Chemical Metrology Division
Applied Sciences Group
Health Sciences Authority
1 Science Park Road, #01-05/06,
The Capricorn, Singapore Science Park II,
Singapore 117528
Tel: 65 6775 1605 Fax: 65 6775 1398
Website: www.hsa.gov.sg
Email: HSA_CML@hsa.gov.sg

Ref. No.: CML-HRM-1008A/06
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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-1008A

L-Leucine

Batch Number
STY-0040-001

Description
A unit of the certified reference material (CRM) consists of 1 g of L-leucine [(*S*)-2-amino-4-methylpentanoic acid] in a screw-capped amber vial. The mass balance approach was adopted to determine the mass fractions (mg/g) of four classes of impurities; namely: structurally-related organic compounds, volatile organic compounds, total non-volatiles and water content, present in the CRM. The mass fraction value of L-leucine was then obtained by subtracting the mass fraction values of the impurities from 1,000.

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

Certified Mass Fraction Value
A certified value is a value for which a laboratory has the highest confidence in its accuracy. The certified mass fraction value given below is based on the results obtained by the mass balance approach:

Certified Mass Fraction Value: 996.9 ± 3.3 mg/g

The final result 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). It was estimated by combining uncertainties in the measurement of mass fractions of the four classes of impurities and the homogeneity and stability of the CRM, in accordance with ISO/IEC Guide 98-3:2008 [4].

Homogeneity
Homogeneity testing on L-leucine and structurally-related organic compounds was performed on two sub-samples taken from eight bottles. The samples were derivatised with ortho-phthalaldehyde and

analysed using LC-DAD. The sample size taken for homogeneity testing was approximately 25 mg. No significant differences in the between and within-bottle variances were found using one-way ANOVA at 95 % confidence level [3]. Thus, the material was regarded to be sufficiently homogeneous.

Stability

The short-term stability of L-leucine and structurally-related organic compounds was studied. The material was stored at 25 °C and 40 °C (maximum allowable transportation temperature) for up to one month. The results showed that L-leucine was stable over the study period.

The long-term stability of L-leucine and structurally-related organic compounds at 4 °C was evaluated on four occasions over a period of up to four months after preparation. The results showed that L-leucine was stable over the study period.

Validity of Certified Mass Fraction Value

The certified mass fraction value is valid within the specified measurement uncertainty until **20 May 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 value has occurred. If necessary, its user will be advised if the CRM can continue to be used or an updated COA may be issued.

Analytical Methods

The sample was analysed by

(i) liquid chromatography with isotope dilution tandem mass spectrometry (LC-IDMS/MS) to determine isoleucine;
(ii) derivatisation with Marfey's reagent followed by LC-DAD to determine D-leucine [5];
(iii) liquid chromatography with tandem mass spectrometry (LC-MS/MS) to determine methionine and phenylalanine;
(iv) gas chromatography with mass spectrometry (GC-MS) and thermogravimetric analyser (TGA) to determine the total volatile organic compounds;
(v) TGA to determine the total non-volatiles; and
(vi) Karl Fisher coulometer to determine the water content.

The Table below summarises the results obtained from the above determinations:

Component(s)	Technique	Mass Fraction (mg/g)	Standard Uncertainty (mg/g)
Structurally-related organic compounds	LC-MS/MS[1] and LC-DAD	2.24	0.06
Total volatile organic compounds	TGA	< 2.3 (LOD[3])	0.66
Total non-volatiles	TGA	< 5 (LOD[3])	1.44
Water	Karl Fisher coulometry[2]	0.85	0.39

[1] Calibrated using amino acid Standard Reference Material (SRM 2389a) from the National Institute of Standards and Technology (NIST), USA.
[2] Validated with water saturated 1-octanol (SRM 2890) from NIST, USA.
[3] LOD: limit of detection

The structurally-related impurities are summarised in the Table below:

Component(s)	Technique	Mass Fraction (mg/g)	Standard Uncertainty (mg/g)
Isoleucine	LC-IDMS/MS	2.20	0.05
Methionine	LC-MS/MS	0.04	0.02
Phenylalanine	LC-MS/MS	0.004	0.002
D-leucine	Derivatisation and LC-DAD	not detected	N/A

Metrological Traceability
The certified mass fraction value is traceable to the International System of Units (SI) through the mass balance method by the Health Sciences Authority (HSA).

Intended Use
The reference material is intended for use as a calibrant for the determination of L-leucine for different purposes, such as peptide and protein determinations.

Instructions for Use
After use, the bottle must be tightly re-capped and protected from moisture and light. The minimum sample size for each use should be 25 mg.

Storage
The material should be properly sealed and stored at refrigerator temperature (2 to 8 ^{o}C) in its original bottle when not in use. Protect the material from moisture and light.

Safety Precautions for Users
Treat the material as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Further Information
Please direct all enquiries regarding this CRM to the contact 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] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).
[5] Szokan, G; Mezo, G; Hudecz, F; Journal of Chromatography, 1988, 444, 115-122.

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

Certificate Revision Records
Certificate of Analysis CML-HRM-1008A/02 replaces Certificate CML-HRM-1008A/01 issued on 12 Jun 2014.

Certificate of Analysis CML-HRM-1008A/03 replaces Certificate CML-HRM-1008A/02 issued on 17 Apr 2015.

Certificate of Analysis CML-HRM-1008A/04 replaces Certificate CML-HRM-1008A/03 issued on 15 May 2017.

Certificate of Analysis CML-HRM-1008A/05 replaces Certificate CML-HRM-1008A/04 issued on 02 May 2019.

Certificate of Analysis CML-HRM-1008A/06 replaces Certificate CML-HRM-1008A/05 issued on 01 Dec 2020.

Dr Teo Tang Lin
Division Director
Chemical Metrology Laboratory
Chemical Metrology Division