.

Preparation of FEBS-1

The otoliths were extracted from the fish, rinsed in ultrapure water, air-dried, and stored in polyethylene bottles. Surface contamination was later removed in a class 100 cleanroom by rest-rinsing all otoliths in 2% ultrapure HNO_3 for 5 minutes, followed by triple-rinsing in ultrapure water. Otoliths were subsequently sonicated in ultrapure water for 10 minutes, triple-rinsed again in ultrapure water and allowed to dry under a laminar flow hood for 72 hours.

The otoliths were crushed using a ball mill and the resulting powder was sieved through 100 and 200 mesh stainless steel sieves (150 µm and 75 µm particle sizes, respectively). The 100+ and 200+ fractions were returned to the mill and ground until all material passed the 200 mesh sieve. The material was then radiation sterilized at a minimum dose of 25 kGy (Neutron Products Inc., Dickerson, MD, USA).

Stability

As a result of interest in only the total amount content of the major, minor and trace elements, coupled with the chemical and physical properties of the matrix, the need for any rigorous stability testing of this material under its recommended conditions of storage was obviated and, as such, contributions to a specific long-term stability component of the uncertainty were not included in the evaluation of the combined uncertainty presented in Table 2.

Expiry

The certified values for FEBS-1 are considered valid until April 2015, provided the CRM is handled and stored in accordance with instructions herein.

Information values

Significant heterogeneity was observed for many elements, precluding their certification. A range of concentration is presented in Table 1 which simply summarizes both the highest and lowest concentrations of the element determined in any given sub-sample by the primary ID-ICP-MS technique along with corroboration by another methodology. These data may be of interest to users/readers and hence are included in this report.

Table 1. Information values.

Element	Range*	Technique
Cd, µg/kg	1.4 - 3.2	ID-ICP-MS, ICP-MS
Cu, mg/kg	4.2 - 6.8	ID-ICP-MS, ICP-MS
Ni, µg/kg	16 - 29	ID-ICP-MS
Pb, mg/kg	0.40 - 0.77	ID-ICP-MS, ICP-MS
Zn, mg/kg	3.2 - 6.3	ID-ICP-MS, INAA

*Extreme values of range of concentrations obtained during sample characterization.

Certified value

The results from a sub-group of participants were used for the certification of FEBS-1. These laboratories were selected based on their previous experience analysing otolith samples.

The certified values were calculated from the unweighted means of the results of the participating laboratories. [3,4]

New guidelines for CRM producers suggest all sources relevant to the user of the material should contribute to the uncertainty of the certified value [2-4]. Included in the overall uncertainty estimate are uncertainties in the batch characterisation (u_{char}), uncertainties related to possible between-bottle variation (u_{hom}) as well as instability derived from effects relating to long-term storage and transport (u_{stab}). Expressed as standard uncertainties, these components can be combined as:

$$u^2_{c(CRM)} = u^2_{char} + u^2_{hom} + u^2_{stab} \qquad (1)$$

The various statistics used to calculate the certified values are shown in Table 2.

Characterisation

The characterisation uncertainties (u_{char}) were calculated in accordance with equation 2, where s is the standard deviation of the means and p is the number of mean results included in the calculation [6].

$$u_{char} = \frac{s}{\sqrt{p}} \qquad (2)$$

Homogeneity

The material was tested for homogeneity at NRC using ICP-MS or ICP-OES. Results from triplicate sub-samples (0.250 g) from at least 8 bottles were evaluated using ANOVA.

For Ba, Li, Mg, Sr and Ca, jue to the inhomogeneity contribution to uncertainty, u_{hom}, was equated to the experimentally determined between-unit standard deviation ($s_{between}$) as the best estimate of the uncertainty due to between-unit heterogeneity. However, for Mn and Na, the situation depicted by equation 3 occurred:

$$s^2_{between} < \frac{s^2_{meas}}{n} \qquad (3)$$

where s_{meas} is the repeatability standard deviation for the method used in the homogeneity assessment and n is the number of replicates per unit. For these cases, u_{hom} was calculated according to equation 4:

$$u_{hom} = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{v_{MS_{within}}}} \qquad (4)$$

where MS_{within} represents the mean squares within groups and $v_{MS_{within}}$ is the number of degrees of freedom. [5]

Table 1 summarizes the resulting uncertainty components for homogeneity for each element at the 250 mg sub-sample size used for the testing.

Stability

As noted above, uncertainty components for long and short term stability were considered negligible and are thus not included in the uncertainty budget.

Table 2. Uncertainty components for FEBS-1.

	Ba, mg/kg	Ca, %	Li, mg/kg	Mg, mg/kg	Mn, mg/kg	Na, mg/kg	Sr, mg/kg
u_{char}	0.039	0.62	0.0211	0.395	0.006	72.9	21
u_{hom}	0.108	0.35	0.0055	0.535	0.0055	23.5	34
u_c	0.115	0.717	0.022	0.665	0.0081	80.3	40
U (k=2)	0.23	1.4	0.044	1.33	0.016	161	79

Updates

It is anticipated that as more data become available, the established values may be updated and reliable values assigned to more elements.

Our web site at http://inms-ienm.nrc-cnrc.gc.ca/calserv/chemical_metrology_e.html will contain any new information.

References

[1] Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9, 1st ed. ISO, Geneva, Switzerland (1993).

[2] J. Pauwels, A. van der Veen, A. Lamberty, H. Schimmel, Evaluation of uncertainty of reference materials.
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[4] A. M.H. van der Veen, T. P.J. Linsinger, H. chimmel, A. Lamberty and J. Pauwels, Uncertainty calculations in the certification of reference materials 4. Characterisation and certification.
Accred Qual Assur (2001) 6:290–294.

[5] T.P.J. Linsinger, J. Pauwels, A.M.H. van der Veen, H. Schimmel and A. Lamberty, Accred. Qual. Assur., 2001, 6, 20 – 25.

The results listed in this certificate are traceable to the SI through gravimetrically prepared standards of established purity and international measurement intercomparisons. As such, they serve as suitable reference materials for laboratory quality assurance programs, as outlined in ISO/IEC 17025.

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