Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

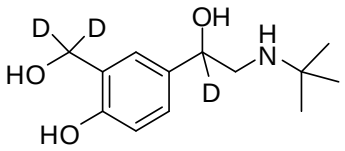
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D939: d3-Salbutamol

Report ID: D939.2018.02 (Ampouled 120424)

Chemical Formula: $C_{13}H_{18}D_3NO_3$

Molecular Weight: 242.3 g/mol (base)



Property value

Batch No.	CAS No.	Mass per ampoule
09-D-03	2294011-36-0 (R isomer) 2294011-78-0 (S-isomer)	938 μg

IUPAC name: 2-(1,1'-^{2}H-Hydroxymethyl)-4-{1—hydroxy-1'-^{2}H-2-[(2-methyl-2-propanyl)amino]ethyl}phenol

Expiration of certification: The property values are valid till 18 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 shelf life does not apply to ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D939. The material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer approximately 938 μg of anhydrous salbutamol (d_3, d_2, d_1 and d_0).

Recommended storage: When not in use, this material should be stored at or below 4 ºC in a closed container in a dry, dark area.

Stability: 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 GC-FID on seven randomly selected ampoules 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.
5 May 2020.

This report supersedes any issued prior to 30 April 2020.

Characterisation Report:

GC-FID:	Instrument:	Varian CP-3800
(*Tris*-TMS)	Column:	HP-1, 30 m × 0.32 mm × 0.25 µm
	Program:	150 °C (1 min), 10 °C/min to 300 °C (6 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 97.5%, s = 0.1% (7 ampoules in duplicate, April 2012)
	Re-analysis:	Mean = 96.1%, s = 0.2% (5 ampoules in duplicate, April 2015)
	Re-analysis:	Mean = 96.7%, s = 0.4% (5 ampoules in duplicate, May 2018)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, 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.

The main component of this material is d_3-salbutamol. d_2-, d_1- and d_0-salbutamol are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_3, d_2 and d_1) and d_0-salbutamol in the material. The isotopic purity, stated below, is an estimate only based on mass spectrometry. The deuterium analysis was carried out on the *tris*-TMS d_3-salbutamol fragment at 372 m/z. Deuterium analysis was not carried out on the parent ion due to its low abundance in the mass spectrum.

The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Isotopic Purity:	$d_3 \approx 98.0\%$ [= (d_3 / d_0 + d_1 + d_2 + d_3) × 100]	
	$d_0 \approx 0\%$ [= (d_0 / d_0 + d_1 + d_2 + d_3) × 100]	
GC-FID:	Instrument:	Varian CP-3800
(Tris-TMS)	Column:	HP-1, 30 m × 0.32 mm × 0.25 µm
		TG-17MS, 30 m × 0.32 mm × 0.25 µm
	Program:	150 °C (1 min), 10 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 97.9%, s = 0.03% (5 sub samples in duplicate, March 2012) (HP-1)
		Mean = 97.7%, s = 0.07% (5 sub samples in duplicate, March 2012) (TG 17MS)
HPLC:	Column:	Waters Symmetry C-18 5 µm (3.9 mm x 150 mm)
	Mobile Phase:	Solvent A: 5mM hexanesulfonic acid in MQ with 1% AcOH Solvent B: methanol Gradient 0 min 90%A, 0-6 min 90-60%A, 6-11 min 60%A, 11-15 min 60-90%A, 15-20 min 90%A
	Flow Rate:	0.9 mL/min
	Detector:	UV at 276 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 96.9%, s = 0.04% (10 sub samples in duplicate, January 2009)
Karl Fischer analysis:		Moisture content 0.42% mass fraction (January 2009) Moisture content 0.61% mass fraction (February 2012)
Thermogravimetric analysis:		Initial volatile content 1.1% and non volatile residue 0.5 % mass fraction (January 2009)

Spectroscopic and other characterisation data

GC-MS:	*Tris*-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm
	Program:	100 °C (1 min), 15 °C /min to 145 °C, 25 °C /min to 300 °C (3 min)
	Injector:	180 °C
	Transfer line temp:	280 °C
	Carrier:	Helium (1.0 mL/min)
	Split ratio:	15/1
	The retention time of d_3-salbutamol *tris*-TMS derivative is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Tris-TMS d_3-salbutamol (6.6 min): 374 (15), 373 (34), 372 (100), 86 (22), 73 (19) m/z	
	The *tris*-TMS derivative of d_3-salbutamol co-elutes with a comparison sample of silylated native salbutamol under these conditions.	
ESI-MS:	Instrument:	Micromass Quatro Micro
	Operation:	Positive ion mode, direct infusion at 5 µL/min
	Ionisation:	ESI spray voltage at 3.2 kV negative ion
	EM voltage:	500 V
	Cone voltage:	20 V
	Peak:	243.1 (M+ H^+) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Methanol
		Single spot observed, R_f = 0.38. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400cm^{-1}, powder
	Peaks:	3179, 2968, 2854, 2705, 2605, 2362, 2135, 2069, 1605, 1487, 1339, 1265, 1107, 1030, 953, 850, 707cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	CD_3OD (3.31 ppm)
	Spectral data:	δ 1.13 (9H, s), 2.68 (1H, d, *J* = 11.0 Hz), 2.79 (1H, d, *J* = 11.0 Hz), 6.76 (1H, d, *J* = 8.2 Hz), 7.12 (1H, dd, *J* = 2.3, 8.2 Hz), 7.30 (1H, d, *J* = 2.2 Hz) ppm.
		^{1}H NMR shows the presence of ethanol, ethyl acetate and toluene in quantities of 1.5%, 0.4% and 0.05% mass fractions respectively (January 2009)
		^{1}H NMR shows the presence of ethanol, ethyl acetate and toluene in quantities of 1.4%, 0.05% and 0.03% mass fractions respectively (February 2012)
^{13}C NMR:	Instrument:	Bruker Avance-400
	Field strength:	100 MHz
	Solvent:	CD_3OD (49.0 ppm)
	Spectral data:	δ 27.3, 49.6, 50.2, 114.5, 125.7, 127.1, 133.5, 154.8 ppm
Melting point:		146-148 °C
Microanalysis:	Found:	C = 64.0 %; H = 8.7 %; N = 5.5% (January 2009)
	Calculated:	C = 64.4 %; H = 10.0 %; N = 5.8% (Calculated for $C_{13}H_{18}D_3NO_3$)

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.