



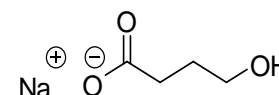
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D812b: Sodium γ -hydroxybutyrate

Report ID: D812b.2023.01

Chemical Formula: C₄H₇O₃Na

Molecular Weight: 126.1 g/mol



Property value

Batch No.	CAS No.	Purity (mass fraction)
12-D-25	502-85-2	99.3 ± 0.7%

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 04 July 2026, 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 °C 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 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.
25 July 2023

This report supersedes any issued prior to 25 July 2023.

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 ^1H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

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)
 Moisture content 0.6% mass fraction (July 2023)

Spectroscopic and other characterisation data

LC-MS:	Instrument: Column: Column temp: Solvent system: Flow rate: Sample prep: Injection volume: Ionisation mode: Capillary voltage: Cone voltage: Source temp: Desolvation gas temperature: Cone gas flow rate: Desolvation gas flow rate:	Waters 2695 (HPLC)/Micromass Quatro Ascentis C-18, 150 mm \times 4.6 mm I.D. \times 2.7 μ m 40 $^{\circ}$ C A 2% Formic acid in MilliQ water [10% v/v] B Acetonitrile [15% v/v] C MilliQ water [75% v/v] 0.15 mL/min 100 μ g/g in MeOH/MilliQ water (25:75) 30 μ L Electrospray negative ion 3.0 kV 25 V 130 $^{\circ}$ C 350 $^{\circ}$ C 27 L/hr 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 ⁺) <i>m/z</i>
HS-GC-MS:	Instrument: Column: Program: Injector: Transfer line temp: Carrier: Split ratio: Solvents detected:	Agilent 6890/5973/G1888 DB-624, 30 m \times 0.25 mm I.D. \times 1.4 μ m 50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min) 150 $^{\circ}$ C 280 $^{\circ}$ C Helium, 1.2 mL/min 50/1 Acetone
IR:	Instrument: Range: Peaks:	Biorad FTS3000MX FT-IR 4000-400 cm^{-1} , KBr powder 3341, 2941, 2875, 1554, 1420, 1069, 1017, 922, 869, 668 cm^{-1}
^1H NMR:	Instrument: Field strength: Solvent: Spectral data:	Bruker Avance-400 400 MHz D ₂ O (4.79 ppm) 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 ^1H NMR.
^{13}C NMR:	Instrument: Field strength: Solvent: Spectral data:	Bruker Avance-400 101 MHz D ₂ O δ 28.4, 34.0, 61.5, 183.1 ppm
Melting point:		146-149 $^{\circ}$ C
Microanalysis:	Found: Calculated:	C = 37.9%; H = 5.5% (March 2013) C = 38.1%; H = 5.6% (Calculated for C ₄ H ₇ O ₃ Na)