



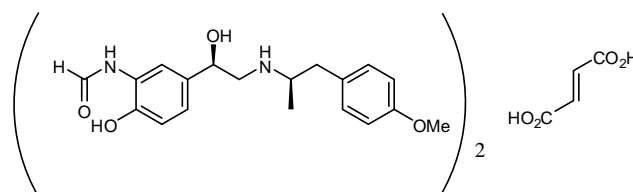
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D1065: Formoterol fumarate

Report ID: D1065.2023.01

Chemical Formula: $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4$

Molecular Weight: 804.9 (salt), 344.4 (free base) g/mol



Property value

| Batch No. | CAS No. | Purity (mass fraction) |
|-----------|------------|------------------------|
| 16-D-04 | 43229-80-7 | 95.5 ± 0.7% |

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

IUPAC name: *N*-{2-Hydroxy-5-[(1*R*)-1-hydroxy-2-[(2*R*)-1-(4-methoxyphenyl)-2-propanyl]amino]ethyl]phenyl}formamide (2*E*)-2-butenedioate

Expiration of certification: The property values are valid till 10 January 2028, 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 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 solid 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 4 °C 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: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials.

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 ten 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.
11 January 2023

This report supersedes any issued prior to 11 January 2023.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS.

The purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include HPLC with UV detection thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

The purity value by qNMR was obtained using a combination of the three-proton doublet at 1.33 ppm and the one-proton multiplet at 5.09 ppm measured against a certified internal standard of maleic acid.

Supporting evidence is provided by headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC: Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler or Waters Model 1525 Binary pump, 717 plus auto sampler or Thermo Scientific Ultimate 3000 RS Pump, RS auto sampler
 Column: X-Bridge C-18, 5.0 μm (4.6 mm x 150 mm)
 Column oven: 32 $^{\circ}\text{C}$
 Mobile Phase: A = MilliQ water; B = Methanol
 0-15 min 40% B; 15-20 min 40-80% B; 20-25 min 80% B; 25-27 min 80-40% B.
 The aqueous phase was buffered at pH 10.8 using 20mM NH_4OAc and NH_3
 Flow rate: 1 mL/min
 Detector: Shimadzu SPD-M20A PDA nm or Waters PDA 2998 or RS Diode Array Detector operating at 310 nm or at 220 nm

Relative mass fraction of the main component:

Initial analysis: Mean = 99.9%, s = 0.03% (10 sub samples in duplicate, January 2017)
 Re-analysis: Mean = 99.6%, s = 0.04% (5 sub samples in duplicate, January 2018)
 Re-analysis: Mean = 100.0%, s = 0.004% (5 sub samples in duplicate, March 2019)
 Re-analysis: Mean = 99.8%, s = 0.03% (5 sub samples in duplicate, March 2020)
 Re-analysis: Mean = 99.8%, s = 0.02% (5 sub samples in duplicate, January 2023)

Thermogravimetric analysis: Volatile content 4.5% and non volatile residue < 0.2% mass fraction (December 2016).

Karl Fischer analysis: Moisture content 4.6% mass fraction (December 2016)
 Moisture content 4.3% mass fraction (December 2017)
 Moisture content 4.5% mass fraction (January 2019)
 Moisture content 4.6% mass fraction (March 2020)
 Moisture content 4.2% mass fraction (October 2022)

qNMR: Instrument: Bruker Avance-III-500
 Field strength: 500 MHz
 Solvent: Acetic acid- d_4 (2.03 ppm)
 Internal standard: Maleic acid (98.7% mass fraction)
 Initial analysis: Mean (1.33 ppm) = 95.1%, s = 0.3% (5 sub samples, January 2017)
 Initial analysis: Mean (5.09 ppm) = 95.1%, s = 0.4% (5 sub samples, January 2017)

Spectroscopic and other characterisation data

| | | |
|----------------------|--------------------|---|
| ESI-MS: | Instrument: | Micromass Quatro LC Micro |
| | Operation: | Positive ion mode, direct infusion at 10 μ L/min |
| | Ionisation: | ESI spray voltage at 2.0 kV positive ion |
| | EM voltage: | 650 V |
| | Cone voltage: | 25 V |
| | Peak: | 345.2 (M+H ⁺) <i>m/z</i> |
| HS-GC-MS: | Instrument: | Agilent 6890/5973/G1888 |
| | Column: | DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m |
| | Program: | 50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min) |
| | Injector: | 150 $^{\circ}$ C Transfer line temp: 280 $^{\circ}$ C |
| | Carrier: | Helium, 1.2 mL/min Split ratio: 50/1 |
| | Solvents detected: | None |
| TLC: | Conditions: | Kieselgel 60F ₂₅₄ . Ethyl acetate/methanol/diethyl amine (10/2/1) Single spot observed, R _f = 0.4. Visualisation with UV at 254 nm |
| IR: | Instrument: | Bruker Alpha FT-IR |
| | Range: | 4000-400 cm^{-1} , neat |
| | Peaks: | 1687, 1515, 1374, 1307, 1275, 1241, 1027, 823, 811, 786, 652 cm^{-1} |
| ¹ H NMR: | Instrument: | Bruker Avance III 500 |
| | Field strength: | 500 MHz |
| | Solvent: | MeOH-d ₄ (3.31 ppm) |
| | Spectral data: | δ 1.20 (3H, d, <i>J</i> = 6.5 Hz), 2.67 (1H, dd, <i>J</i> = 9.5, 13.5 Hz), 3.04-3.16 (3H, m), 3.40 (1H, m), 3.77 (3H, s), 4.84 (1H, dd, <i>J</i> = 4.5, 8.5 Hz), 6.69 (1H, s), 6.86-6.89 (3H, m), 7.04 (1H, dd, <i>J</i> = 2.0, 8.0 Hz), 7.13 (2H, d, <i>J</i> = 8.5 Hz), 8.11 (1H, d, <i>J</i> = 2.0 Hz), 8.31 (1H, s) ppm The data reported is for the major rotamer only. Ethanol estimated at 0.08% mass fraction was observed in the ¹ H NMR. |
| ¹³ C NMR: | Instrument: | Bruker Avance III 500 |
| | Field strength: | 126 MHz |
| | Solvent: | MeOH-d ₄ (49.0 ppm) |
| | Spectral data: | δ 16.1, 40.0, 52.8, 55.7, 56.9, 70.6, 115.2, 116.1, 120.3, 123.8, 126.9, 129.6, 131.4, 133.5, 137.0, 148.5, 160.3, 162.0, 174.1 ppm |
| Melting point: | | 147-149 $^{\circ}$ C |
| Microanalysis: | Found: | C = 60.2%; H = 6.8%; N = 6.7% (November 2016) |
| | Calculated: | C = 62.7%; H = 6.5%; N = 7.0% (Calculated for (C ₁₉ H ₂₄ N ₂ O ₄) ₂ .C ₄ H ₄ O ₄) |