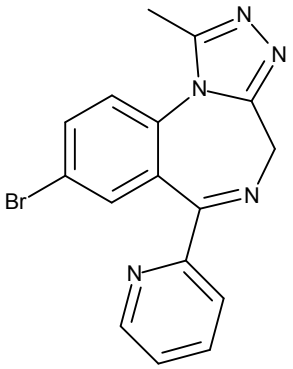
LoGiCal®
produced by LGC

Certificate of Analysis

Reference Material - Primary Standard

Product Name: Pyrazolam 1.0 mg/ml in Methanol

Catalogue Number: LGCAMP1441.00-01
Lot Number: 77201



CAS Number: 39243-02-2
Molecular Formula: $C_{16}H_{12}BrN_5$
Molecular Weight: 354.20
Solvent: Methanol
Volume per Ampoule: Not less than 1 ml [1]

Long-term Storage: -18 °C, dark

Expiry Date: July-2017
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, September 2015

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".

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Verification of Concentration and Homogeneity				
Lot Number	Verified Concentration (mg/ml)		% RSD - Homogeneity	
	Result	*Acceptance Criteria*	*Result*	*Acceptance Criteria*
77201	0.996	± 3 %	1.852	≤ 3 %
Concentration verified by HPLC				

Solution Standard Assay Parameters		**External Calibration (100 % amount)**
Analysis Method	HPLC	
Column:	Cortecs UPLC C18+, 1.6 µm, 75 x 2.1 mm	Number of Measurements: 6
Injector:	Auto; 0.1 µl; 1.0 mg/ml in Methanol	
Flow:	0.5 ml/min, 40 °C	Detector: DAD, 225 nm
Conditions:	mob. Phase A: Water + 0.1 % HCOOH, mob. Phase B: Acetonitrile + 0.1 % HCOOH 0-1 min A/B 98/2, 1-4 min A/B to 2/98, 4-5 min A/B to 98/2, 5-6 min A/B 98/2 (v/v)	

Neat Material Data		
Product Name:	Pyrazolam	
CAS Number:	39243-02-2	
Molecular Formula:	$C_{16}H_{12}BrN_5$	
Molecular Weight:	354.20	
Compound Lot:	77199	
Test	**Method**	**Result**
Melting Point (DSC) (°C)*	SOP 06-038	255 °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
Assay by carbon titration ("as is")*	**Elementary Analysis**	**99.33 %**
The expanded uncertainty according to the assay is U = 0.36 % (about 95 % level of confidence using a coverage factor of k = 2).		

*: Validated method performed by ISO/IEC 17025 accredited testing lab

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

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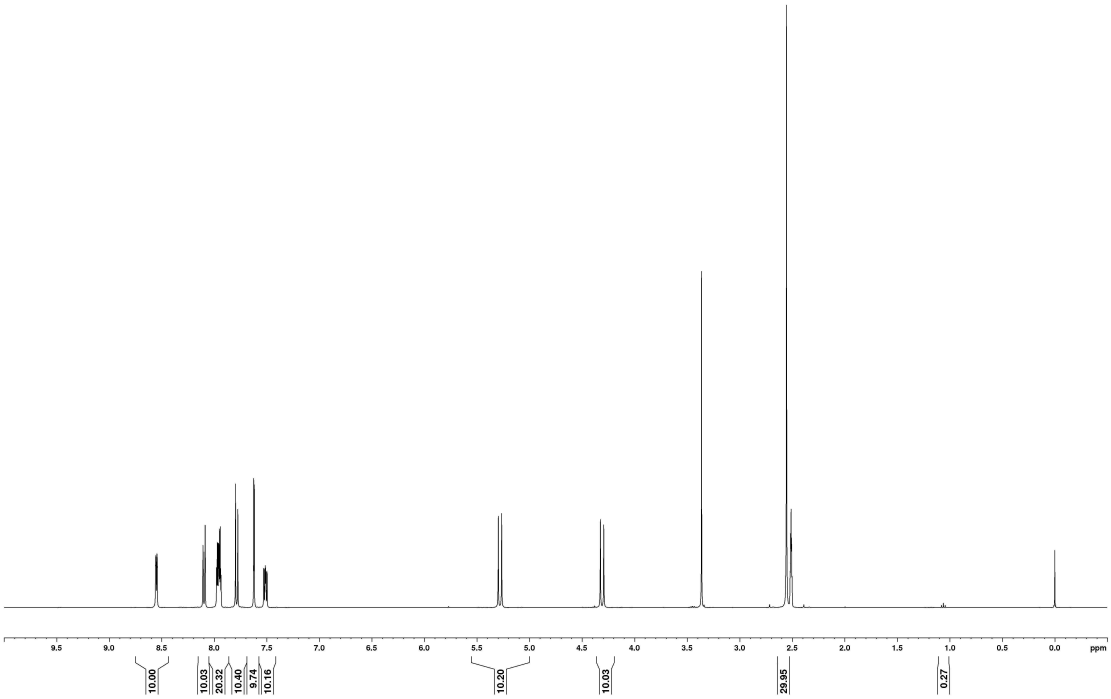
I. Identity

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.



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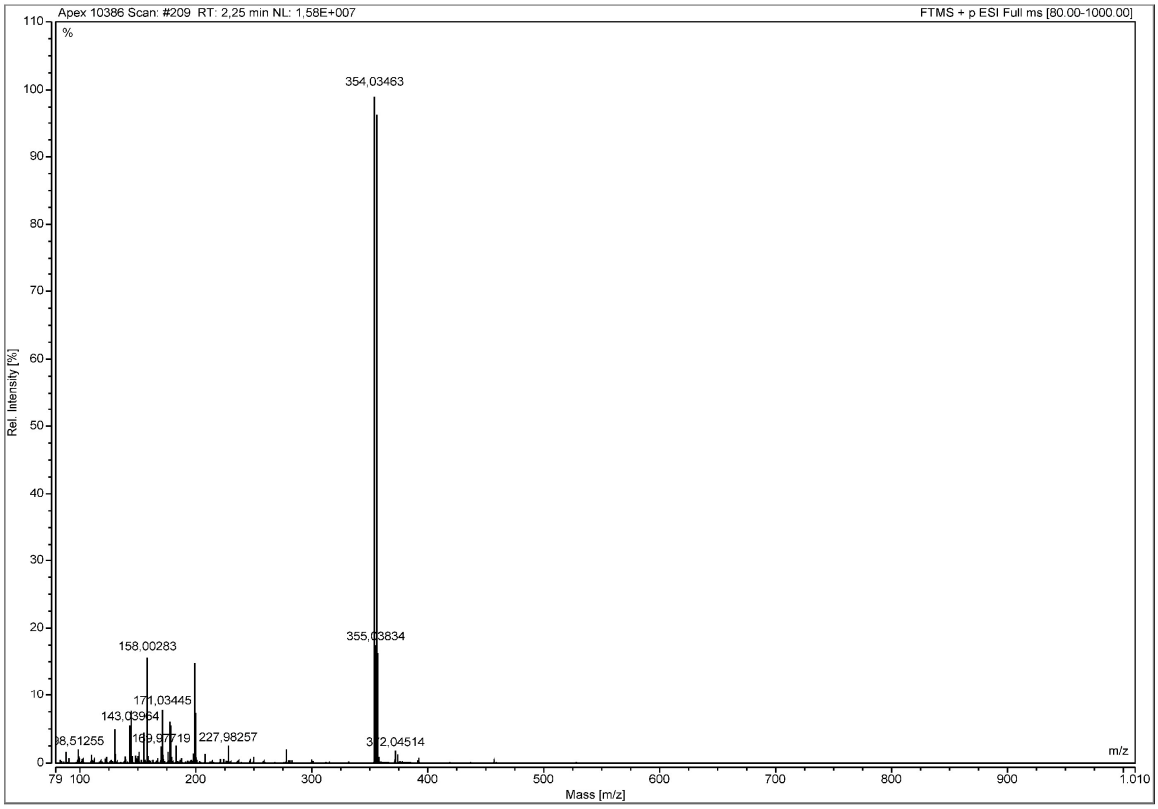
Ib. Mass Spectrum

Method: 3.5 kV ESI+; capillary temperature: 269 °C



Theoretical value: 354.03488

The signal of the MS spectrum is consistent with the theoretical value and its interpretation is consistent with the structural formula.

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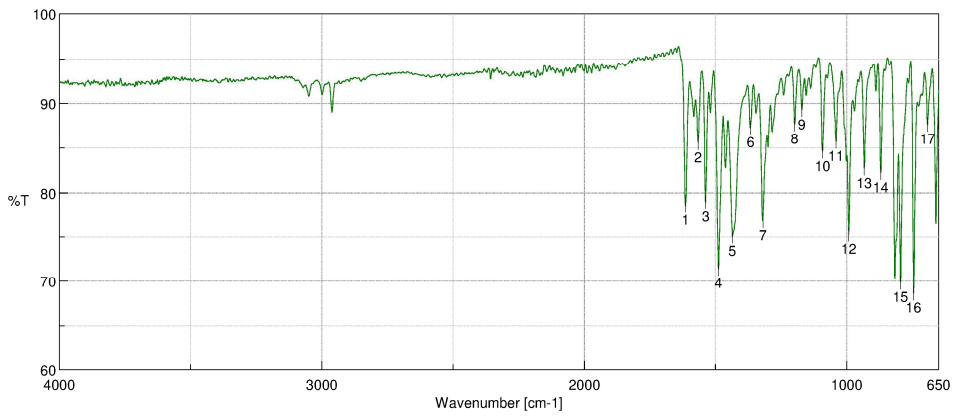
Ic. IR Spectrum

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



Results of Peak Find

No.	Position	Intensity
1	1615.09	78.5385
2	1566.88	85.529
3	1537.95	78.9228
4	1488.78	71.4194
5	1435.74	75.0614
6	1367.28	87.2229
7	1320.04	76.7783
8	1198.54	87.6208
9	1170.58	89.2591
10	1092.48	84.5757
11	1041.37	85.7046
12	992.196	75.2797
13	933.378	82.7219
14	870.703	82.2157
15	795.493	69.8094
16	745.352	68.609
17	692.32	87.577

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

Id. Melting Point (DSC)

255 °C

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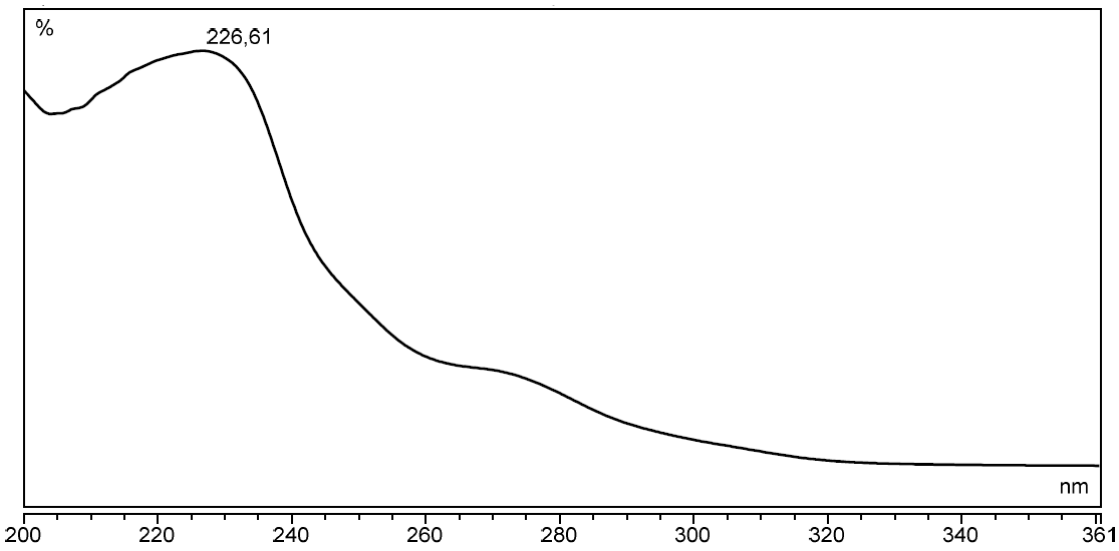
Ie. UV Spectrum

Method: HPLC (DAD-detection)



II. Assay by Elementary Analysis (Carbon Titration – neat material)

Method: percentage carbon found in relation to percentage carbon as calculated for molecular formula

Results:

Arithmetic mean (n=3)	99.33 % (mass fraction)
Uncertainty U	0.36 %

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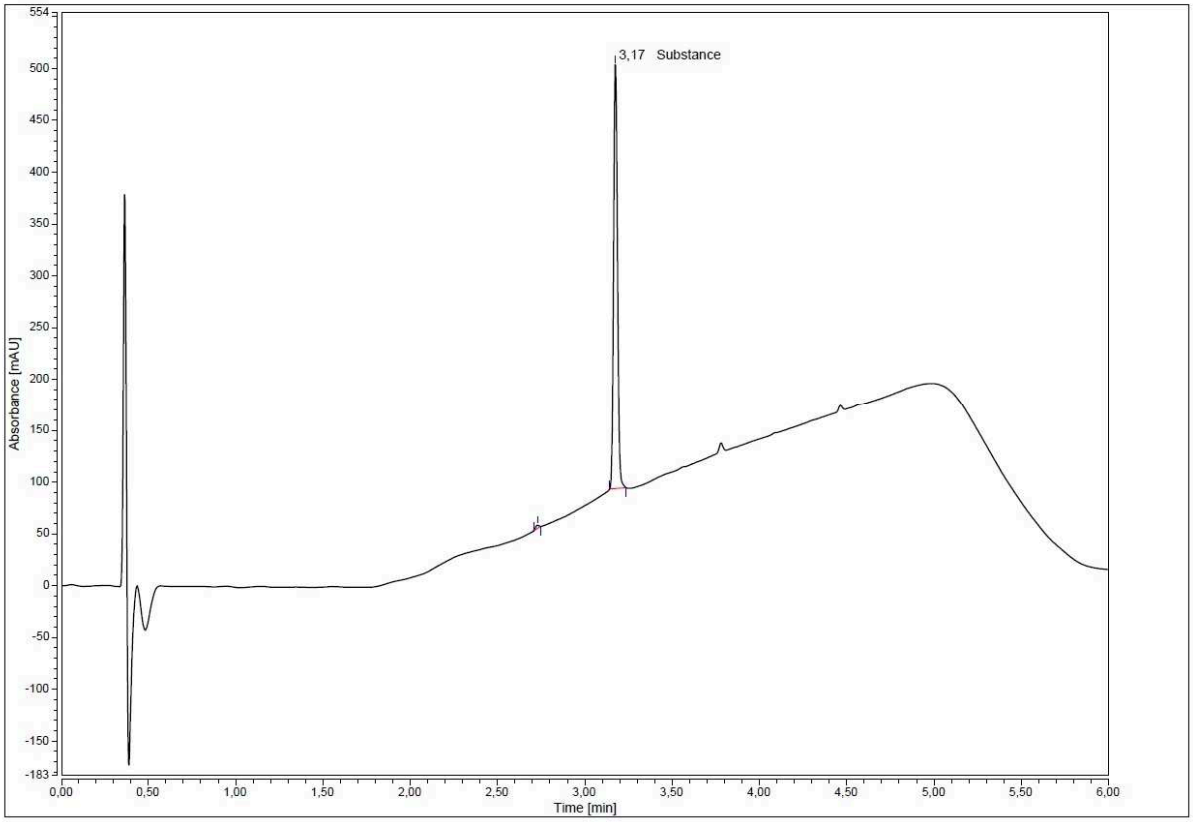
III. Purity

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	2.730	0.0721	0.68
2	3.173	10.5823	99.32
Totals		10.6544	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 %.

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HPLC Conditions:

Column:	Conditions:	Detector:	Injector:
Cortecs UPLC C18+ 1.6 µm, 75 x 2.1 mm	0.5 ml/min, 40 °C 0-1 min Water/Acetonitrile 98/2 1-4 min Water/Acetonitrile to 2/98 4-5 min Water/Acetonitrile to 98/2 5-6 min Water/Acetonitrile 98/2 (v/v); 0.1 % HCOOH	DAD 225 nm	Auto 2 µl; 0.05 mg/ml in Acetonitrile

Results:

Arithmetic mean (n=3) 99.32 %

IIIb. Water Content

Method: coulometric Karl Fischer titration

Results:

Arithmetic mean (n=3) 0.48 % (mass fraction)

IIIc. Residual Solvents

Method: ^{1}H-NMR

Result: 0.12 % Ethanol

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).

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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).

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