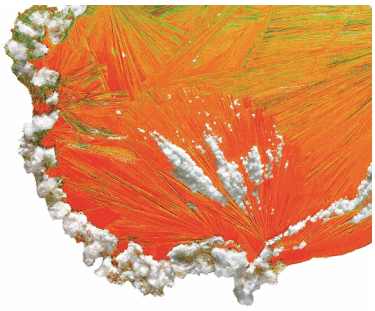
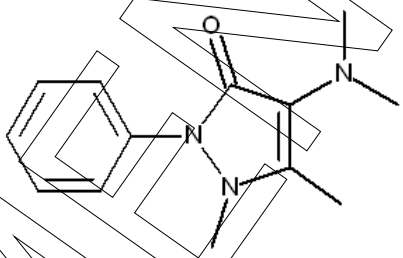


Certificate of Analysis ISO 17034

Reference Material

Product name

4-Dimethylamino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (4,4-Dimethylaminophenazone)



Product code MM0052.05	**Lot number** G1061700
CAS number 58-15-1	**Appearance** white solid
Molecular weight 231.29	**Melting point (DSC)** 107 °C
Molecular formula $C_{13}H_{17}N_3O$	**Long-term storage** 2 to 8 °C, dark

Assay[1] "as is"	Uncertainty[2] U
99.8 %	**0.4 %**

Intended Use: Use for identification and quantification. The assay is verified by a second testing method. Due to the homogeneity studies, the minimum amount of sample to be used is 10 mg.

Date of shipment: **27 Nov 2020**

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:**		
Dr. Sabine Schröder	Luckenwalde, 23 Apr 2020		Product Release

[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.

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Important 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 production of this RM was undertaken in accordance with the requirements of ISO 17034. 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.

Further content

Assigned value
Purity
Identity
Stability and homogeneity
Revision table

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Assigned value

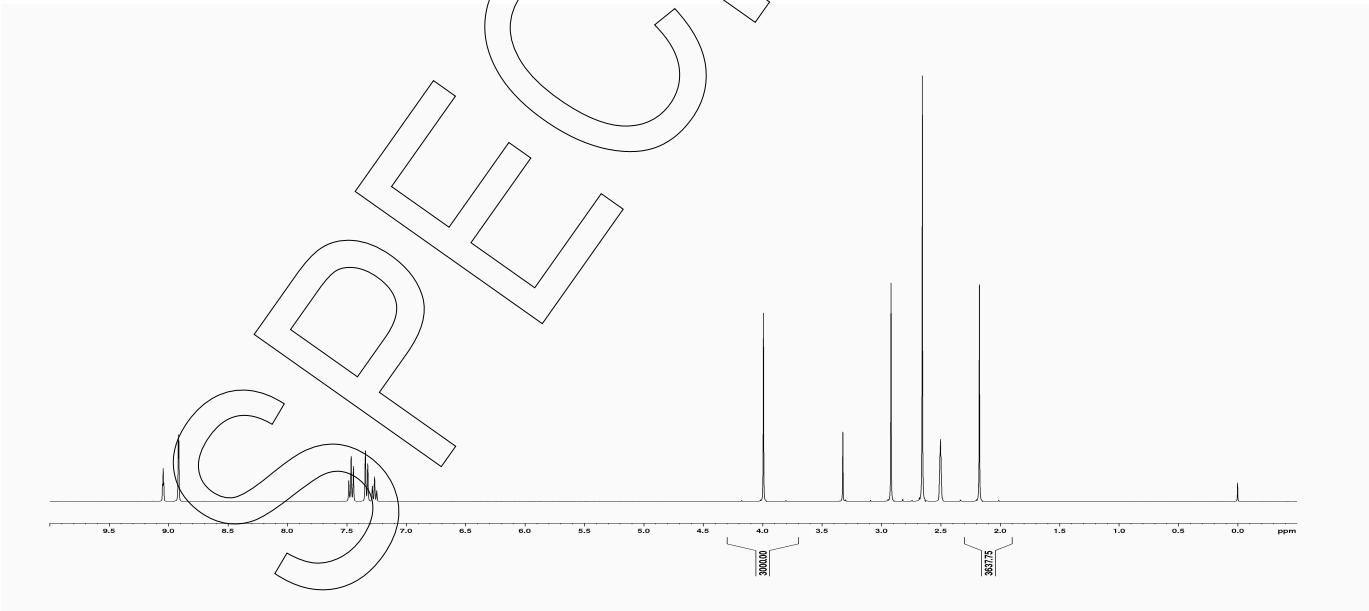
Assay "as is": **99.84 %; U = 0.44 %**

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-d_6
Internal Standard	Methyl 3,5-dinitrobenzoate (certified reference material), signal 3.7 - 4.3 ppm, 3 H
Result (mass fraction, **n = 6**)	99.84 %; U = 0.44 %

Quantitative NMR spectrum



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Method 2: Value verifying technique - 100% method	
100% method (mass balance) with chromatographic purity by HPLC	
Result	100.00 %

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.

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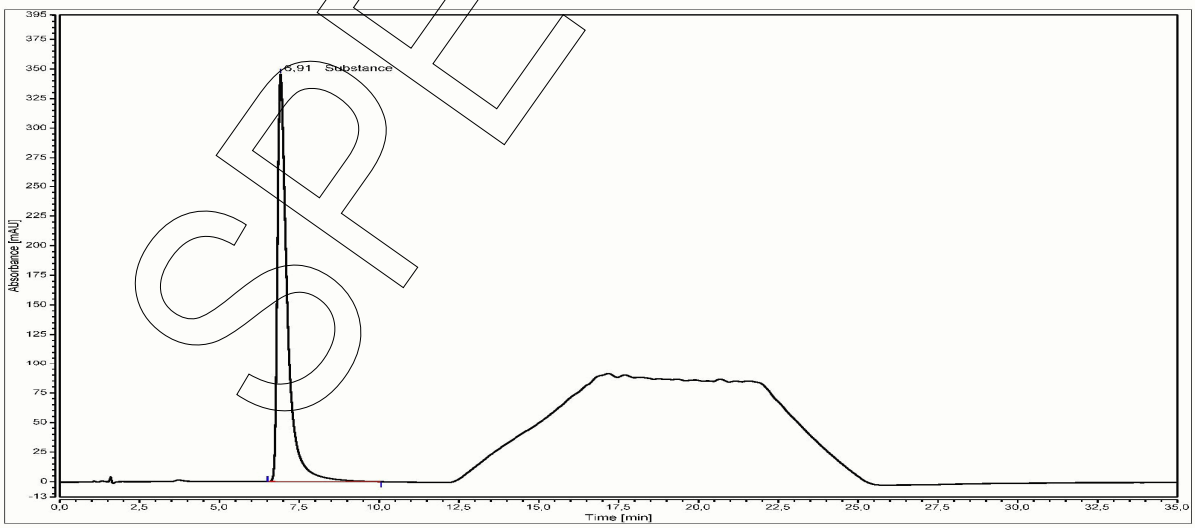
Purity

Purity by high performance liquid chromatography (HPLC)

HPLC Conditions:	
Column	LiChrospher 60 RP-select B, 5 µm, 125 x 4.0 mm
Column temperature	40 °C
Detector	DAD, 200 nm
Injector	Auto 4 µl, 0.0706 mg/ml in Acetonitrile/Water 50/50 (v/v)
Flow rate	1.0 ml/min
Phase A	Water, 0.1 % H_3PO_4
Phase B	Acetonitrile, 0.1 % H_3PO_4
Gradient program	0-10 min A/B 95/5 10-15 min A/B to 60/40 15-20 min A/B 60/40 20-23 min A/B to 95/5 23-35 min A/B 95/5 (v/v)

HPLC chromatogram and peak table



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Area percent report - sorted by signal				
Pk #	**Retention time**	**Area**	**Area %**	
1	6.911	124.1349	100.00	
Totals		**124.1349**	**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 = 6)	100.00 %; U = 0.18 %

Volatile content

Water content	
Method	Karl Fischer titration
Result (n = 3)	No significant amounts of water were detected (< 0.05 %).*

*not accredited testing method

Residual solvents	
Method	^{1}H-NMR
Result (n = 1)	No significant amounts of residual solvents were detected (< 0.05 %).*

*not accredited testing method

Inorganic residues

Method:	Sulphated ash, EP 8.2 chapter 2.4.14

According to the available data, the presence of inorganic impurities in the reference material other than those detectable by sulphated ash is highly unlikely. Inorganic residues can be excluded by results of the sulphated ash. Therefore, no assay correction was performed for inorganic impurities.

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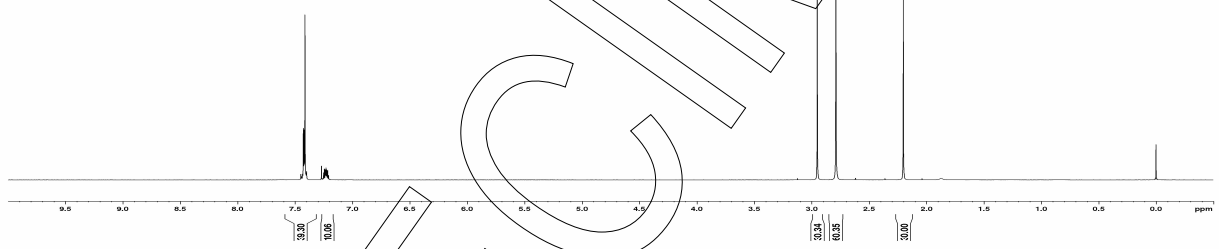
Identity

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

Method	Conditions	Result
^{1}H-NMR	400 MHz, $CDCl_3$	Structure confirmed



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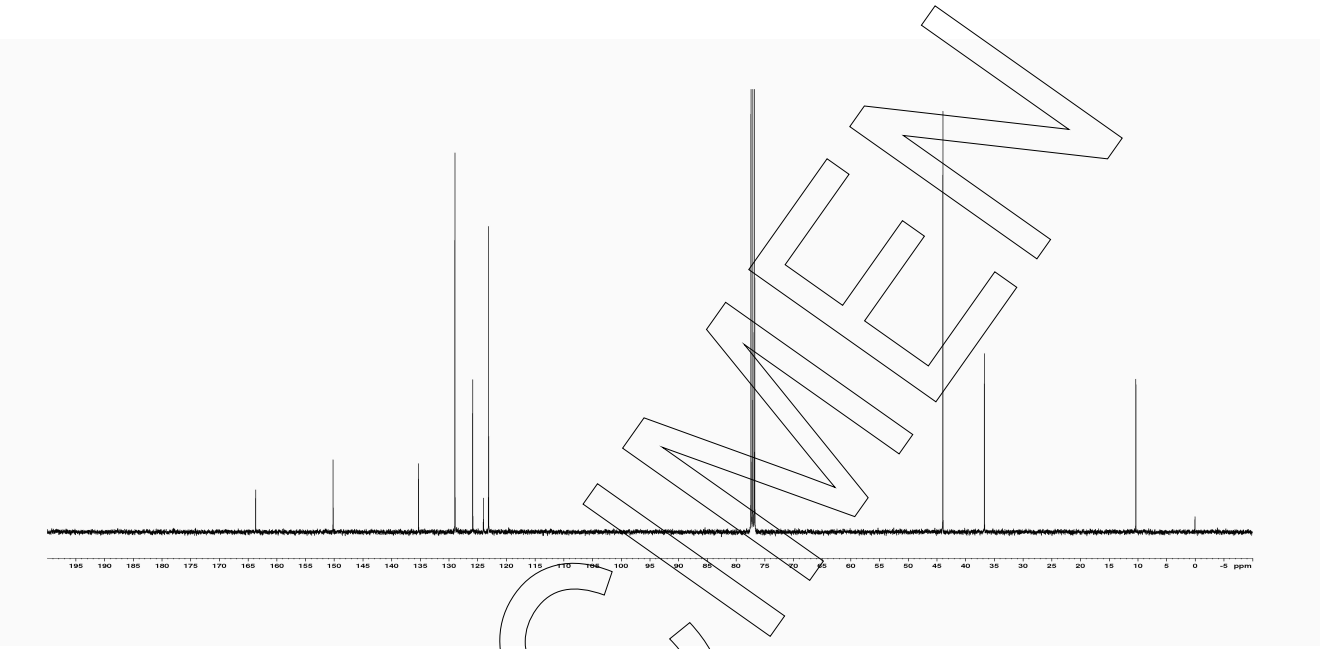
Method	Conditions	Result
^{13}C-NMR	100 MHz, $CDCl_3$	Structure confirmed



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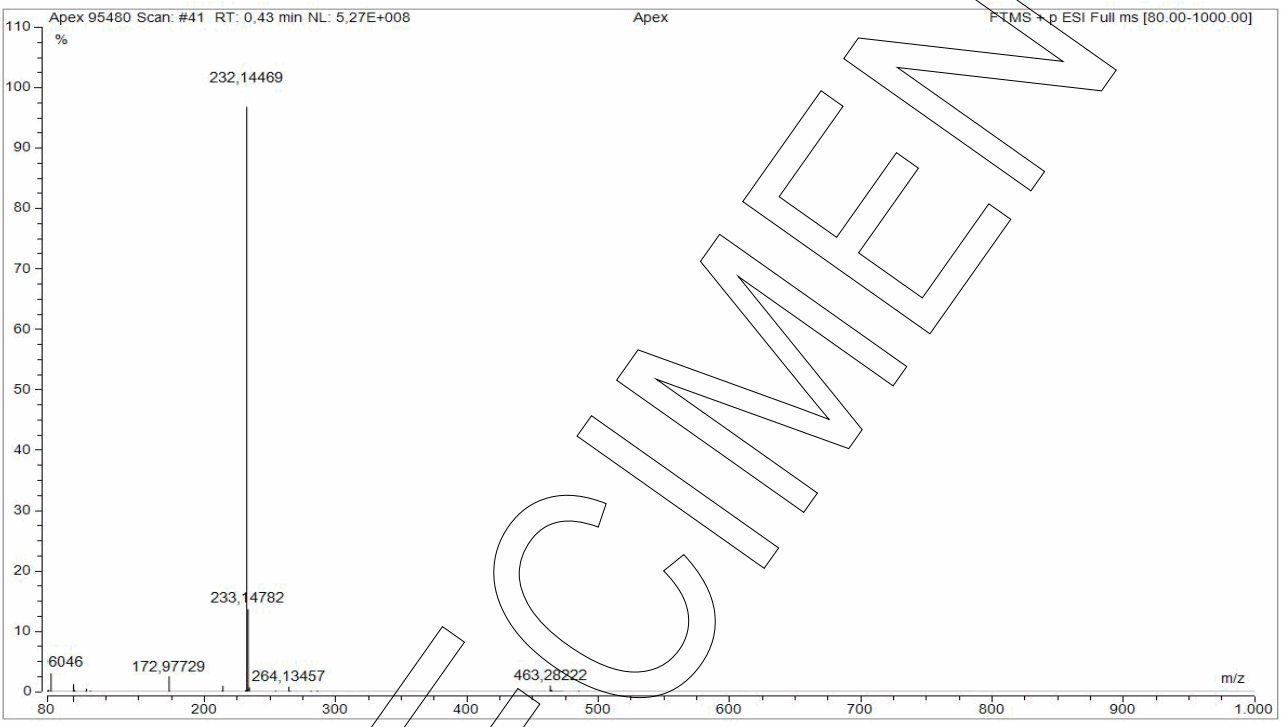
Method	Conditions	Result
MS	3.5 kV ESI+; capillary temperature: 269 °C Theoretical value: 232.14444	Structure confirmed



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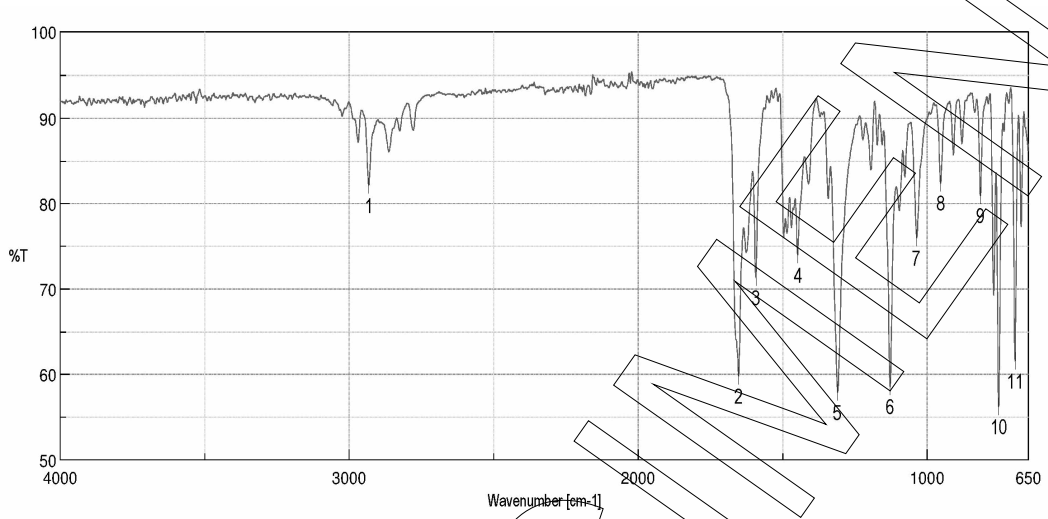
Method	Conditions	Result
IR	Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy	Structure confirmed



Results of Peak Find

No.	Position	Intensity
1	2932.23	82.0792
2	1652.7	59.7697
3	1592.91	71.3101
4	1448.28	73.9562
5	1310.39	57.8579
6	1128.15	58.5524
7	1036.55	75.9278
8	953.627	82.3359
9	815.742	80.8378
10	753.066	56.2192
11	696.177	61.5291

Stability and Homogeneity

Accelerated stability studies indicate no significant instability. The given validity period is based on this data. This is backed up by additional stability testing and historical data over the range of several years.

RM quality is controlled by regularly performed quality control tests (re tests). Homogeneity assured by qualified process of preparation and verified by homogeneity testing.

Revision table

Revision	Date	Reason for revision
00	23 Apr 2020	Release of the Certificate of Analysis - initial version

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

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