Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

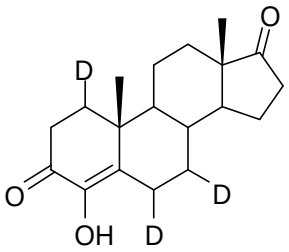
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA S046: 1,6,7-d_3-4-Hydroxyandrostendione

Report ID: S046.2022.01 (Ampouled 191017)

Chemical Formula: $C_{19}H_{23}D_3O_3$

Molecular Weight: 305.4 g/mol



Property value

Batch No.	CAS No.	Purity estimate by HPLC-UV
18-S-04	**N/A**	**98.5%**

Synonyms: 1,6,7-d_3-Formestane

Expiration of certification: The property values are valid till 17 May 2025, 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 shelf life does not apply to bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White powder prepared by synthesis, and certified for identity and purity by NMIA. The main component of this material is d_3-4-hydroxyandrostendione. d_2-, d_1- and d_0-4-Hydroxyandrostendione are also present. The stated purity of the analyte represents the combined masses of deuterated (d_3, d_2 and d_1) and d_0-4-hydroxyandrostendione in the material.

Intended use: This material has not been fully characterized and 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). Each ampoule contains approximately 200 μg of S046.

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: 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 UV 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.
28 July 2022.

This report supersedes any issued prior to 28 July 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, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (55:45 v/v)
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 276 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 98.9%, s = 0.011% (7 sub samples in duplicate, October 2019)
	Re-analysis:	Mean = 98.9%, s = 0.02% (5 sub samples in duplicate, May 2022)

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 organic purity value was obtained by HPLC with UV detection.

Supporting evidence is provided by ^{1}H NMR spectroscopy.

The main component of this material is d_3-4-hydroxyandrostendione. d_2-, d_1- and d_0-4-Hydroxyandrostendione are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_3, d_2 and d_1) and d_0-4-hydroxyandrostendione 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:		$d_3 \approx 48\%$ [= d_3 / (d_0 + d_1 + d_2 + d_3) × 100]
		$d_0 < 3.0\%$ [= d_0 / (d_0 + d_1 + d_2 + d_3) × 100]
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (60:40 v/v)
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 276 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.02% (7 sub samples in duplicate, August 2018)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	DB-5, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention time of the parent compound 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.	
	Parent (11.9 min):	305 (M^+, 75), 263 (19), 262 (15), 164 (16), 160 (16), 148 (100), 140 (32), 114 (75), 93 (27), 68 (20), 55 (27) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (65:35) Single spot observed, R_f = 0.3. Visualisation with UV at 254 n
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3380, 2945, 2915, 1735, 1661, 1382, 1257, 1178, 1043, 1011, 709, 651, 616, 585 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	MeOH-*d*$_4$ (3.31ppm)
	Spectral data:	δ 0.93 (3H, s), 1.01 (1.4H, m), 1.23 (3H, s), 1.24-1.37 (2H, m), 1.48 (1H, dddd, *J* = 13.6, 13.6, 13.6, 4.1 Hz), 1.59-1.82 (4.3H, m), 1.96-2.13 (4.3H, m), 2.37-2.49 (2H, m), 2.56 (1H m), 3.08 (0.4H, m) ppm
		Ethyl acetate estimated at 0.4% mass fraction was observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	MeOH-*d*$_4$ (49.0 ppm)
	Spectral data:	δ 14.1, 17.4, 21.4, 22.7, 23.5, 31.0, 32.6, 33.2, 35.9, 36.7, 38.9, 52.2, 52.3, 55.8, 141.3, 143.0, 195.3, 223.6 ppm
Melting point:		197-202 °C