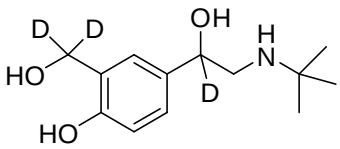
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

Report ID: D939.2018.01 (Ampouled 120424)
This batch of ampoules was prepared from the bulk material on 24th April 2012.

Compound Name: **d_3-Salbutamol**
Collection Number: D939
Chemical Formula: $C_{13}H_{18}D_3NO_3$
CAS Number: N/A
Structure:

Description: Off white solid
Batch Number: 09-D-03
Molecular Weight: 242.3
Release Date: 4th May 2012



Synonyms: d_3-albuterol
d_3-α[1]-[[(1,1-dimethylethyl)-amino]methyl]-4-hydroxy-1,3-benzenedimethanol
d_3-α[1]-[(*tert*-butyl-amino)methyl]-4-hydroxy-*m*-xylene-α,α′-diol
d_3-2-(*tert*-butylamino)-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol
d_3-4-hydroxy-3-hydroxymethyl-α-[(*tert*-butylamino)methyl]benzyl alcohol

The main component of this material is d_3-salbutamol. Also present are d_2-, d_1- and d_0-salbutamol. The stated chemical purity represents the combined mass fraction of deuterated (d_3, d_2 and d_1) and d_0-salbutamol.

The material is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D939. Each ampoule contains approximately 938 µg of anhydrous Salbutamol (d_3, d_2, d_1 and d_0). Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (chloroform).

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

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.

Isotopic Purity: $d_3 \approx 98.0\%$ [$= (d_3 / d_0 + d_1 + d_2 + d_3) \times 100$]
$d_0 \approx 0\%$ [$= (d_0 / d_0 + d_1 + d_2 + d_3) \times 100$]

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

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 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 response of main component:		
	Initial analysis:	Mean = 96.9%, s = 0.04% (10 sub samples in duplicate, January 2009)	
Thermogravimetric analysis:		Initial volatile content 1.1% and non volatile residue 0.5 % mass fraction (January 2009)	
Karl Fischer analysis:		Moisture content 0.42% mass fraction (January 2009)	
		Moisture content 0.61% mass fraction (February 2012)	

Spectroscopic and other characterisation data

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
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.

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) Calc: 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.

Expiration of certification
The property values are valid till 18th 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 ampoules 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 been given a shelf life of five years from the date of re-certification.

In the absence of stability data the measurement uncertainty at the 95% coverage interval has been expanded 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 10 years.

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.

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.

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: 22 May, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 22nd May 2018.