Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

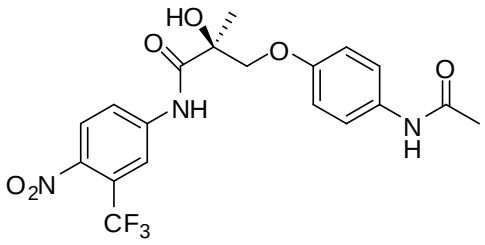
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D1074: Andarine

Report ID: D1074.2023.01

Chemical Formula: $C_{19}H_{18}F_3N_3O_6$

Molecular Weight: 441.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
20-D-03	**401900-40-1**	**99.4 ± 0.9%**

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

IUPAC name: (2S)-3-(4-Acetamidophenoxy)-2-hydroxy-2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide. The stereochemistry of the main component detailed in the structure above and the IUPAC name is based on literature precedents and has not been verified for this material. The enantiomeric purity of this material has also not been verified.

Expiration of certification: The property values are valid till 15 March 2026, 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. 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.

Description: Yellow powder sourced from an external supplier, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 25 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 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 1-2 mg sub samples 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.
17 March 2023

This report supersedes any issued prior to 17 March 2023.

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

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained from a combination of traditional analytical techniques used in the mass balance approach 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 elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT or Waters alliance 2695
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40°C
	Mobile Phase:	A = Milli-Q water; B = Acetonitrile 0-20 min 40% B; 20-25 min 40-80% B; 25-28 min 80% B; 28-30 min 80-40% B, 30-45 min 40% B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA or Waters 2998 PDA operating at 240 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.04% (7 sub samples in duplicate, August 2020)
	Re-analysis:	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, July 2021)
	Re-analysis:	Mean = 99.5%, s = 0.20% (5 sub samples in duplicate, May 2022)
	Re-analysis:	Mean = 99.5%, s = 0.03% (5 sub samples in duplicate, March 2023)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (August 2020, June 2021, May 2022 and March 2023)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (September 2020)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters Alliance/ Micromass Quattro TQ Detector
	Column:	Alltima C-18, 150 mm x 4.6 mm I.D. x 5 μm
	Column temp:	40 °C
	Solvent system:	A = 0.1% formic acid; B = Acetonitrile 0-20 min 40% B; 20-25 min 40-80% B; 25-28 min 80% B; 28-30 min 80-40% B.
	Flow rate:	0.2 mL/min
	Sample prep:	50 μg/g in acetonitrile/Milli-Q water (40:60)
	Injection volume:	10 μL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	2.5 kV
	Cone voltage:	30 V
	Source temp:	120 ºC
	Desolvation gas temp:	396 ºC
	Cone gas flow rate:	1 L/hr
	Desolvation gas flow:	600 L/hr
	The retention time of andarine is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	12.98 min:	442.01 $(M+H)^+$ *m/z*
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3340, 3262, 1688, 1638, 1508, 1325, 1276, 1233, 1144, 1044, 920, 857, 821, 754, 637, 580, 446 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 1.43 (3H, s), 1.99 (3H, s), 3.95 (1H, d, *J* = 9.5 Hz), 4.18 (1H, d, *J* = 9.5 Hz), 6.27 (1H, br s), 6.85 (2H, d, *J* = 9.0 Hz), 7.44 (2H, d, *J* = 9.0 Hz), 8.19 (1H, d, *J* = 9.0 Hz), 8.36 (1H, dd, *J* = 2.0, 9.0 Hz), 8.57 (1H, d, *J* = 2.0 Hz), 9.76 (1H, s), 10.62 (1H, br s) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	DMSO-d_6 (39.52 ppm)
	Spectral data:	δ 23.1, 23.8, 73.9, 74.9, 114.7, 118.3 (J_{CF} = 6.3 Hz), 120.5, 122.1 (J_{CF} = 272.8 Hz), 122.7 (J_{CF} = 32.7 Hz) 123.1, 127.4, 132.8, 141.7 (J_{CF} = 1.5 Hz), 143.3, 154.3, 167.8, 174.7 ppm
Melting point:		152-154 °C
Microanalysis:	Found:	C = 51.7%; H = 3.9%; N = 9.5% (June, 2020)
	Calculated:	C = 51.7%; H = 4.1%; N = 9.5% (Calculated for $C_{19}H_{18}F_3N_3O_6$)