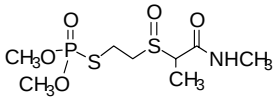
REFERENCE MATERIAL ANALYSIS REPORT

Compound Name: **Vamidothion sulfoxide**
Collection No: P1729
Chemical Formula: $C_8H_{18}NO_5PS_2$
CAS Number: 20300-00-9
Structure:



Description: Colourless oil
Batch No: 01-AV-04
Molecular Weight: 303.3
Synthesised: August 2001

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

Purity (mass fraction): 95.9 ± 1.2% (95% confidence interval)
Purity estimate obtained by subtraction from 100% of total impurities by HPLC-UV, TGA, and ^{1}H NMR.

HPLC:
- Column: Alltech Prevail C18, 3µm (100 mm × 2.1 mm)
- Mobile Phase: 5% Acetonitrile/95% water, raise to 25% acetonitrile in 7 minutes, elute for 10 minutes
- Flow Rate: 0.3 mL/min
- Detector: UV 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)
Current re-analysis: Mean = 98.5%, s = 0.02 (5 sub samples in duplicate, October 2006)

ESI-MS:
- Instrument: Finnigan MAT TSQ 700 with electrospray interface
- Operation: Negative ion mode and positive ion mode, direct infusion at 13 µl/min
- Solvent: ammonium acetate buffer (7.5 mM, pH 7.5) / methanol, 1:1
- 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 a.m.u. from negative ion mode 326 ($M+Na^+$), 321 ($M+NH_4^+$), 304 ($M+H^+$, 100) a.m.u. from positive ion mode

IR:
- Instrument: FT-IR, Biorad FTS3000MX
- Range: 4000-400 cm^{-1}, KBr powder
- Peaks: 3300, 3086, 2954, 1673, 1560, 1454, 1256, 1183, 1028, 773 cm^{-1}

The IR spectrum shows a very strong absorbance at 1028 cm^{-1} due to S=O stretching.

^{1}H 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 ^{1}H and ^{13}C NMR spectra. The two phosphorothioate methyl ester resonances appear as two pair of doublets due to phosphorus-hydrogen coupling. An estimate of 1.6% (m/m) of residual chloroform was observed.

^{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% Calc: C = 31.7%, H = 6.0%, N = 4.6%, S = 21.1% The microanlaysis supports the presence of chloroform in 1.6% mass fraction.
HRMS:	Found m/z 304.0453 (MH^+) for $C_8H_{18}NO_5PS_2$; requires m/z 304.0442 (-3.5 ppm)
Thermogravimetric analysis:	Volatile content < 2.6% and non-volatile residue = 0.3% mass fraction (December 2001 & September 2006)
Homogeneity:	The homogeneity of the material was assessed using purity assay by HPLC on ten randomly selected 1 mg samples of the material. The material was judged to be homogeneous as the variation between samples was not significantly different from the analytical variation observed on repeat analysis of the same sample.
Expiration of certification:	The property values are valid till 6th October 2011, i.e. five years from the date of re-certification, provided the 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 long-term stability of the compound in solution has not been examined.
Recommended storage:	When not in use store at or below 4 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:	 Dr Laurie Besley, General Manager, Chemical & Biological Metrology, NMI Dated: 11 October, 2006.
Report ID:	P1729.2006.01 Characterisation data and property values specified in this report supercede those in all reports issued prior to 6th October 2006.

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