

Certificate of Analysis

Characterisation methods are accredited according to

ISO 17025

Reference Material

Product name

3,4-Dehydrocilostazol
(6-[4-(1-Cyclohexyl-1H-tetrazol-5-yl)-butoxy]-1H-quinolin-2-one)

Product code

MM0796.01

CAS number

73963-62-9

Molecular weight

367.44

Molecular formula

C₂₀H₂₅N₅O₂

Lot number

W1017697

Appearance

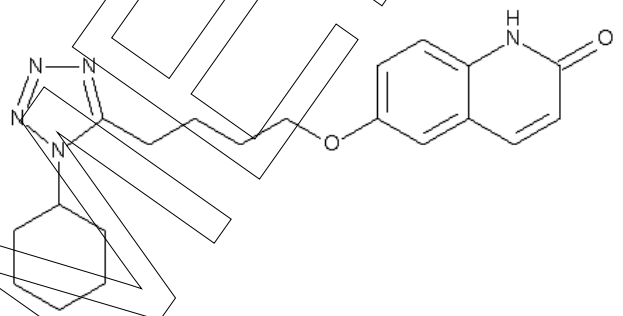
light pink solid

Melting point (DSC)

183 °C

Long-term storage

2 to 8 °C, dark



Assay¹ "as is"
90.4 %

Uncertainty² U
0.4 %

Intended Use: Use for identification and quantification. The assay is verified by a second testing method.

Date of shipment: **13 Sep 2019**

Producer confirms that this reference material (RM) meets the specification detailed on this Certificate of Analysis for **two years** from the date of shipment, provided the substance is stored under the recommended conditions unopened in the original container.

Release by:	Date of Release:		Product Release
Dr. Sabine Schröder	Luckenwalde, 12 Sep 2019		

¹ Calibration and verification were carried out using standards traceable to SI-units. The value is expressed on an "as is" basis.

² The uncertainty "U" is the expanded uncertainty of the testing method for the assigned value estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM). It corresponds to a level of confidence of about 95%. Coverage factor k=2.



Product information

This RM is intended for laboratory use only and is not suitable for human or animal consumption.

This RM conforms to the characteristics of a primary standard as described in the ICH Guidelines. The values quoted in this Certificate of Analysis are the producer's best estimate of the true values within the stated uncertainties and based on the techniques described in this Certificate of Analysis. The characterisation of this material was undertaken in accordance with the requirements of ISO/IEC 17025. The identity is verified by data from international scientific literature.

Storage and handling

Before usage of the RM, it should be allowed to warm to room temperature. No drying is required, as assigned values are already corrected for the content of water and other volatile materials.

Reference Material quality is controlled by regularly performed quality control tests (retests).

Further content

Assigned value

Purity

Identity

Revision table

SPECIMEN



Assigned value

Assay "as is": 90.37 %; U = 0.41 %

The assay "as is" is assessed by quantitative NMR spectroscopy and is equivalent to the assay based on the not-anhydrous and not-dried substance. The assay is verified by 100% method (mass balance).

The verified result lies inside our acceptance criteria, i.e. less than 1.0 % difference to assay assigning technique.

For quantitative applications, use the assay as a calculation value on the "as is basis". The uncertainty of the assay can be used for estimation/calculation of measurement uncertainty.

Method 1: Value assigning technique - quantitative NMR spectroscopy

Conditions

400 MHz, DMSO-d₆

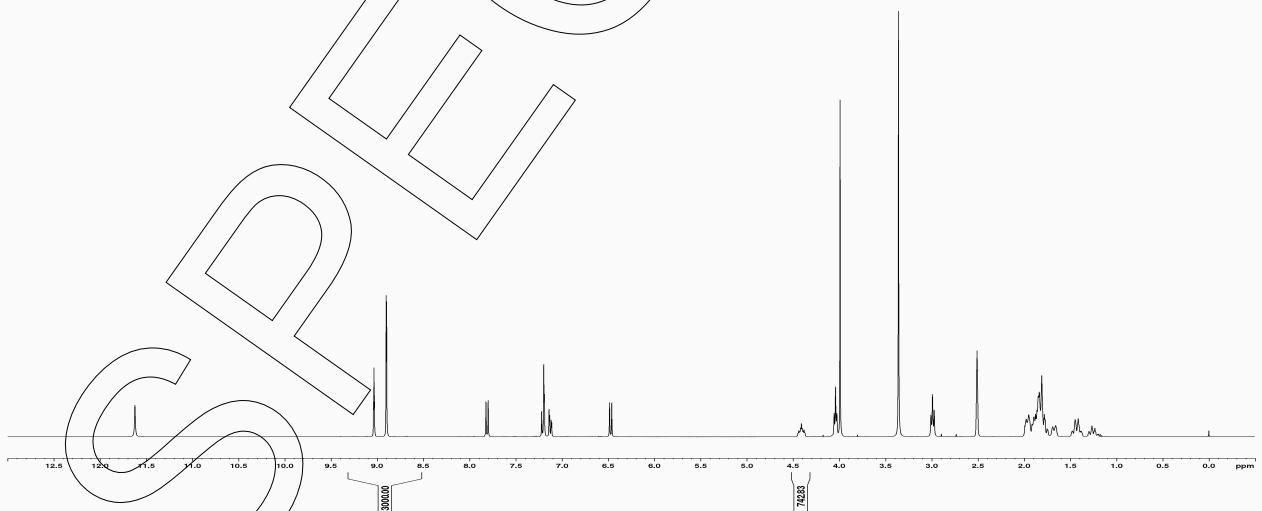
Internal standard

Methyl 3,5-dinitrobenzoate (certified reference material), signal 8.5 - 9.3 ppm, 3 H

Result (mass fraction, n = 6)

90.37 %; U = 0.41 %

Quantitative NMR spectrum





Method 2: Value verifying technique - 100% method

100% method (mass balance) with chromatographic purity by HPLC

Result 89.46 %

The calculation of the 100% method follows the formula:

$$\text{Assay (\%)} = (100 \% - \text{volatile contents (\%)}) * \frac{\text{Purity (\%)}}{100 \%}$$

Volatile contents are considered as absolute contributions and purity is considered as relative contribution. Inorganic residues are excluded by additional tests.

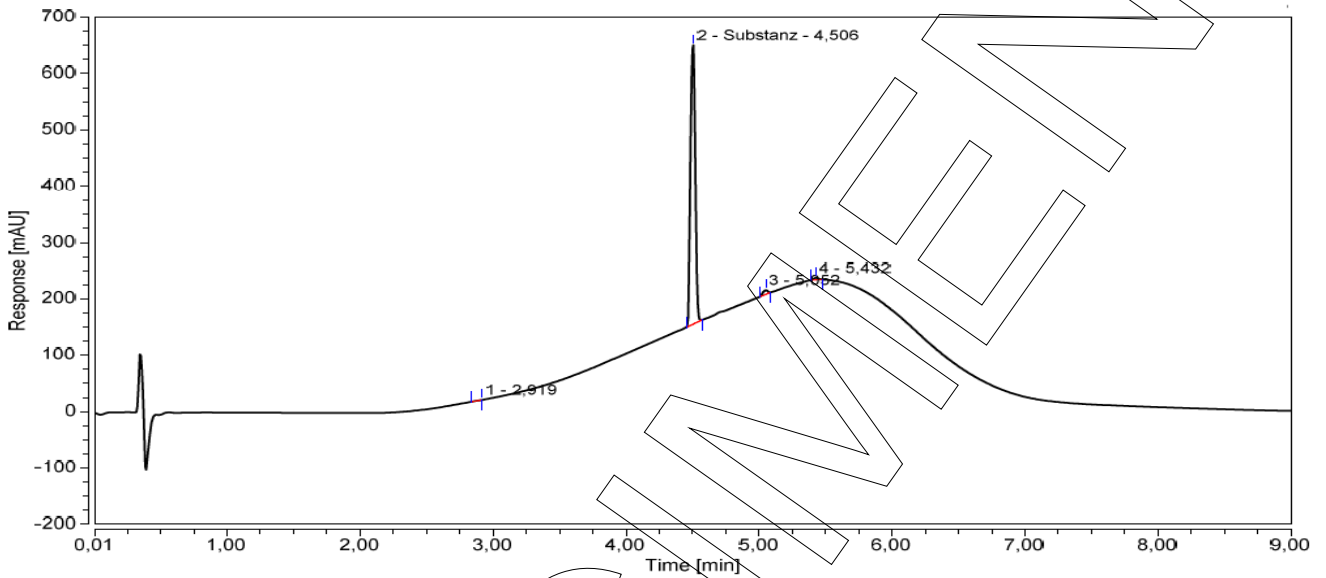
Purity

Purity by High Performance Liquid Chromatography (HPLC)

HPLC Conditions:	
Column	Cortecs UPLC C18 +; 1.6 µm, 75 x 2.1 mm
Column temperature	40 °C
Detector	DAD, 210 nm
Injector	Auto 2.00 µl; 0.056 mg/ml in Acetonitrile
Flow rate	0.5 ml/min
Phase A	Water, 0.1 % HCOOH
Phase B	Acetonitrile, 0.1 % HCOOH
Gradient program	0-1 min A/B 98/2 1-4 min A/B to 2/98 4-5 min A/B to 98/2 5-9 min A/B 98/2 (v/v)



HPLC chromatogram and peak table



Area percent report - sorted by signal

Pk #	Retention time	Area	Area %
1	2.919	0.012	0.06
2	4.506	20.856	97.67
3	5.052	0.319	1.50
4	5.432	0.165	0.77
Totals		21.352	100.00

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 %. System peaks were ignored in calculation.

Result (n = 3)

97.66 %; U = 0.19 %



Volatile content

Water content

Method	Karl Fischer titration
Result (n = 3)	8.21 %*; SD = 0.04 %

*not accredited testing method

Residual solvents

Method	¹ H-NMR
Result (n = 1)	Sum: 0.19 %* 0.19 % Dimethylformamide

*not accredited testing method

Inorganic residues

Method: Elementary analysis

Inorganic residues can be excluded by elementary analysis (CHN).

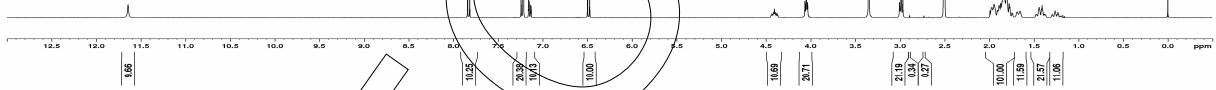
SPECIMEN



Identity

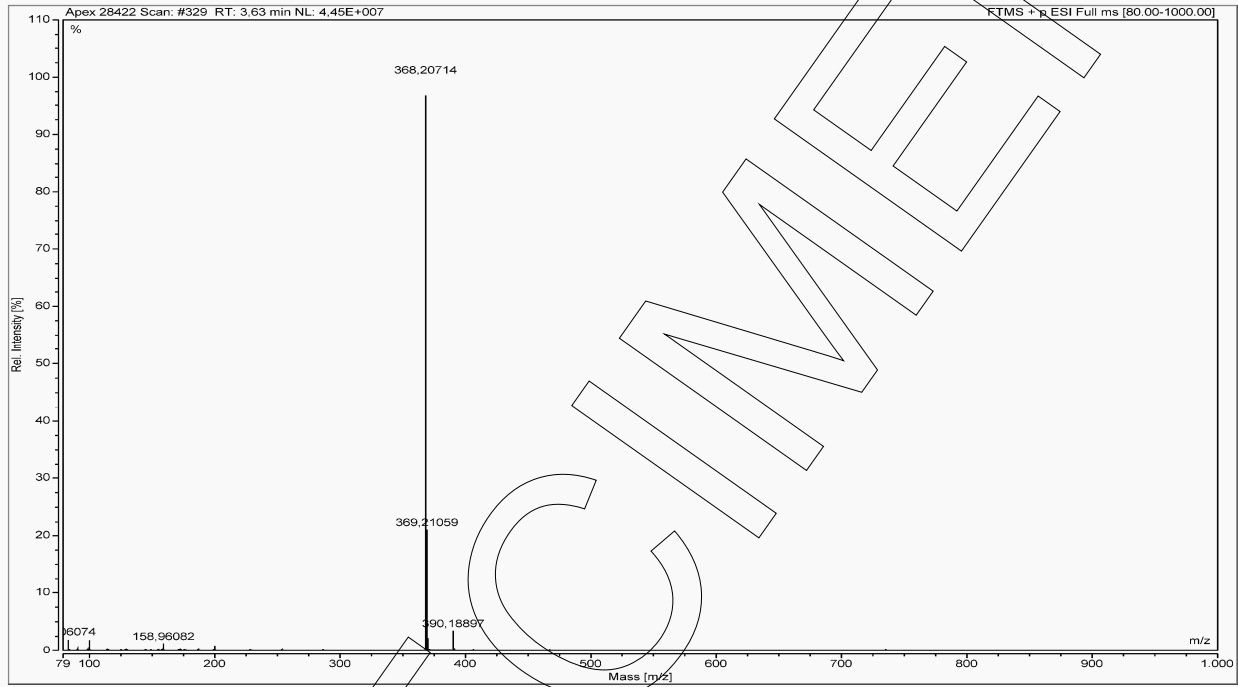
The identity is assessed by ISO/IEC 17025 accredited testing methods.

Method	Conditions	Result
¹ H-NMR	400 MHz, DMSO-d ₆	Structure confirmed





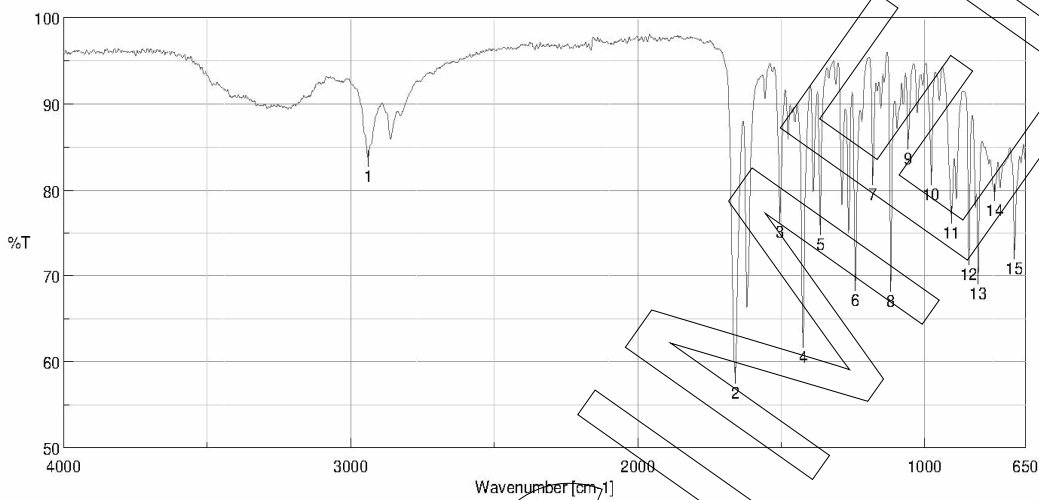
Method	Conditions	Result
MS	3.5 kV ESI+; capillary temperature: 269 °C Theoretical value: 368.20810	Structure confirmed



SPE
SAMPLE



Method	Conditions	Result
IR	Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy	Structure confirmed



No.	Position	Intensity
1	2939.95	83.7212
2	1661.37	58.4487
3	1504.2	77.2203
4	1424.17	62.5844
5	1364.39	75.7563
6	1241.93	69.127
7	1181.19	81.5451
8	1118.51	69.0619
9	1057.76	85.7582
10	977.733	81.5748
11	907.344	77.1018
12	846.597	72.2157
13	813.813	69.9687
14	756.923	79.6709
15	687.498	72.8965

Revision table

Revision	Date	Reason for revision
00	12 Sep 2019	Release of the Certificate of Analysis - initial version

Product warranties for the RM are set out in the terms and conditions of purchase.