National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

BBET-1

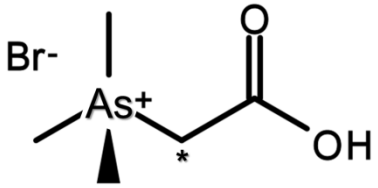
Certified Reference material for carbon-13 labelled arsenobetaine bromide

BBET-1 is a high-purity certified reference material (CRM) of carbon-13 labelled arsenobetaine bromide from the National Research Council of Canada (NRC), intended for analytical method development and accurate quantitation of arsenobetaine. A unit of this material consists of approximately 30 mg. Certified and information values (Table 1) are based on results from data generated at NRC using quantitative proton nuclear magnetic resonance spectroscopy (^{1}H-qNMR) and chemical specifications.

Table 1: Certified and information values and expanded uncertainties (*k* = 2) for BBET-1

Compound	Mass fraction, g/g	Type of value
[$^{13}CH_2$]-arsenobetaine bromide (a)	0.9902 ± 0.0086	certified
[$^{13}CH_2$]-arsenobetaine (cation) (a)	0.6858 ± 0.0060	certified
ethanol (a)	0.0006	information

Refer to the sections below for additional explanations



[$^{13}CH_2$]-arsenobetaine bromide
InChIKey: MJHCMTTVEWKRNC-VZHAHHFWSA-N
Molecular formula: $^{13}C_1C_4H_{12}AsO_2Br$
Molar mass: 259.956 ± 0.006 g/mol

Period of validity: until March 2036
Storage conditions: +4 °C under typical refrigerator conditions

DOI: doi.org/10.4224/crm.2016.bbet-1

Intended use

This reference material is primarily intended for use in the calibration of procedures and the development of methods for the determination of arsenobetaine. A minimum sample mass of 20 mg is recommended.

Preparation of material

Arsenobetaine was synthesized at NRC from commercially-available substrates. A single-pot macro-scale synthesis of arsenobetaine bromide was performed using trimethylarsane and isotopically labeled 2-bromoacetic acid following the procedure of Minhas et al. [1,2].

Characterization of material

The explanatory list of letters next to each compound (Table 1) refers to the instrumental method used for measurements:

a) Proton nuclear magnetic resonance spectroscopy (^{1}H-qNMR)

Isotopic enrichment (abundance) of the methylene-carbon of arsenobetaine, $x(^{13}C) = 0.9891 \pm 0.0006$ ($k = 2$) and it was obtained by quantitative ^{1}H-NMR in experiments that rely on the signal splitting caused by the magnetic ^{13}C nucleus on the ^{1}H signals [2]. When mathematically convoluted with the remainder of the AsBet, the following isotopic abundances are obtained for the molecular ion ($C_5H_{12}AsO_2$) for use in mass spectrometry: $x_{179} = 0.0104 \pm 0.0004$, $x_{180} = 0.9420 \pm 0.0048$, $x_{181} = 0.0428 \pm 0.0048$ with the ratio $x_{179}/x_{180} = 0.0110 \pm 0.0004$ ($k = 2$).

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically-prepared standards of established purity (high-purity benzoic acid, NIST SRM 350b and NIST PS1, obtained from NIST) and international measurement intercomparisons. As such, they serve as suitable reference materials for laboratory quality assurance programs, as outlined in ISO/IEC 17025.

Homogeneity

The material was tested for homogeneity at NRC using ^{1}H-qNMR [3]. Results from sub-samples were evaluated using the DerSimonian-Laird random effects model and included in the calculation of the certified values [4].

Stability

Unlike the hydroxide salt, the arsenobetaine bromide is non-hygroscopic. The purity of arsenobetaine bromide has not changed over the fifteen-year period at +4 °C temperature and the material is deemed stable [3].

Uncertainty

The expanded uncertainty (U) for all values is equal to $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the Joint Committee for Guides in Metrology (JCGM) [3] and k is the coverage factor. A coverage factor of $k = 2$ was applied which corresponds to a level of confidence of approximately 95 %.

All reasonable sources of uncertainty related to the certified values in Table 1 were considered [3]. Included in the combined uncertainty estimate are uncertainties in the batch characterization, uncertainties related to possible between-unit variation, and uncertainties related to stability.

Storage

It is recommended that the material be stored in a cool, clean location. Vials may be stored at room temperature for short-term only. A long-term storage temperature of +4 °C is recommended.

Instructions for handling and use

Users shall take responsibility for demonstrating that their sub-sampling and storage procedures do not impact certified values.

Health and safety information

Only qualified personnel should handle the material and appropriate disposal methods should be used. A Safety Data Sheet (SDS) is available at doi.org/10.4224/crm.2016.bbet-1. For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

Period of validity

The certified values are valid until March 2036, provided the storage and instructions for handling and use specified in this certificate are followed.

Quality Management System

The NRC is Canada's National Metrology Institute (NMI) and is a signatory of the International Committee for Weights and Measures Mutual Recognition Arrangement (CIPM MRA). The CIPM MRA was developed in a response to a growing need for an open, transparent, and comprehensive scheme to give users reliable quantitative information on the comparability of national metrology services and to provide the technical basis for wider agreements negotiated for international trade, commerce, and regulatory affairs. Our Quality Management System for measurement services and certified reference materials conforms to the requirements of ISO/IEC 17025 and ISO 17034.

Description of terms

Certified values are those for which the NRC has the highest confidence and that all known and suspected sources of bias have been considered by the NRC and are reflected in the stated expanded uncertainties.

Information values are those that may be of interest to users, but for which the NRC has not established sufficient information to provide an estimate of uncertainty, or that reflect a lack of agreement between different methods of analysis.

References

1. Minhas R, Forsyth DS, Dawson B (1998) Synthesis and characterization of arsenobetaine and arsenocholine derivatives. *Appl Organometal Chem*, 12: 635-641. https://doi.org/10.1002/(sici)1099-0739(199808/09)12:8/9<635::aid-aoc772>3.0.co;2-j
2. Le PM, Ding J, Leek DM, Mester Z, Robertson G, Windust A, Meija J (2016) Determination of chemical purity and isotopic composition of natural and carbon-13-labeled arsenobetaine bromide standards by quantitative ^{1}H-NMR. *Anal Bioanal Chem*, 408: 7413-7421. https://doi.org/10.1007/s00216-016-9827-y.
3. JCGM-100:2008 (2008) Evaluation of measurement data – Guide to the expression of uncertainty in measurement. Joint Committee for Guides in Metrology (JCGM). doi.org/10.59161/JCGM100-2008E
4. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. *Controlled Clinical Trials* 7: 177-188. doi.org/10.1016/0197-2456(86)90046-2

Authorship

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This certificate is only valid if the corresponding material was obtained directly from the NRC or an authorized reseller. Users should ensure that the certificate they have is current. For updates, please refer to doi.org/10.4224/crm.2016.bbet-1

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