Australian Government
Department of Industry, Science and Resources

National Measurement Institute

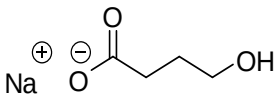
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D812b: Sodium γ-hydroxybutyrate

Report ID: D812b.2021.03

Chemical Formula: $C_4H_7O_3Na$

Molecular Weight: 126.1 g/mol



Property value

Batch No.	CAS No.	Purity (mass fraction)
12-D-25	**502-85-2**	**99.6 ± 0.5%**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: Sodium 4-hydroxybutanoate

Expiration of certification: The property values are valid till 7 April 2024, i.e. three years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

This material has been given a shelf life of three years from the date of re-certification.

Description: White powder prepared by synthesis or sourced from an external supplier, certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 25 ºC in a closed container in a dry, dark area.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. The long-term stability of the compound in solution has not been examined.

Warning: Sodium γ-hydroxybutyrate is extremely hygroscopic. In a closed container this material has been shown to absorb more than 10% moisture and in some cases the sample has become liquid.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC-ELSD on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

Safety: Treat as a hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
15 September 2022

This report supersedes any issued prior to 15 September 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance using a combination of traditional analytical techniques including HPLC with ELS detection, Karl Fischer analysis, and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

Supporting evidence is provided by quantitative NMR, headspace GC-MS analysis of occluded solvents and elemental microanalysis. The purity value by qNMR was obtained using a combination of the two-proton triplet at 3.57 ppm, two-proton triplet at 2.21 ppm and the two proton multiplet at 1.79 ppm against a certified internal standard of potassium hydrogen maleate.

QNMR:	Instrument:	Bruker Avance-III-400
	Field strength:	400 MHz
	Solvent:	D_2O (4.79 ppm)
	Internal standard:	Potassium hydrogen maleate (99.4% mass fraction)
	Re-analysis:	Mean (3.57 ppm) = 99.3%, s = 0.3% (3 sub samples, March 2014)
	Re-analysis:	Mean (1.79 ppm) = 99.3%, s = 0.3% (3 sub samples, March 2014)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	D_2O (4.79 ppm)
	Internal standard:	Potassium hydrogen maleate (100% mass fraction)
	Re-analysis:	Mean (3.56 ppm) = 99.5%, s = 0.3% (6 sub samples, April 2015)
	Re-analysis:	Mean (2.21 ppm) = 99.5%, s = 0.3% (6 sub samples, April 2015)
	Re-analysis:	Mean (1.79 ppm) = 99.5%, s = 0.3% (6 sub samples, April 2015)
Karl Fischer analysis:		Moisture content 1.2% mass fraction (April 2015) Moisture content 1.4% mass fraction (March 2018) Moisture content 1.1% mass fraction (January 2021) Moisture content 0.3% mass fraction (April 2021)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro
	Column:	Ascentis C-18, 150 mm × 4.6 mm I.D. × 2.7 μm
	Column temp:	40 °C
	Solvent system:	A 2% Formic acid in MilliQ water [10% v/v] B Acetonitrile [15% v/v] C MilliQ water [75% v/v]
	Flow rate:	0.15 mL/min
	Sample prep:	100 μg/g in MeOH/MilliQ water (25:75)
	Injection volume:	30 μL
	Ionisation mode:	Electrospray negative ion
	Capillary voltage:	3.0 kV
	Cone voltage:	25 V
	Source temp:	130 ºC
	Desolvation gas temperature:	350 ºC
	Cone gas flow rate:	27 L/hr
	Desolvation gas flow rate:	750 L/hr
	The retention time of γ–hydroxybutyrate anion is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	9.80min:	102.9 ($M-Na^+$) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Acetone
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3341, 2941, 2875, 1554, 1420, 1069, 1017, 922, 869, 668 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 1.76 (2H, quintet, *J* = 6.8 Hz), 2.20 (2H, d, *J* = 7.9 Hz), 3.57 (2H, d, *J* = 6.8 Hz) ppm
		Methanol (0.02%), butyrolactone (0.08%) and an impurity of related structure (0.04%) were estimated using ^{1}H NMR.
^{13}C NMR:	Instrument:	Bruker Avance-400
	Field strength:	101 MHz
	Solvent:	D_2O
	Spectral data:	δ 28.4, 34.0, 61.5, 183.1 ppm
Melting point:		146-149 °C
Microanalysis:	Found:	C = 37.9%; H = 5.5% (March, 2013)
	Calculated:	C = 38.1%; H = 5.6% (Calculated for $C_4H_7O_3Na$)