Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

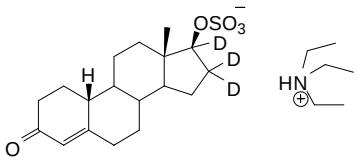
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D782b: d_3-Nandrolone sulfate (triethylammonium salt)

Report ID: D782b.2023.01 (Ampouled 090107)

Chemical Formula: $C_{24}H_{38}D_3NO_5S$

Molecular Weight: 458.7 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
08-S-04	Not available	637 ± 74 µg

IUPAC name: Triethylammonium (17β)-3-Oxo(16,16,17-$^2H_3$)estr-4-en-17-yl sulfate.

Expiration of certification: The property values are valid till 30 May 2028, 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 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 RM is intended for a single use to prepare a standard solution containing D782b. The material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: 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). This will transfer approximately 637 ± 74 µg of anhydrous nandrolone sulfate anion (d_3, d_2, d_1 and d_0). 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.

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 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.
11 August 2023.

This report supersedes any issued prior to 11 June 2023.

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:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/ MilliQwater
		The aqueous phase was buffered at pH 4.2 using 10mM NH4OAc
		Gradient 0-5 min 75% A, 10-18 min 40% A, 20-26 min 75% A
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 2998 operating at 242 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 95.97%, s = 0.18% (7 ampoules in duplicate, January 2009)
	Re-analysis:	Mean = 90.1%, s = 0.5% (5 ampoules in duplicate, February 2012)
	Re-analysis:	Mean = 84%, s = 0.9% (5 ampoules in duplicate, January 2015)
	Re-analysis:	Mean = 81.4%, s = 1.0% (6 ampoules in duplicate, November 2017)
	Re-analysis:	Mean = 79.6%, s = 2.5% (5 ampoules in duplicate, August 2020)
	Re-analysis:	Mean = 79.4%, s = 3.7% (7 ampoules in duplicate, May 2023)

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 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.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

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

Supporting evidence is provided by qualitative elemental microanalysis.

The main component of this material is d_3-nanadrolone sulfate. d_2-, d_1- and d_0-nandrolone sulfate 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-nandrolone 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:	$d_4 \approx 92\%$ [= $d_3/(d_3 + d_2 + d_1 + d_0) \times 100$]	
	$d_0 < 0.5\%$ [= $d_0/(d_3 + d_2 + d_1 + d_0) \times 100$]	
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/MilliQ water
		The aqueous phase was buffered at pH 4.2 using 10mM NH_4OAc
		Gradient 0-5 min 75% A, 10-18 min 40% A, 20-26 min 75% A
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 at 254 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 96.6%, s = 0.06% (10 sub samples in duplicate, May 2008)
Thermogravimetric analysis:		Initial volatile content < 0.1% and non volatile residue < 0.2%mass fraction (July 2008)
Karl Fischer analysis:		Moisture content 0.3% mass fraction (February 2008)

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:	30 V
	Peak:	356 ($M\text{-}Et_3NH^+$) *m/z*
TLC:	Conditions:	Kieselgel $60F_{254}$. Chloroform/methanol (17/3) Single spot observed, R_f =0.3. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3034, 2909, 2518, 2361, 1680, 1622, 1472, 1260, 1196, 602 cm^{-1}
^1H NMR:	Instrument:	DMX 600
	Field strength:	600 MHz
	Solvent:	$CDCl_3$
	Spectral data:	δ 0.85 (3H, s), 0.8-0.9 (1H, m), 1.0-1.1 (2H, m), 1.2-1.4 (4H, m) 1.41 (“6.7” H, t, *J* = 7.3 Hz), 1.51 (1H, m), 1.62 (1H, dd, J = 7.3, 12.3 Hz), 1.80 (2H, m), 1.97 (1H, m), 2.07 (1H, m), 2.2-2.3 (3H, m), 2.40 (1H, m), 2.48 (1H, dd, *J* = 1.5, 7.9 Hz), 3.05 (“4.7”H, t, *J* = 7.3 Hz) 5.83 (1H, s) ppm
^{13}C NMR:	Instrument:	DMX 600
	Field strength:	150 MHz
	Solvent:	MeOH-d_4 (48.0 ppm)
	Spectral data:	δ 10.6, 11.1, 22.9, 26.1, 26.7, 30.9, 35.5, 36.3, 36.7, 40.4, 42.6, 42.7, 42.9, 49.5, 50.0, 123.8, 169.8, 201.9 ppm