



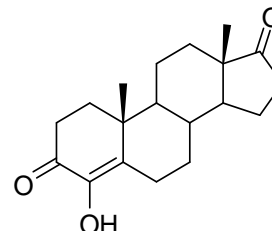
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D852b: 4-Hydroxyandrostenedione

Report ID: D852b.2018.02 (Ampouled 120206)

Chemical Formula: C₁₉H₂₆O₃

Molecular Weight: 302.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
11-S-11	566-48-3	984 ± 14 µg

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

IUPAC name: 4-Hydroxyandrost-4-ene-3,17-dione.

Expiration of certification: The property values are valid till 19 December 2023, 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 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 is intended for a single use to prepare a standard solution containing D852b. 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. methanol). This will transfer 984 ± 14 µg of anhydrous 4-hydroxyandrostenedione. 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 °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 approach all impurities are quantified as a mass fraction and subtracted from 100%.

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 stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last ten years. 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 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.
5 May 2020

This report supersedes any issued prior to 22 April 2020.

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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

Characterisation Report:

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
 Column: Grace Alltima C-18, 5 µm (4.6 mm x 150 mm)
 Column oven: 30 °C
 Mobile Phase: MilliQ water/methanol (40:60)
 Flow rate: 1.0 mL/min
 Detector: Waters PDA 2998 operating at 276 nm
 Relative mass fraction of the main component:
 Initial analysis: Mean = 98.6%, s = 0.02% (7 ampoules in duplicate, February 2012)
 Re-analysis: Mean = 98.7%, s = 0.6% (5 ampoules in duplicate, January 2013)
 Re-analysis: Mean = 98.0%, s = 0.02% (5 ampoules in duplicate, January 2016)
 Re-analysis: Mean = 98.4%, s = 0.07% (5 ampoules in duplicate, December 2018)

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 HPLC with UV detection, thermogravimetric analysis, 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 qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
 Column: Grace Alltima C-18, 5 µm (4.6 mm x 150 mm)
 Column oven: 30 °C
 Mobile Phase: MilliQ water/methanol (40:60)
 Flow rate: 1.0 mL/min
 Detector: Waters PDA 2998 operating at Max plot
 Relative mass fraction of the main component:
 Initial analysis: Mean = 98.7%, s = 0.05% (10 sub samples in duplicate, January 2012)
 Detector: Waters PDA 2998 operating at 276 nm
 Relative mass fraction of the main component:
 Initial analysis: Mean = 99.6%, s = 0.02% (10 sub samples in duplicate, January 2012)

Karl Fischer analysis: Moisture content < 0.2% mass fraction (November 2011)

Thermogravimetric analysis: Volatile content 0.2% and non-volatile residue < 0.2% mass fraction (November 2011)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μ m
	Program:	180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (2 min)
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ 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 (11.1 min):	302 (M^+ , 77), 260(22), 163 (15), 147 (100), 145 (28), 140 (22), 138 (33), 123 (22), 113 (48), 105 (22), 91 (31), 81 (20), 79 (32), 77 (21), 67 (27), 55 (26) <i>m/z</i>
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
	Program:	50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C /min to 220 $^{\circ}$ C (8.3 min)
	Injector:	150 $^{\circ}$ C
	Carrier:	Helium, 1.2 mL/min
	Transfer line temp:	280 $^{\circ}$ C
	Split ratio:	50/1
	Solvents detected:	No solvents detected
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate (65:35) Single spot observed, R_f = 0.27. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1} , KBr powder
	Peaks:	3406, 3384, 2945, 2884, 1736, 1662, 1628, 1450, 1382, 1239, 1162, 1098, 1024, 958, 901, 831, 788 cm^{-1}
¹ H NMR:	Instrument:	Bruker Avance-III-400
	Field strength:	400 MHz
	Solvent:	CDCl ₃ (7.26 ppm)
	Spectral data:	δ 0.91 (3H, s), 1.19 (3H, s), 3.08 (1H, m), 6.10 (1H, s) ppm
	Dichloromethane estimated at 0.07% mass fraction was observed in the ¹ H NMR	
¹³ C NMR:	Instrument:	Bruker Gyro-300
	Field strength:	75 MHz
	Solvent:	CDCl ₃ (77.2 ppm)
	Spectral data:	δ 13.7, 17.1, 20.3, 21.8, 22.7, 29.8, 31.4, 31.7, 34.7, 34.8, 35.8, 37.9, 47.5, 51.0, 54.3, 139.0, 141.3, 193.4, 220.4 ppm
Melting point:	202-205 $^{\circ}$ C	
Microanalysis:	Found:	C = 75.8%; H = 8.9% (December, 2011)
	Calculated:	C = 75.5%; H = 8.7% (Calculated for C ₁₉ H ₂₆ O ₃)