Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA
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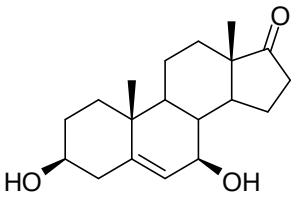
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D865b: 7β-Hydroxy DHEA

Report ID: D865b.2020.01 (Ampouled 160811)

Chemical Formula: $C_{19}H_{28}O_3$

Molecular Weight: 304.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
13-S-04	2487-48-1	985 ± 18 μg

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

IUPAC name: (3β,7β)-3,7-Dihydroxyandrost-5-en-17-one.

Expiration of certification: The property values are valid till 15 July 2023, 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 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 under an atmosphere of argon. The CRM intended for a single use to prepare a standard solution containing D865b. This material was prepared by sourced from an external supplier and certified for identity and purity by NMIA.

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

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. acetonitrile). This will transfer 985 ± 18 μg of 7β-hydroxy DHEA. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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.

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%.

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.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by GC-FID 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 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.
28 July 2020

This report supersedes any issued prior to 28 July 2020.

Characterisation Report:

Warning: **This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250°C) into a GC instrument.**

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1 or HP-5, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.07% (7 ampoules in duplicate, September 2016)
	Re-analysis:	Mean = 98.7%, s = 0.03% (5 ampoules in duplicate, August 2017)
	Re-analysis:	Mean = 98.8%, s = 0.03% (5 ampoules in duplicate, July 2020)

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 certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

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

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents andelemental microanalysis.

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1 or HP-5, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.9%, s = 0.02% (10 sub samples in duplicate, May 2013)
	Re-analysis:	Mean = 98.9%, s = 0.09% (5 sub samples in duplicate, April 2014)
Karl Fischer analysis:		Moisture content ≤ 0.2% mass fraction (May 2013 and 2014)
Thermogravimetric analysis:		Volatile content 0.2% and non volatile residue < 0.2% mass fraction (June 2013)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973
Column:	TG1-MS, 30 m x 0.25 mm I.D. x 0.25 µm
Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)
Injector:	250 °C
Transfer line temp:	280 °C
Carrier:	Helium, 1.0 mL/min
Split ratio:	20/1

Tris-TMS derivative:

Instrument:	Agilent 6890/5973
Column:	TG1-MS, 30 m x 0.25 mm I.D. x 0.25 µm
Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (3 min)
Injector:	250 °C
Transfer line temp:	280 °C
Carrier:	Helium, 1.0 mL/min
Split ratio:	20/1

The retention times of the parent compound and *tris*-TMS derivative is/are reported along with the major peaks in the mass spectrum/spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (10.5 min): 304 (M^+, 2), 286 (100), 268 (12), 145 (11), 141 (10), 129 (13), 128 (13), 119 (23), 105 (19), 91 (25), 79 (16), 77 (12), 67 (10), 55 (13) *m/z*

Tris-TMS (12.3 min): 520 (M^+, 1), 505 (1), 430 (39), 415 (19), 358 (14), 325 (19), 235 (7), 221 (7), 181 (14), 169 (45), 129 (12), 105 (6), 75 (100), 73 (89) *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:	Ethyl acetate and a trace amount of hexane

TLC:

Conditions: Kieselgel 60F_{254}. Ethyl acetate
Single spot observed, R_f = 0.43. Visualisation with vanillin

IR:

Instrument:	Biorad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3474, 3447, 2948, 1726, 1456, 1376, 1349, 1285, 1053, 1038, 1028, 951, 833 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance-400
Field strength:	400 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.89 (3H, s), 1.04-1.14 (2H, m), 1.07 (3H, s), 1.25 (1H, dt, *J* = 4.3, 13.1 Hz), 1.33 (1H, d, *J* = 7.4 Hz), 1.40-1.73 (6H, m), 1.80-1.90 (4H, m), 2.10 1H, m), 2.21-2.37 (3H, m), 2.47 (1H, dd, *J* = 8.6, 19.1 Hz), 3.55 (1H, m), 3.95 (1H, m), 5.31 (1H, t, *J* = 1.9 Hz) ppm

Ethyl acetate estimated at 1.2% mass fraction was observed in the ^{1}H NMR.
Hexane content could not be quantified by ^{1}H NMR.

^{13}C NMR:

Instrument:	Bruker Advance 300
Field strength:	75.5 MHz
Solvent:	$CDCl_3$ (77.2 ppm)
Spectral data:	δ 13.6, 19.2, 20.4, 24.2, 31.2, 31.5, 36.0, 36.7, 36.9, 40.5, 41.7, 47.8, 48.2, 51.2, 71.3, 72.9, 125.5, 143.7, 221.0 ppm

Melting point: 210-213 °C

Microanalysis:

Found:	C = 75.1%; H = 9.3% (June, 2013)
Calculation:	C = 75.0%; H = 9.3% (Calculated for $C_{19}H_{28}O_3$)