Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

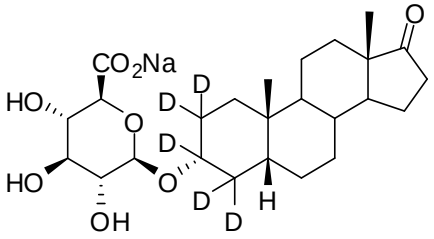
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S020: d_5-Etiocholanolone glucuronic acid sodium salt

Report ID: S020.2019.03 (Ampouled 160714)

Chemical Formula: $C_{25}H_{32}D_5O_8Na$

Molecular Weight: 493.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
13-S-01	**Not available**	**869 ± 25 μg**

IUPAC name: Sodium (3α,5β)-17-Oxo(2,2,3,4,4-$^2H_5$)androstan-3-yl β-D-glucopyranosiduronate

Expiration of certification: The property values are valid till 21 June 2024, i.e. five 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 shelf life does not apply to ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing S020. The material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer approximately 896 μg of anhydrous etiocholanolone glucuronic acid sodium salt (d_5, d_4, d_3, d_2, d_1 and d_0).

Recommended storage: When not in use, this material should be stored at or below 4 ºC in a closed container in a dry, dark area.

Stability: In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The long-term stability of the compound in solution has not been examined.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with ELS detection on seven randomly selected ampoules 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.
16 November 2022.

This report supersedes any issued prior to 16 November 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:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5µm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (65:35) 0.5% Formic acid was present in the aqueous phase.
	Flow Rate:	1.0 mL/min
	Detector:	Shimadzu ELS detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.08% (7 ampoules in duplicate, July 2016)
	Re-analysis:	Mean = 99.7%, s = 0.06% (5 sub samples in duplicate, June 2019)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with ELS detection, thermogravimetric analysis, 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 qualitative elemental microanalysis.

The main component of this material is d_5-etiocholanolone-β-glucuronic acid sodium salt. d_4, d_3-, d_2-, d_1- and d_0- Etiocholanolone-β- glucuronic acid sodium salt are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0- etiocholanolone-β- glucuronic acid sodium salt in the material.

The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Isotopic Purity:	$d_5 \approx 93\%$ [= $d_5/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	$d_0 < 0.2\%$ [= $d_0/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (65:35) 0.5% Formic acid was present in the aqueous phase.
	Flow rate:	1 mL/min
	Detector:	Waters 2424 ELS detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.1% (7 sub samples in duplicate, January 2013)
	Re-analysis:	Mean = 99.6%, s = 0.1% (5 sub samples in duplicate, January 2016)
Thermogravimetric analysis:		Volatile content 7.8% and non volatile residue 7.1% mass fraction (February 2013)
Karl Fischer analysis:		Moisture content 10.4% mass fraction (March 2013) Moisture content 10.4% mass fraction (January 2016)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Waters Acquity UPLC/TQD
	Ionisation mode:	Electrospray negative ion, direct infusion at 5 µL/min
	Capillary voltage:	3.0 kV
	Cone voltage:	55 V
	Source temp:	80 ºC
	Desolvation gas temp:	150 ºC
	Cone gas flow rate:	2 L/hr
	Desolvation gas flow:	550 L/hr
	Peak:	470.3 (M-Na+) *m/z*
GC-MS:	The free steroid was liberated upon treatment with β-glucuronidase enzyme (E. Coli K12) and derivatised with MSTFA.	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm
	Program:	180 °C, 3 °C /min to 240 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C
	Injector:	260 ºC
	Transfer line temp:	300 ºC
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	14/1
	The retention time of the *bis*-TMS derivative of d_5-etiocholanolone is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Bis-TMS (9.4 min):	439 (M+, 23), 424 (36), 334 (20), 244 (12), 182 (12), 169 (20), 73 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (4/1) Single spot observed, R_f = 0.17. Visualisation with vanillin
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm-1, KBr powder
	Peaks:	3615, 3520, 3454, 2934, 2923, 2866, 2853, 2200, 2130, 1730, 1612, 1408, 1376, 1295, 1167, 1087, 1070, 1042, 1027 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-400
	Field strength:	400 MHz
	Solvent:	CD_3OD (3.31 ppm)
	Spectral data:	δ 0.87 (3H, s), 0.98 (3H, s), 1.01 (1H, s), 1.20-1.45 (6H, m), 1.50-1.67 (5H, m), 1.75-1.84 (2H, m), 1.89-1.99 (2H, m), 2.08 (1H, m), 2.44 (1H, dd, *J* = 8.5, 19.2 Hz), 3.18 (1H, dd, *J* = 8.0, 8.9 Hz), 3.39 (1H, t, *J* = 8.8 Hz), 3.43 (1H, t, *J* = 8.7 Hz) 3.55 (1H, d, *J* = 9.4 Hz), 4.40 (1H, d, *J* = 7.7 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-400
	Field strength:	101 MHz
	Solvent:	CD_3OD (49.0 ppm)
	Spectral data:	δ 14.2, 21.2, 22.8, 23.8, 26.5, 28.0, 33.0, 35.9, 36.1, 36.7, 36.8, 42.1, 43.4, 49.17, 52.8, 73.3, 75.0, 76.2, 77.9, 101.8, 176.9, 224.5 ppm
Melting point:		> 220 ºC decomposition
Microanalysis:	Found:	C = 55.0%; H = 7.7% (February, 2013)
	Calculated:	C = 60.8%; H = 7.7% (Calculated for $C_{25}H_{32}D_5O_8Na$)
	Calculated:	C = 54.8%; H = 8.0% (Calculated for $C_{25}H_{32}D_5O_8Na$ + 10% H_2O)