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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-1006A

L-Valine

Batch Number
STY-0031-001

Description
A unit of the certified reference material consists of 1 g of L-valine [(*S*)-2-amino-3-methylbutanoic 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 reference material. The mass fraction value of L-valine 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 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: 991.4 ± 3.4 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 reference material, in accordance with ISO/IEC Guide 98-3:2008 [4].

Homogeneity
Homogeneity testing on L-valine and structurally-related organic compounds was performed on two sub-samples taken from eight bottles using LC-MS/MS. The sample size taken for homogeneity

testing was approximately 30 mg to produce a solution with L-valine concentration of about 50 µg/g. 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-valine 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-valine was stable over the study period.

The long-term stability of L-valine and structurally-related organic compounds at 4 °C was evaluated on three occasions over a period of up to three months after preparation. The results showed that L-valine 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 **13 Nov 2027**, provided that the reference material is subjected to the same handling and storage conditions as stated below.

The reference material 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 material can continue to be used or an updated *Certificate of Analysis* may be issued.

Analytical Methods

The sample was analysed by

(i) liquid chromatography with isotope dilution tandem mass spectrometry (LC-IDMS/MS) to determine alanine, isoleucine, and leucine;
(ii) derivatisation with ortho-phthalaldehyde (OPA) followed by liquid chromatography with diode array detection (LC-DAD) to determine 2-aminobutanoic acid;
(iii) derivatisation with Marfey's reagent followed by LC-DAD to determine D-valine [5];
(iv) liquid chromatography with tandem mass spectrometry (LC-MS/MS) to determine other structurally-related organic compounds;
(v) gas chromatography with mass spectrometry (GC-MS) and thermogravimetric analyser (TGA) to determine the total volatile organic compounds;
(vi) TGA to determine the total non-volatiles; and
(vii) 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	7.16	0.12
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]	1.40	0.57

[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)
Alanine	LC-IDMS/MS	2.62	0.07
Isoleucine	LC-IDMS/MS	1.93	0.07
Leucine	LC-IDMS/MS	1.97	0.06
2-Aminobutanoic acid	Derivatisation and LC-DAD	0.60	0.02
Methionine	LC-MS/MS	0.03	0.02
Glycine	LC-MS/MS	0.005	0.003
Lysine	LC-MS/MS	0.006	0.003
Phenylalanine	LC-MS/MS	0.0007	0.0004
Threonine	LC-MS/MS	0.0006	0.0003
Tyrosine	LC-MS/MS	0.0010	0.0006
D-valine	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-valine 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 30 mg.

Storage
The reference 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 reference material to the contact above.

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.

Certificate Revision Records

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-1006A/01	13 Nov 2013	Issuance of first certificate
CML-HRM-1006A/02	03 Nov 2014	Extension of expiry date
CML-HRM-1006A/03	08 Sep 2016	Extension of expiry date
CML-HRM-1006A/04	04 Oct 2017	Extension of expiry date
CML-HRM-1006A/05	02 May 2019	Revision of certificate
CML-HRM-1006A/06	01 Dec 2020	Revision of certificate
CML-HRM-1006A/07	21 Oct 2021	Extension of expiry date
CML-HRM-1006A/08	28 Oct 2024	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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