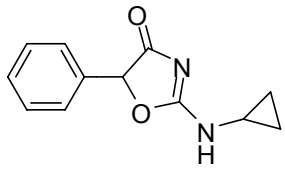
CERTIFIED REFERENCE MATERIAL
CERTIFICATE OF ANALYSIS

Report ID: D914.2014.01

Compound Name: **Cyclazodone**
Collection Number: D914
Chemical Formula: $C_{12}H_{12}N_2O_2$
CAS Number: 14461-91-7
Structure:



Description: White solid
Batch Number: 06-D-015
Molecular Weight: 216.2
Release Date: 4th April 2007

Synonyms: 5-Phenyl-2-cyclopropylamino-4-oxazolinone
4(5H)-Oxazolone, 2-(cyclopropylamino)-5-phenyl-
Cyclopropylpemoline

Purity (mass fraction): 99.6 ± 1.4 % (95 % confidence interval)

The purity value was obtained from a combination of traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by GC-FID thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890N	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	100 °C (1 min), 15 °C/min to 300 °C (3 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area response of main component:		
	Initial analysis:	Mean = 99.9%, s = 0.01% (7 sub samples in duplicate, January 2007)	
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, January 2010)	
	Re-analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, November 2014)	
Thermogravimetric analysis:		Volatile content < 0.1% and non volatile residue < 0.2 % mass fraction	
Karl Fischer analysis:		Moisture content < 0.3% mass fraction (January 2008, 2009 & 2010) Moisture content 0.2% mass fraction (October 2014)	

Spectroscopic and other characterization data

GC-MS:	Instrument:	HP 6890/5973
	Column:	ZB-5, 30 m × 0.25 mm I.D. × 0.25 μm
	Program:	100 °C (1 min), 15 °C/min to 300 °C, (2 min)
	Injector:	200°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 to charge ratios and (in brackets) as a percentage relative to the base peak.	
	10.9 min: 216 (M^+, 6), 189 (6), 132 (17), 118 (100), 90 (26), 77 (25), 68 (24), 55 (25) m/z	
TLC:	Conditions:	Kieselgel 60F_{254}. Dichloromethane / methanol (90/10) Single spot observed, R_f = 0.58. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3251, 3036, 2945, 1745, 1651, 1485, 1387, 1342, 1256, 1199, 1013, 816, 715cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz Solvent: DMSO-d_6 (2.5 ppm)
	Spectral data:	δ 0.64 (2H, m), 0.75 (2H, m), 2.82 (1H, m), 5.73 (0.58H, s), 5.79 (0.42H, s), 7.28 – 7.43 (5H, m), 9.13 (0.59H, bs), 9.44 (0.41H, bs) ppm. (2 rotational isomers observed)
^{13}C NMR:	Instrument:	Bruker DMX600
	Field strength:	125 MHz Solvent: DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 5.88, 5.91, 6.0, 23.0, 24.9, 82.1, 82.8, 126.2, 126.3, 128.7, 134.4, 134.5, 176.4, 185.4, 185.9 ppm. (2 rotational isomers observed)
Melting point:		138-143°C
Microanalysis:		Found: C = 67.0 %; H = 5.7 %; N = 13.0 % (December 2006) Calc: C = 66.7 %; H = 5.6 %; N = 13.0 % (Calculated for $C_{12}H_{12}N_2O_2$)

The Synthesis and Certification of this Reference Material is supported by the Australian Government through the *Anti-Doping Research Program (ADRP)* of the Department of Communications, Information Technology and the Arts.

Expiration of certification
The property values are valid till 11th November 2019, i.e. 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 sample bottles 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 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.

Homogeneity assessment
The homogeneity of the material was assessed using purity assay by GC-FID on seven randomly selected 1-2 mg samples of the material. The material was judged to be 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 25 ºC in a closed container in a dry, dark area.

Intended Use
For ***in vitro*** laboratory analysis only.

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: 8 December, 2014.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 8th December 2014.