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: d3-Salbutamol
Collection Number: D939
Chemical Formula: C13H18D3N2O3
Molecular Weight: 242.3
CAS Number: N/A

Structure:

\[
\text{HO} - \text{D} - \text{NH} - \text{OH} - \text{HO} - \text{H}
\]

Description: Off white solid

Batch Number: 09-D-03
Release Date: 4th May 2012

Synonyms:
- d3-albuterol
- d3-α1-[(1,1-dimethylethyl)-aminomethyl]-4-hydroxy-1,3-benzenedimethanol
- d3-α1-[(tert-butyl-aminomethyl]-4-hydroxy-m-xylene-a,a'-diol
- d3-2-(tert-butylamino)-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol
- d3-4-hydroxy-3-hydroxymethyl-a-[(tert-butylamino)methyl]benzyl alcohol

The main component of this material is d3-salbutamol. Also present are d2-, d1- and d0-salbutamol. The stated chemical purity represents the combined mass fraction of deuterated (d3, d2 and d1) and d0-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 (d3, d2, d1 and d0). 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 d3-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:
- \[d_3 = 98.0\% \left(\frac{d_3}{d_0 + d_1 + d_2 + d_3}\right) \times 100\]
- \[d_0 = 0\% \left(\frac{d_0}{d_0 + d_1 + d_2 + d_3}\right) \times 100\]

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 1H NMR spectroscopy. Supporting evidence is provided by elemental microanalysis.

<table>
<thead>
<tr>
<th>Analytical Technique</th>
<th>Instrument</th>
<th>Column</th>
<th>Program</th>
<th>Injector</th>
<th>Carrier</th>
<th>Relative Peak Area of Main Component</th>
<th>Initial Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-FID: (Tris-TMS)</td>
<td>Varian CP-3800</td>
<td>HP-1, 30 m × 0.32 mm × 0.25 μm</td>
<td>150 °C (1 min), 10 °C/min to 300 °C (3 min)</td>
<td>250 °C</td>
<td>Helium</td>
<td>Mean = 97.9%, s = 0.03% (5 sub samples in duplicate, March 2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG-17MS, 30 m × 0.32 mm × 0.25 μm</td>
<td></td>
<td></td>
<td>Split ratio: 20/1</td>
<td>(TR17MS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean = 97.7%, s = 0.07% (5 sub samples in duplicate, March 2012)</td>
<td></td>
</tr>
<tr>
<td>HPLC:</td>
<td>Waters Symmetry C-18 5 μm (3.9 mm x 150 mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobile Phase:</td>
<td>Solvent A: 5mM hexanesulfonic acid in MQ with 1% AcOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solvent B: methanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>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</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flow Rate: 0.9 mL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Detector: UV at 276 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermogravimetric analysis:</td>
<td>Initial volatile content 1.1% and non volatile residue</td>
<td>0.5 % mass fraction (January 2009)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karl Fischer analysis:</td>
<td>Moisture content 0.42% mass fraction (January 2009)</td>
<td>Moisture content 0.61% mass fraction (February 2012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 d₃-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 d₃-salbutamol (6.6 min): 374 (15), 373 (34), 372 (100), 86 (22), 73 (19) m/z

The tris-TMS derivative of d₃-salbutamol co-elutes with a comparison sample of silylated native salbutamol under these conditions.

TLC: Conditions: Kieselgel 60F₂₅₄, Methanol
Single spot observed, Rf = 0.38. Visualisation with UV at 254 nm

IR: Instrument: Biorad FTS300MX FT-IR
Range: 4000-400 cm⁻¹, powder
Peaks: 3179, 2968, 2854, 2705, 2605, 2362, 2135, 2069, 1487, 1339, 1265, 1107, 1030, 953, 850, 707 cm⁻¹

¹H NMR: Instrument: Bruker Avance-400
Field strength: 400 MHz
Solvent: CD₂OD (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.
¹H 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)
¹H 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)

¹³C NMR: Instrument: Bruker Avance-400
Field strength: 100 MHz
Solvent: CD₂OD (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 C₁₃H₁₈D₃N₃O₃)

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 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 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 °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:

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.

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