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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM – 2019A

Inorganic Elements in Infant Formula

Batch Number
STY-0169-001

Description
The certified reference material (CRM) was produced by spiking a batch of infant formula with solutions of cadmium nitrate tetrahydrate, mercury (II) chloride, lead nitrate, sodium arsenate dibasic heptahydrate, along with a stabiliser and preservatives to ensure stability.

Through a spray-drying process, the liquid-reconstituted infant formula was converted into powder. The powder was freeze-dried overnight and sieved at 850 µm to achieve uniform particle size. The sieved powder was homogenised in a drum mixer before being packed into mylar pouches. Each pouch was flushed with Argon and sealed inside a glove box. Following that, the pouches were heat-sealed and exposed to γ-irradiation at about 14 kGy[1] to prolong their shelf-life. A unit of the CRM consists of 10 mylar pouches, each containing approximately 2 g of material.

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO Guide 35:2017 [3].

Certified Mass Fraction Values
A certified value is a value for which a laboratory has the highest confidence in its accuracy, in that all known or suspected sources of biases have been investigated and accounted for. The certified mass fraction values for the seven analytes in the CRM are listed below. The certified mass fraction values for cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu) and potassium (K) were determined by inductively coupled plasma mass spectrometer using isotope dilution mass spectrometry (ICP-IDMS) [4]. The certified mass fraction value for arsenic (As) was determined by inductively coupled plasma high resolution mass spectrometer (ICP-HR-MS) using standard addition method. The certified mass fraction value for iodine (I) was determined by inductively coupled plasma mass spectrometer (ICP-MS) using standard addition method [5].

[1] The irradiation work was performed by a subcontractor.

Analyte	Mass fraction	Unit
Cadmium	0.1939 ± 0.0083	mg/kg
Mercury	0.1069 ± 0.0031	mg/kg
Lead	0.0534 ± 0.0068	mg/kg
Copper	4.99 ± 0.17	mg/kg
Potassium	6767 ± 232	mg/kg
Arsenic	0.327 ± 0.073	mg/kg
Iodine	1.045 ± 0.083	mg/kg

The mass fraction value is expressed as the certified value ± the expanded uncertainty.

The uncertainty listed with the certified value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence). The certified value has an associated measurement uncertainty attributed to uncertainty contribution from characterisation of the material (u_{char}), uncertainty in the homogeneity of the material (u_{bb}) and uncertainty in the stability of the material (u_{stab}). The u_{char} was evaluated by combining uncertainties from method precision, the concentration of calibration solution, weighing, different ion pairs used (for Cd, Hg and Pb), isotope ratios (for Cd, Hg, Pb, Cu and K) and the relative atomic mass (for Pb only), in accordance with ISO/IEC Guide 98-3:2008 [6].

Homogeneity

Homogeneity testing on the analytes in the material was performed on at least ten pouches with two sub-samples taken from each pouch. ICP-MS was employed for the determination of the analytes. The sample size taken for homogeneity testing was about 0.5 g. No significant differences in the between- and within-bottle variances were found for Hg, Pb, Cu, K and I using one-way ANOVA at 95 % confidence level [3]. For Cd and As, significant differences in the between- and within-bottle variances were observed using one-way ANOVA at 95 % confidence level [3]. However, the between-bottle standard deviations were sufficiently small compared to the standard uncertainties of the certified mass fraction values [3]. Thus, the material was regarded to be sufficiently homogeneous. The u_{bb} was evaluated from the uncertainty due to between-pouch inhomogeneity.

Stability

Short-term stability testing on the analytes in the material at 50 °C (maximum allowable transportation temperature) showed that they were stable up to 21 days.

The long-term stability of the analytes at storage temperature (18 °C to 25 °C) was evaluated on three occasions over a period of up to 6 months. The results showed that the analytes were stable over the study period. The u_{stab} was evaluated from the standard error of the slope.

Validity of Certified Mass Fraction Values

The certified mass fraction values are valid within its measurement uncertainty until **28 Mar 2026**, provided that the CRM is subjected to the same handling and storage conditions as stated in this Certificate of Analysis (COA).

The CRM will be continuously monitored during the validity period to determine if any substantive change to the certified values has occurred. If necessary, its user will be advised or an updated COA will be issued when the property value of the CRM is found to have changed.

Analytical Methods

The certified mass fractions of Cd, Hg, Pb, Cu and K in the material were determined by exact-matching ICP-IDMS. Standard reference materials (SRMs) from the National Institute of Standards and Technology (NIST, USA) (SRM 3108 for Cd, SRM 3133 for Hg, SRM 3128 for Pb, SRM 3114 for

Cu and SRM 918c for K) were used as calibration standards for IDMS measurements. Enriched isotopes ^{111}Cd, ^{201}Hg, ^{206}Pb, ^{65}Cu and ^{41}K from Oak Ridge National Laboratory (USA) were used as the internal standards. The calibration blends were prepared gravimetrically by mixing appropriate amount of calibration standard solutions and internal standard solutions. The sample blends were prepared by spiking appropriate amount of internal standard into the material.

The certified mass fraction of As was determined by ICP-HR-MS using standard addition method. Gallium SRM from NIST (SRM 3119a) was added to the sample digest as internal standard. Different amounts of As SRM from NIST (SRM 3103a) were then spiked into the sample digest to produce sample blends.

The certified mass fraction of I was determined by ICP-MS using standard addition method. Tellurium SRM from NIST (SRM 3156) was added to the sample extract as internal standard. Different amounts of I CRM from the National Institute of Metrology (NIM), China (GBW06110f) were then spiked into the sample extract to produce sample blends.

Quality control blends were also prepared and analysed concurrently. For Cd, Hg, Pb, Cu, K and As, microwave acid digestion was employed on both sample and quality control blends using 5 mL HNO_3, 0.2 mL HF and 2 mL H_2O_2 to give clear digests. For I, both sample and quality control blends underwent alkaline extraction to stabilise iodine in the solution and minimising volatility. The extraction method involved adding 25 % tetramethylammonium hydroxide (TMAH) to the blends, allowing the mixtures to stand for at least 16 h, followed by heating for 3 h in a calibrated oven set to 90 °C [7].

Metrological Traceability

The certified mass fraction values are traceable to the International System of Units (SI) through the use of SRMs from NIST or CRM from NIM.

Intended Use

For the validation of methods or as quality controls used to determine the mass fraction of Cd, Hg, Pb, Cu, K, As and I in food products with high protein and fat content, such as infant formula and similar matrices.

Instruction for Use

Each pouch is intended for a single analysis. Any remaining sample in the pouch should be discarded, if not used within the same day. To reduce moisture absorption, weighing needs to be performed as quickly as possible and the pouch should not be left open after sampling. The minimum sample size for each use should be about 0.5 g.

Storage

The material should be stored at 18 °C to 25 °C in its original pouch. Exposure to direct intense light and ultraviolet radiation should be avoided.

Health and Safety Information

Treat the material as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. The Safety Data Sheet (SDS) for this material is provided separately and contains essential safety and handling information.

Further Information

Please direct all enquiries regarding this CRM to the contact provided in this COA.

References

1. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.

2. ISO 17034:2016 General requirements for the competence of reference material producers.
3. ISO Guide 35:2017 Reference materials – Guidance for characterisation and assessment for homogeneity and stability.
4. Sargent, M.; Harrington, C.; Harte, R.; *Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry*, RSC Publishing, 2002.
5. Abbyad, P.; Tromp, J.; Lam, J.; Salin, E.; *Optimization of the technique of standard additions for inductively coupled plasma mass spectrometry*, J. Anal. At. Spectrom. (2001) 16: 464 – 469.
6. ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).
7. Fecher, P. A., Goldmann, I., & Nagengast, A,; *Determination of iodine in food samples by inductively coupled plasma mass spectrometry after alkaline extraction,* J. Anal. At. Spectrom (1998) 13, 977-982.

Certificate Revision Record

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-2019A/01	28 Mar 2024	Issuance of first certificate
CML-HRM-2019A/02	28 Mar 2025	Extension of expiry date

Note

HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the reference material by the customer.

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