Australian Government

National Measurement Institute

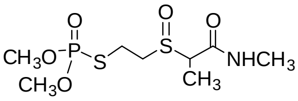
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

Report ID: P1729.2018.01

Compound Name: **Vamidothion sulfoxide**
Collection Number: P1729
Chemical Formula: $C_8H_{18}NO_5PS_2$
CAS Number: 20300-00-9

Description: Colourless oil
Batch Number: 01-AV-04
Molecular Weight: 303.3
Release Date: 15 November 2004

Structure:



Synonym: *O, O*-Dimethyl S-[2-[[1-methyl-2-(methylamino)-2-oxoethyl] sulfinyl] ethyl] phosphorothioate

Purity (mass fraction): 85.0 ± 2.6% (95% coverage interval)

Purity estimate obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR. The purity estimate by qNMR was obtained using a certified internal standard of triphenylphosphine oxide. Supporting evidence is provided by headspace GC-MS analysis of occluded solvent, elemental microanalysis and NMR analysis.

QNMR:
Instrument: Bruker Avance-500
Field strength: 162 MHz Solvent: d_6-DMSO
Internal standard: Triphenylphosphine oxide (100% mass fraction)
Initial analysis: Mean (29.2 ppm) = 89.12%, s = 0.49% (5 sub samples, January 2012
Re-analysis: Mean (29.2 ppm) = 90.56%, s = 1.21% (5 sub samples, June 2012)
Re-analysis: Mean (29.1 ppm) = 91.32%, s = 0.23% (5 sub samples, May 2013)
Re-analysis: Mean (29.2 ppm) = 85.01%, s = 0.13% (5 sub samples, May 2018)

HPLC:
Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: Alltech Prevail C-18, 3μm (100 mm × 2.1 mm)
Mobile Phase: Solvent A: acetonitrile
Solvent B: water
Gradient, 0 min 95% B, 0-7 min 75% B, 7-17 min 75% B
Flow rate: 0.3 mL/min
Detector: Waters PDA 996 operating at 218 nm
Relative peak area response of main component:
Initial analysis: Mean = 99.1%, s = 0.28% (10 sub samples in duplicate, December 2001)
Re-analysis: Mean = 98.5%, s = 0.02% (5 sub samples in duplicate, March 2006)

HPLC:
Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
Column: Waters X-bridge C-18, 5μm (150 mm × 4.6 mm)
Mobile Phase: 30% Acetonitrile/70% water, isocratic
Flow rate: 0.3 mL/min
Detector: Shimadzu SPD-M20A PDA operating at 200 nm
Relative peak area response of main component:
Initial analysis: Mean = 95.7%, s = 0.1% (5 sub samples in duplicate, April 2011)

Thermogravimetric analysis: Volatile content < 2.6% and non volatile residue 0.3% mass fraction (December 2001 and September 2006)

Karl Fischer analysis:
Moisture content 1.6% mass fraction (March 2011)
Moisture content 2.8% mass fraction (April 2012)
Moisture content 2.9 % mass fraction (May 2013)
Moisture content 4.8% mass fraction (May 2018)

Spectroscopic and other characterisation data

LC/ESI -MS:	Instrument:	Micromass Quatro LC Micro	
	Operation:	Negative ion mode and positive ion mode, direct infusion at 13 μL/min	
	Ionisation:	ESI spray voltage at 3.5 kV for negative ion mode and 4.5 kV for positive ion mode	
	Peak:	362 ($M+CH_3COO^-$, 100), 288 ($M-CH_3^+$), 141 m/z from negative ion mode 326 ($M+Na^+$), 321 ($M+NH_4^+$), 304 ($M+H^+$, 100) m/z from positive ion mode	
HRMS:		Found m/z 304.0453 (MH^+) for $C_8H_{18}NO_5PS_2$; requires m/z 304.0442 (-3.5 ppm)	
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:	Chloroform	
IR:	Instrument:	Biorad FTS3000MX FT-IR	
	Range:	4000-400 cm^{-1}, KBr powder	
	Peaks:	3300, 3086, 2954, 1673, 1560, 1454, 1256, 1183, 1028, 773 cm^{-1}	
^1H NMR:	Instrument:	Bruker DMX-300	
	Field strength:	300 MHz	Solvent: d_6-benzene
	Spectral data:	δ 1.19, 1.28 (3H, d, J = 7.2 Hz), 2.56, 2.59 (3H, d, J = 4.9 Hz), 2.85-3.20 (5H, m), 3.31, 3.33, 3.35, 3.38 (6H, d, J_{PH} = 0.8 Hz), 6.83, 6.88 (1H, s) ppm This material exists as a pair of inseparable diastereoisomers and as a consequence, all resonances are duplicated in both the ^1H and ^{13}C NMR spectra. The two phosphorothioate methyl ester resonances appear as two pair of doublets due to phosphorus-hydrogen coupling. Chloroform estimated at 1.6% mass fraction was observed in the ^1H NMR	
^{13}C NMR:	Instrument:	Bruker DMX-300	
	Field strength:	75.5 MHz	Solvent: d_6-benzene
	Spectral data:	δ 10.7, 12.1; 24.8; 26.4, 26.5; 49.1, 51.1; 53.92; 53.97; 59.4, 59.5; 167.5, 168.4 ppm Resonances are duplicated due to the diastereoisomers.	
Microanalysis:		Found: C = 31.4%, H = 6.0%, N = 4.6%, S = 20.8% (September, 2001) Calc: C = 31.7%, H = 6.0%, N = 4.6%, S = 21.1% (Calculated for $C_8H_{18}NO_5PS_2$ + 1.6% $CHCl_3$)	

Expiration of certification

The property values are valid till 15th May 2023, 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% coverage 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 HPLC with UV detection on ten 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.

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. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

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.

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: 1 June, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 1st June 2018.

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