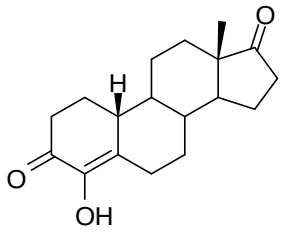
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: S043.2018.01 (Ampouled 170921)
This batch of ampoules was prepared from the bulk material on 21st September 2017.

Compound Name: **4-Hydroxyestrendione**
Collection Number: S043
Chemical Formula: $C_{18}H_{24}O_3$
CAS Number: 76251-16-6
Structure:

Description: White solid
Batch Number: 17-S-01
Molecular Weight: 288.4
Release Date: 1st August 2017



Synonyms: 4-Hydroxyestr-4-ene-3,17-dione

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 S043. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. acetonitrile). This will transfer 1002 ± 14 µg of anhydrous 4-hydroxyestrendione. The uncertainty is stated at the 95% coverage interval.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	X-Bridge C-18, 5 µm (4.6 mm x 250 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-22 min 40% B; 22-23 min 40-80% B; 23-25 min 80% B; 25-26 min 80-40% B; 26-30 min 40% B
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 272 nm
	Relative mass fraction of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (7 ampoules in duplicate, October 2017)
	Re-analysis:	Mean = 99.9%, s = 0.0004% (5 ampoules in duplicate, October 2018)

The following analytical data were obtained on the bulk material subsequently used in the preparation of the ampoules.
The purity value was obtained from a combination of traditional analytical techniques by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. Supporting evidence is provided by headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Waters Symmetry C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-22 min 40% B; 22-23 min 40-80% B; 23-25 min 80%B; 25-26 min 80-40%B; 26-30 min 40%B.
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 272 nm
	Relative mass fraction of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (10 sub samples in duplicate, May 2017)
Thermogravimetric analysis:		Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (May 2017)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (May 2017)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 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 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 (8.7 min):	288 (M^+, 100), 270 (10), 246 (11), 163 (13), 159 (19), 147 (19), 124 (37), 107 (23), 91 (28), 79 (28), 67 (20), 55 (21) *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 hexane
TLC:	Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (1/1) Single spot observed, R_f = 0.5. Visualisation with UV at 254 nm
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3387, 2956, 2922, 2859, 1726, 1649, 1619, 1376, 1344, 1335, 1207, 1157, 1082, 1041, 611, 582 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz Solvent: MeOH-d_4 (3.31ppm)
	Spectral data:	δ 0.88 (1H, m), 0.95 (3H, s), 1.07 (1H, m), 1.28-1.42 (3H, m), 1.43-1.56 (2H, m), 1.63 (1H, m), 1.79 (1H, m), 1.89-2.03 (4H, m), 2.10 (1H, ddd, *J* = 9.0, 9.0, 19.1 Hz), 2.21-2.29 (2H, m), 2.36 (1H, m), 2.44-2.50 (2H, m), 3.20 (1H, m) ppm Ethyl acetate estimated at 0.2% mass fraction was observed in the ^{1}H NMR.
^{13}C NMR:	Instrument:	Bruker Avance III 500
	Field strength:	126 MHz Solvent: MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 14.2, 22.6, 26.5, 26.7, 27.4, 30.1, 32.6, 36.1, 36.7, 40.7, 42.7, 51.1, 51.5, 136.8, 143.8, 196.0, 223.7 ppm
Melting point:		206-209 °C
Microanalysis:	Found:	C = 75.1%; H = 8.4 (May 2017)
	Calc:	C = 75.0%; H = 8.4% (Calculated for $C_{18}H_{24}O_3$)

Expiration of certification
The property values are valid till 8th October 2021, 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.

The long-term stability of the compound in solution has not been examined.

This material has been given a shelf life of three years from the date of re-certification. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

In the absence of 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 10 years.

Homogeneity assessment
The homogeneity of the material was assessed using purity assay by HPLC with UV detection on seven randomly selected 1 mg 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.

Metrological traceability
The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis.

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.

Intended use
This certified reference material may be used for instrument calibration.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 27 November, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 27th November 2018.