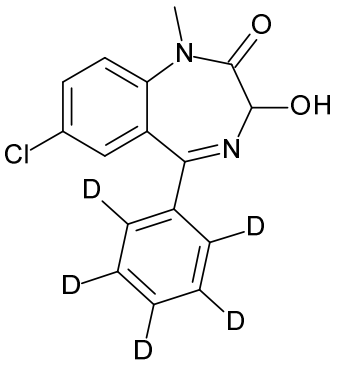
LoGiCal®
produced by LGC

Certificate of Analysis

Reference Material - Primary Standard

Product Name: Temazepam-D_5 1.0 mg/ml in Methanol

Catalogue Number: LGCAMP0087.80-01
Lot Number: 49716



CAS Number: 136765-51-0
Molecular Formula: $C_{16}H_8D_5ClN_2O_2$
Molecular Weight: 305.77
Solvent: Methanol
Volume per Ampoule: Not less than 1 ml [1]

Long-term Storage: - 18 °C, dark

Expiry Date: June-2016
Intended Use: The primary aim of this material is for identification, calibration and quantification.

Component	Concentration ("as is")	Uncertainty
see product name	1.000 mg/ml [2]	U = 0.004 mg/ml [3]

Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and Guide 34 at the about 95 % level of confidence using a coverage factor of k = 2 and has been calculated by statistical analysis of our production system and incorporates uncertainty of the purity, material density and balance and weighing technique.
Concentration based on material weighings and material purity factor (assay of the neat material).

The solution's concentration and homogeneity are verified by independent method.

LGC certifies that this standard meets the specification stated in this certificate and warrants this product to meet the stated acceptance criteria through the retest date when stored unopened as recommended. Product should be used shortly after opening to avoid concentration changes due to evaporation. Warranty does not apply to ampoules stored after opening.

Release Date:
Luckenwalde, June 2014

Signed:

Dr. Sabine Schröder
Unit for Reference Materials

[1] Ampoules are overfilled to ensure a minimum 1 ml volume fill. We advise laboratories to use measured volumes of this standard solution before diluting to the desired concentration.
[2] The value is based on the results of analytical techniques, which calibration and verification was carried out with standards traceable to SI-units. The value is expressed on an "as is" basis.
The concentration with its uncertainty is valid in the range between 19 °C and 25 °C.
The identity is verified by data from international scientific literature.
Gravimetrically prepared using qualified balances calibrated annually by accredited calibration service. Calibration verification performed daily prior to use utilizing weights traceable to SI via other mass standards.
[3] The uncertainty "U" is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM). It is corresponding to a level of confidence of about 95 %. Standard uncertainties are indicated with "u".

Verification of Concentration and Homogeneity				
Lot Number	Verified Concentration (mg/ml)		% RSD - Homogeneity	
	Result	*Acceptance Criteria*	*Result*	*Acceptance Criteria*
49716	1.025	± 3 %	2.417	≤ 3 %
Concentration verified by HPLC				

Solution Standard Assay Parameters		**External Calibration (100 % amount)**	
Analysis Method	HPLC		
Column:	Hypersil Gold (C18), 5 µm, 150 x 4.6 mm	Number of Measurements:	6
Injector:	Auto; 1 µl; 1.0 mg/ml in Methanol		
Flow:	1.0 ml/min, 40 °C	Detector:	230 nm
Conditions:	mob. Phase A: Water + 0.1 % H_3PO_4, mob. Phase B: Acetonitrile + 0.1 % H_3PO_4 0-12 min A/B 67/33, 12-15 min A/B to 10/90, 15-17 min A/B to 67/33, 17-22 min A/B 67/33 (v/v)		

Neat Material Data	
Product Name:	Temazepam-D_5
CAS Number:	136765-51-0
Molecular Formula:	$C_{16}H_8D_5ClN_2O_2$
Molecular Weight:	305.77
Compound Lot:	49715

Test	**Method**	**Result**
Melting Point (°C)*	SOP 06-010	101 °C
^{1}H-NMR Spectrum*	SOP 06-053	conform / complies to structure
IR Spectrum*	SOP 06-036	conform / complies to structure
Mass Spectrum (ESI)*	SOP 06-022	conform / complies to structure
Isotopic Purity by HRMS		0.01 % D_3, 2.96 % D_4, 97.03 % D_5
Assay by quantitative NMR (as is)*	**Quant. NMR**	**96.77 %**
The expanded uncertainty according to the assay is U = 0.40 % (about 95 % level of confidence using a coverage factor of k = 2).		

*: Validated method performed by ISO/IEC 17025 accredited testing lab. Purity factor does not include adjustment for chiral and/or isotopic purity.

The assay of the neat material is verified by the 100 % method using HPLC, corrected with water (KFT) and residual solvents.

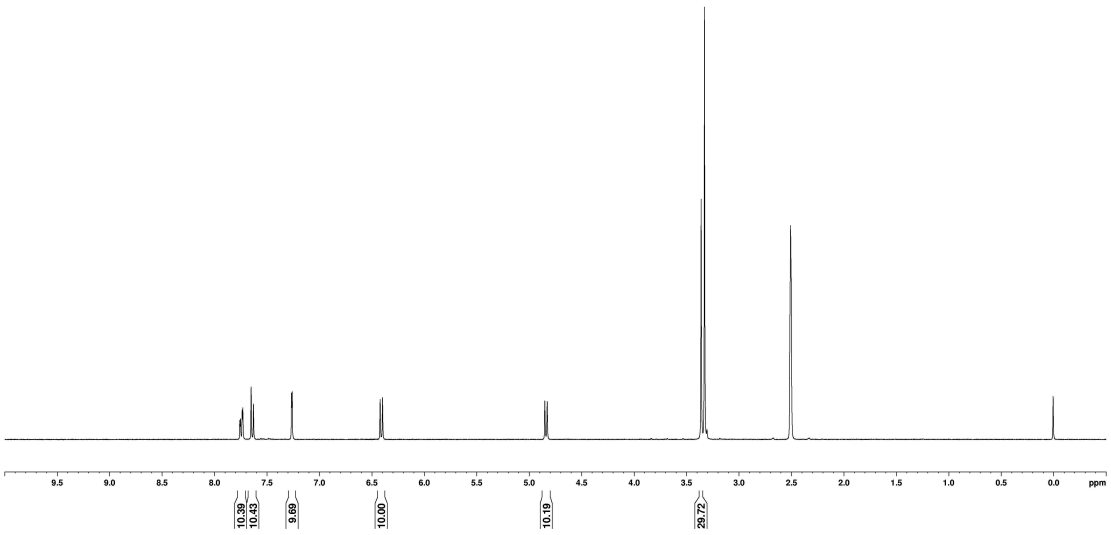
I. Identity

The identity of the reference substance (neat material) was established by the following analyses.

Ia. ^{1}H-NMR Spectrum

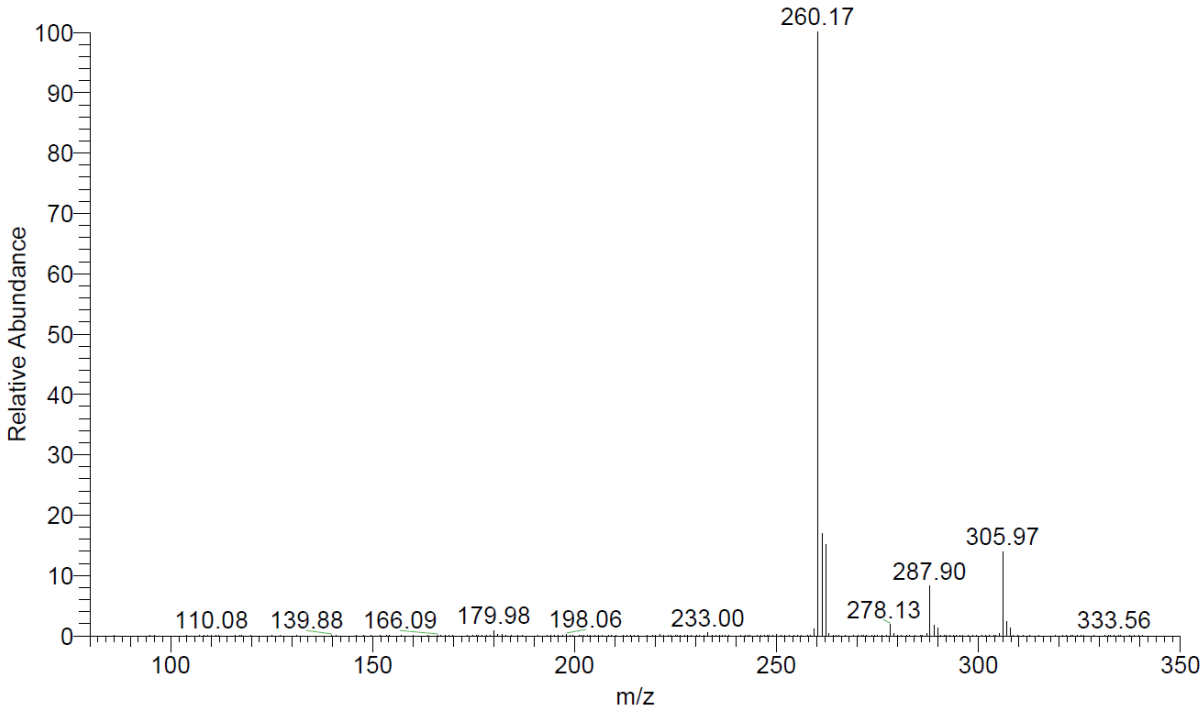
Conditions: 400 MHz, DMSO-d_6

The structure is confirmed with the signals of the spectrum and their interpretation.



Ib. Mass Spectrum

Method: 4.5 kV ESI; vaporization temperature: 200 °C, direct inlet

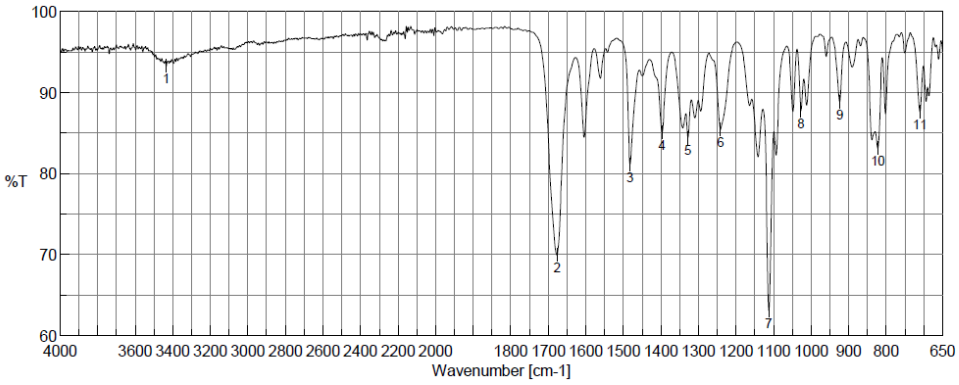


m/z	fragments
305.97	[MH]
287.90	[MH – H_2O]
260.17	[MH – H_2O – CO]

The signals of the mass spectrum and their interpretation are consistent with the structural formula.

Ic. IR Spectrum

Method: attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy



Result of Peak Picking

No.	Position	Intensity
1	3433.64	93.2665
2	1676.8	69.9275
3	1482.03	81.1161
4	1397.17	85.0829
5	1327.75	84.4238
6	1241.93	85.4404
7	1112.73	63.0919
8	1026.91	87.7168
9	923.736	88.8445
10	822.491	83.1135
11	710.64	87.6363

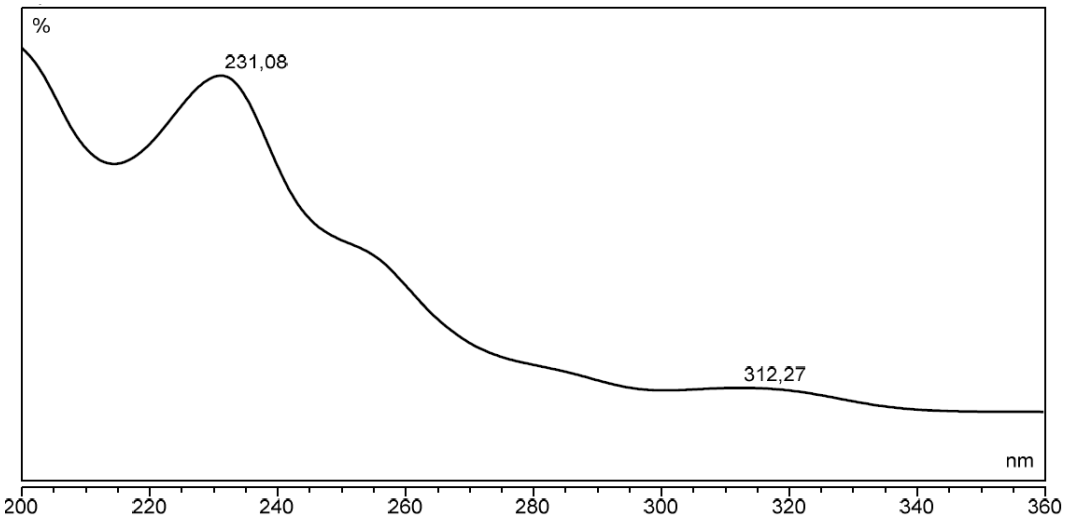
The signals of the IR spectrum and their interpretation are consistent with the structural formula.

Id. Melting Point

101 °C

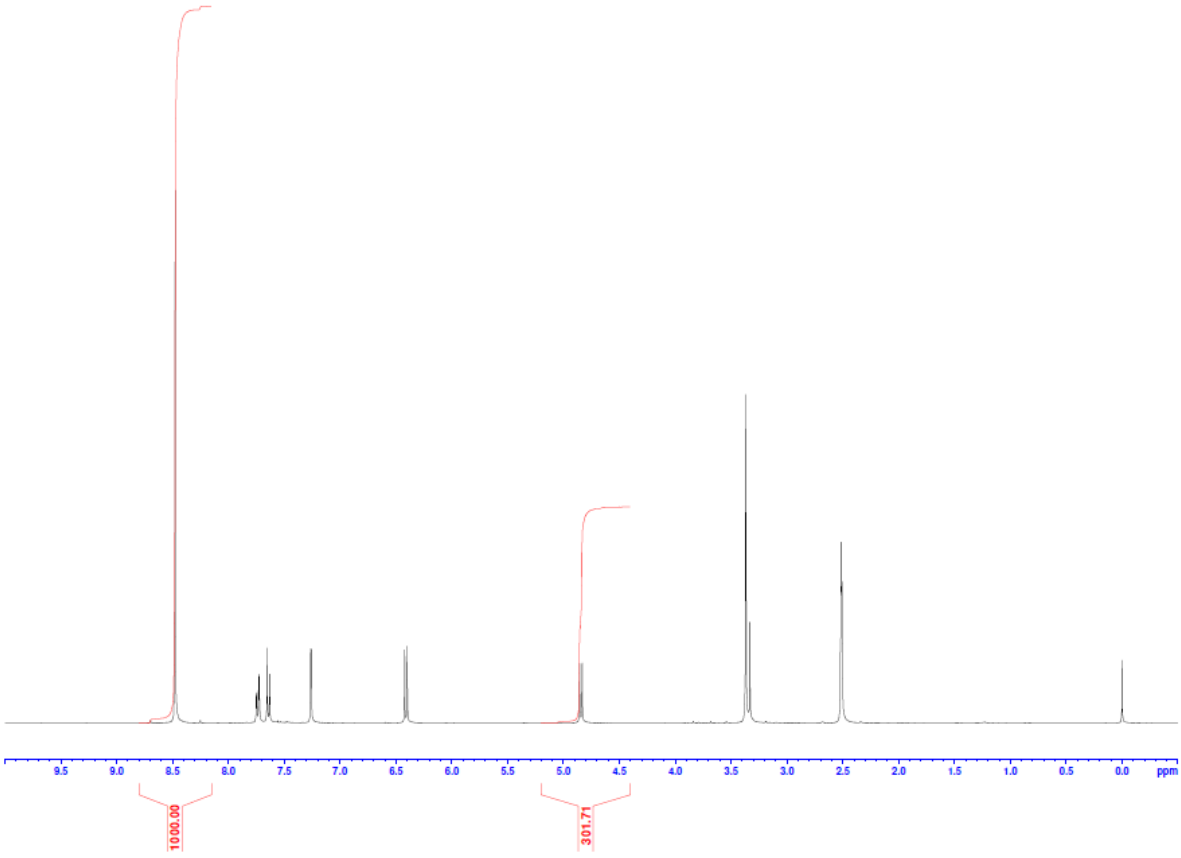
Ie. UV Spectrum

Method: HPLC (DAD-detection)



II. Assay by quantitative NMR spectroscopy

The assay of the reference substance was established by quantitative NMR spectroscopy using DMSO-d_6 as the solvent and with 2,3,5,6-Tetrachloro-1-nitrobenzene (certified reference material, signal 8.15 – 8.80 ppm, 1 H) as internal standard.



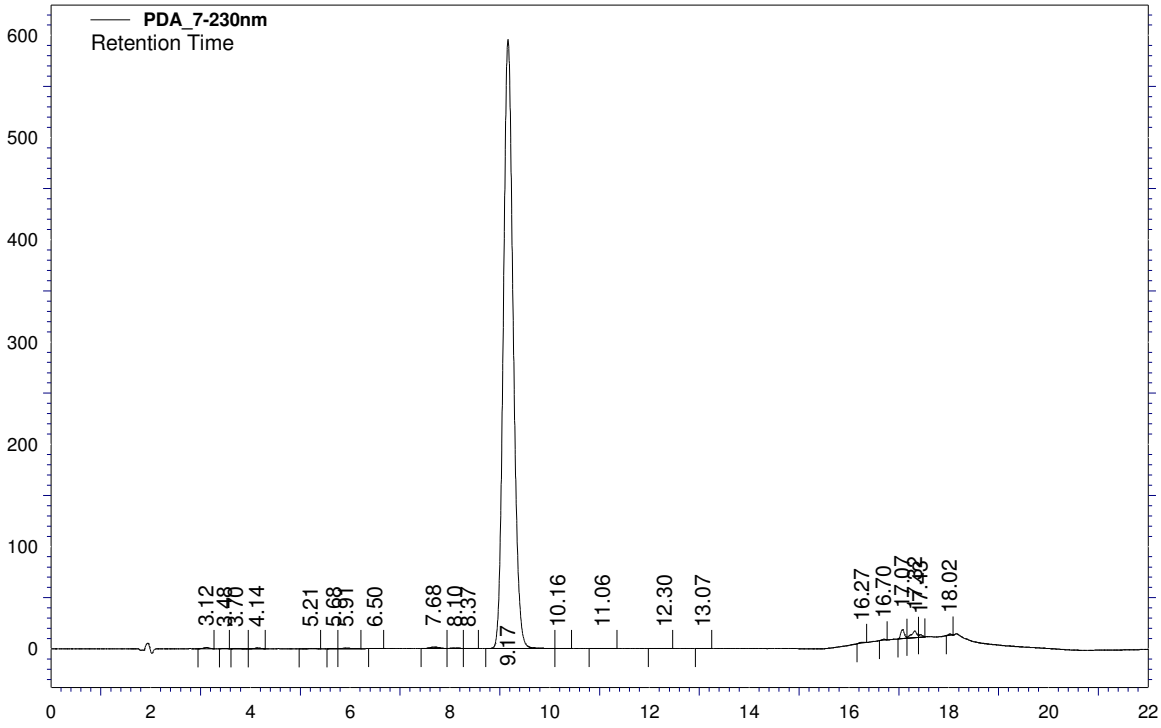
Results:

Average	96.77 %
Number of results	n=6
Uncertainty U (expanded)	0.40 %

III. Purity

IIIa. High Performance Liquid Chromatography (HPLC)

The purity of the reference substance (neat material) was analysed by high performance liquid chromatography (HPLC).



Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	3.12	7047	0.08
2	3.48	1181	0.01
3	3.70	2010	0.02
4	4.14	4714	0.06
5	5.21	732	0.01
6	5.68	864	0.01
7	5.91	6180	0.07
8	6.50	1034	0.01
9	7.68	19055	0.23
10	8.10	5896	0.07
11	8.37	2177	0.03
12	9.17	8163115	97.89
13	10.16	1080	0.01
14	11.06	1888	0.02
15	12.30	1907	0.02

16	13.07	801	0.01
17	16.27	5415	0.06
18	16.70	4395	0.05
19	17.07	44508	0.53
20	17.32	48624	0.58
21	17.43	11354	0.14
22	18.02	5126	0.06
Totals		8339103	100.00

For the calculation the system peaks were ignored. The content of the analyte was determined as ratio of the peak area of the analyte and the cumulative areas of the purities, added up to 100 %.

HPLC Conditions:

Column:	**Conditions:**	**Detector:**	**Injector:**
Hypersil Gold (C18)	1.0 ml/min, 40 °C	DAD	Auto
5 µm, 150 x 4.6 mm	0-12 min Water/Acetonitrile 67/33 12-15 min Water/Acetonitrile to 10/90 15-17 min Water/Acetonitrile to 67/33 17-22 min Water/Acetonitrile 67/33 (v/v); 0.1 % H_3PO_4	230 nm	0.5 µl; 0.471 mg/ml in Methanol

Results:

Arithmetic mean (n=3) 97.88 %

IIIb. Water Content

Method: coulometric Karl Fischer titration

Results:

Arithmetic mean (n=3) 0.29 % (mass fraction)

IIIc. Residual Solvents

Method: ^{1}H-NMR

No significant amounts of residual solvents were detected (< 0.05 %).

IV. Stability and Homogeneity

Accelerated stability studies indicate no significant instability. The given validity period is based on this data. This is backed up by historical data over the range of several years for the neat substance. Homogeneity assured by validated process of preparation (incl. ampoulation), verified by homogeneity testing (HPLC).

V. Further Information

General

For laboratory use only. Not suitable for human or animal consumption.

This material conforms to the characteristics of a primary standard as described within ISO Guide 30 (Terms and definitions used in connection with reference materials).
The certified values quoted in this certificate are LGC's best estimate of the true values within the stated uncertainties and based on the techniques described in this certificate.

Handling of the RM

Before usage of the RM, it should be allowed to warm to room temperature. The concentration with its uncertainty is guaranteed in the range between 19 °C and 25 °C. The uncertainty accounts for the temperature-dependent density in this range.

Quality Control Assessment

The product quality is controlled by regularly performed quality control tests (retests).