



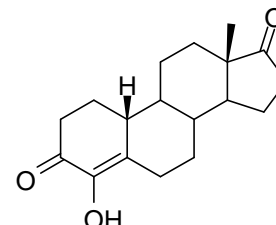
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA S043: 4-Hydroxyestrendione

Report ID: S043.2020.01 (Ampouled 170921)

Chemical Formula: C<sub>18</sub>H<sub>24</sub>O<sub>3</sub>

Molecular Weight: 288.4 g/mol



### Certified value

Batch No.	CAS No.	Mass per ampoule
17-S-01	76251-16-6	1002 ± 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-Hydroxyestr-4-ene-3,17-dione.

**Expiration of certification:** The property values are valid till 21 August 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 sample bottles 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 S043. This material was prepared by synthesis, 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 1002 ± 14 µg of anhydrous 4-hydroxyestrendione.

**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:** 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 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.  
30 September 2020

This report supersedes any issued prior to 30 September 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.

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### Characterisation Report:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters alliance 2695 separations Module
	Column:	X-Bridge C-18, 5 µm (4.6 mm x 250 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-18 min 40 % B; 18-19 min 40-80 % B; 19-24 min 80 % B; 24-25 min 80-40 % B; 25-30 min 40 % B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A or Waters 2998 PDA operating at 272 nm
	Relative mass fraction of the 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)
	Re analysis:	Mean = 99.98%, s = 0.004% (5 ampoules in duplicate, October 2019)
	Re analysis:	Mean = 99.98%, s = 0.004% (5 ampoules in duplicate, August 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 HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

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

Supporting evidence is provided by qualitative 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:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 272 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (10 sub samples in duplicate, May 2017)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (May 2017)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (May 2017)

## Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	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,
	Split ratio:	20/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 <i>m/z</i>
	The retention time of the parent compound is reported along with the major peaks in the mass spectra. 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) <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 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 <sub>254</sub> . 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 $cm^{-1}$
<sup>1</sup> H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	MeOH- <i>d</i> <sub>4</sub> (3.31 ppm)
	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, <i>J</i> = 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 <sup>1</sup> H NMR.
<sup>13</sup> C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH- <i>d</i> <sub>4</sub> (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)
	Calculated:	C = 75.0%; H = 8.4% (Calculated for C <sub>18</sub> H <sub>24</sub> O <sub>3</sub> )