Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

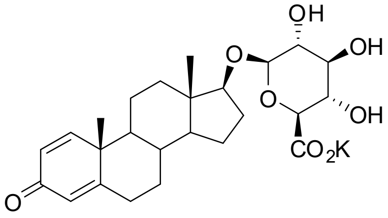
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D862: Boldenone glucuronide (potassium salt)

Report ID: D862.2024.02 (Ampouled 201126)

Chemical Formula: $C_{25}H_{33}O_8K$

Molecular Weight: 500.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
03-S-17	**Not available**	**998 ± 15 μg**

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

IUPAC name: (17β)-3-Oxoandrosta-1,4-dien-17-yl-β-D-glucupyranosiduronic acid potassium salt.

Expiration of certification: The property values are valid till 10 October 2029, 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 D862. This material was 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 998 ± 15 μg of anhydrous boldenone glucuronide (potassium salt). 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 all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of five 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.
10 December 2025

This report supersedes any issued prior to 10 December 2025.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Hichrom C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water (20 mM NH_4OAc buffer, pH 4.2); B = Acetonitrile 0-10 min 23% B; 10-17 min 23-80% B; 17-20 min 80%B; 20-22 min 80-23%B; 22-33 min 23%B
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 246 nm.
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.9%, s = 0.01% (7 sub samples in duplicate, December 2020)
	Re analysis:	Mean = 98.8%, s = 0.04% (5 ampoules in duplicate, October 2024)

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 ^{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 elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler Waters alliance 2695
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm) Hichrom C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water (20 mM NH_4OAc buffer, pH 4.2); B = Acetonitrile 0-10 min 23% B; 10-17 min 23-80% B; 17-20 min 80%B; 20-23 min 80-23%B; 23-28 min 23%B
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 246 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.03% (5 sub samples in duplicate, January 2004)
	Re-analysis:	Mean = 98.9%, s = 0.07% (5 sub samples in duplicate, June 2005)
	Re-analysis:	Mean = 98.9%, s = 0.05% (5 sub samples in duplicate, July 2007)
	Re-analysis:	Mean = 98.8%, s = 0.02% (7 sub samples in duplicate, June 2010)
	Re-analysis:	Mean = 98.8%, s = 0.01% (7 sub samples in duplicate, December 2020)

Karl Fischer analysis:	Moisture content 3.3% mass fraction (July 2006 & July 2007) Moisture content 3.6% mass fraction (March 2010) Moisture content 4.4% mass fraction (November 2020)
Thermogravimetric analysis:	Volatile content 4.7% mass fraction (March 2004, December 2005 & July 2006)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro Micro
	Operation:	Negative ion mode, direct infusion at 5 μL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	40 V
	Peak:	461.3 (M-K) *m/z*
TLC:	Conditions:	Kieselgel $60F_{254}$. Ethyl acetate/methanol/acetic acid (67:30:3) Single spot observed, R_f = 0.3. Visualization with vanillin
IR:	Instrument:	BioRad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3429, 3284, 2936, 1666, 1612, 1407, 1295, 1159, 1061, 886 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	D_2O (4.79 ppm)
	Key spectral data:	δ 0.86 (3H, s), 1.25 (3H, s), 2.39 (1H, bd), 2.54 (1H, ddd), 3.75 (1H, t), 4.45 (1H, d), 6.13 (1H, s) 6.28 (1H, d), 7.42 (1H, d) ppm ^{1}H NMR confirmed the presence of methanol in 0.8% mass fraction (February 2021)
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz
	Solvent:	D_2O
	Spectral data:	δ 11.4, 18.1, 22.9, 23.2, 28.2, 33.1, 33.8, 35.0, 37.5, 43.4, 45.3, 50.3, 53.4, 72.4, 73.7, 76.1, 76.8, 88.9, 102.7, 122.5, 126.2, 161.7, 175.9, 176.5, 189.7 ppm
Melting point:		286 ºC (decomposition)
Microanalysis:	Found:	C = 57.2%; H = 6.8% (December 2003)
	Calculated:	C = 57.2%; H = 6.9% (Calculated based on the original observation of 2% mass fraction methanol and 4% mass fraction water in 2004 and 2006)