Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

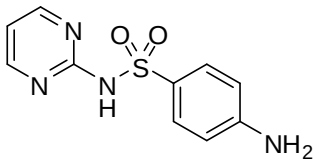
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA M317: Sulfadiazine

Report ID: M317.2017.02

Chemical Formula: $C_{10}H_{10}N_4O_2S$

Molecular Weight: 250.3 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
16-AV-01	**68-35-9**	**99.7 ± 0.4%**

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

IUPAC name: 4-Amino-N-(2-pyrimidinyl)benzenesulfonamide

Expiration of certification: The property values are valid till 13 July 2020, 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.

Description: Off-white 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 or screw top 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%. 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).

Stability: In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last ten years. 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 nine 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.
28 April 2020

This report supersedes any issued prior to 28 April 2020

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified 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 headspace GC-MS analysis of occluded solvents, elemental microanalysis and qNMR obtained using a combination of the one-proton doublet at 7.62 ppm and the one-proton doublet at 8.22 ppm measured against a certified internal standard of potassium hydrogen maleate.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	ambient
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-5 min 5% B; 35-40 min 10% B; 50-55 min 80%B; 56-69 min 5%B. 0.1 % formic acid was present in aqueous phase.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 265 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.8%, s = 0.1% (10 sub samples in duplicate, May 2016)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	ambient
	Mobile Phase:	A =0.1 % formic acid in MilliQ water, B = Acetonitrile 0-25 min 5%-25% B, 25-28 min 25%-80% B, 28-32 min 80% B, 32-33 min 80%-5% B, 33-40 min 5% B
	Flow rate:	1 mL/min
	Detector:	Waters 2998 PDA operating at 265 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, July 2017)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (May 2016 and March 2017)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (May 2016)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	D_2O/NaOD (4.79 ppm)
	Internal standard:	Potassium hydrogen maleate (100.0% mass fraction)
	Initial analysis:	Mean (7.62 ppm) = 99.9%, s = 0.5% (5 sub samples, April 2016)
	Initial analysis:	Mean (8.22 ppm) = 100.0%, s = 0.4% (5 sub samples, April 2016)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters Acquity/Waters TQ Detector
	Column:	Acquity BEH C-18, 150 mm × 2.1 mm I.D. × 1.7 μm
	Column temp:	40 °C
	Solvent system:	A = 0.1% formic acid in MilliQ water; B = Acetonitrile 0-5 min 5% B; 5-35 min 5-10% B; 35-40 min 10% B; 41-50 min 10-80% B.
	Flow rate:	0.3 mL/min
	Sample prep:	20 μg/g in 0.025% ammonia
	Injection volume:	10 μL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	3.0 kV
	Cone voltage:	35 V
	Source temp:	120 ºC
	Desolvation gas temp:	350 ºC
	Cone gas flow rate:	1 L/hr
	Desolvation gas flow:	600 L/hr
	The retention time of sulfadiazine is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	5.94 min:	251.04 ($M+H^+$) *m/z*
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:	None
IR:	Instrument:	Bruker Alpha FT-IR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3422, 3353, 1578, 1489, 1440, 1325 1153, 939, 796, 681, 548 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III 500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 6.01 (2H, s), 6.56 (2H, m) 7.00 (1H, t, *J* = 5.0 Hz), 7.61 (2H, m), 8.47 (2H, d, *J* = 4.9 Hz), 11.27 (1H, br s) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 112.2, 115.6, 124.9, 129.9, 153.1, 157.3, 158.3 ppm
Melting point:		254-256 °C
Microanalysis:	Found:	C = 48.2%; H = 4.1%; N = 22.5%; S% = 12.6% (May, 2016)
	Calculated:	C = 48.0%; H = 4.0%; N = 22.4%; S% = 12.8% (Calculated for $C_{10}H_{10}N_4O_2S$)