

Certificate of Analysis

Characterisation methods are accredited according to

ISO 17025

Reference Material

Product name

(2Z)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylprop-2-enamide

Product code

MM1221.02-0025

CAS number

145195-63-7

Molecular weight

305.29

Molecular formula

C₁₄H₁₅N₃O₅

Lot number

W1125535

Appearance

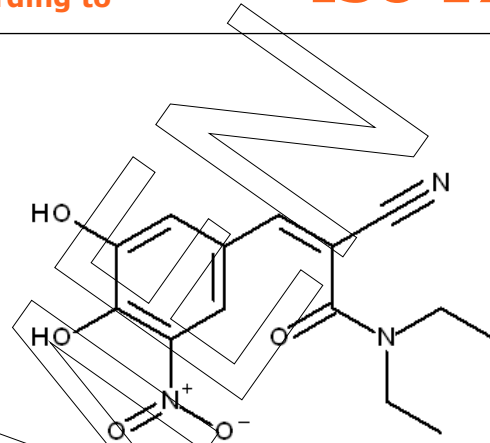
orange solid

Melting point (DSC)

152 °C

Long-term storage

2 to 8 °C, dark



Assay¹ "as is"
99.2 %

Uncertainty² U
0.2 %

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

Date of shipment: **05 Nov 2021**

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, 13 Sep 2021		

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": 99.18 %; U = 0.20 %

The assay "as is" is assessed by 100% method (mass balance) and is equivalent to the assay based on the not-anhydrous and not-dried substance. The assay is verified by carbon titration of elemental analysis. 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 - 100% method	
100% method (mass balance) with chromatographic purity by HPLC	
Result	99.18 %; U = 0.20 %

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.

Method 2: Value verifying technique - carbon titration of elemental analysis	
Method	percentage carbon found in relation to percentage carbon as calculated for molecular formula
Result (mass fraction, n = 3)	100.02 %

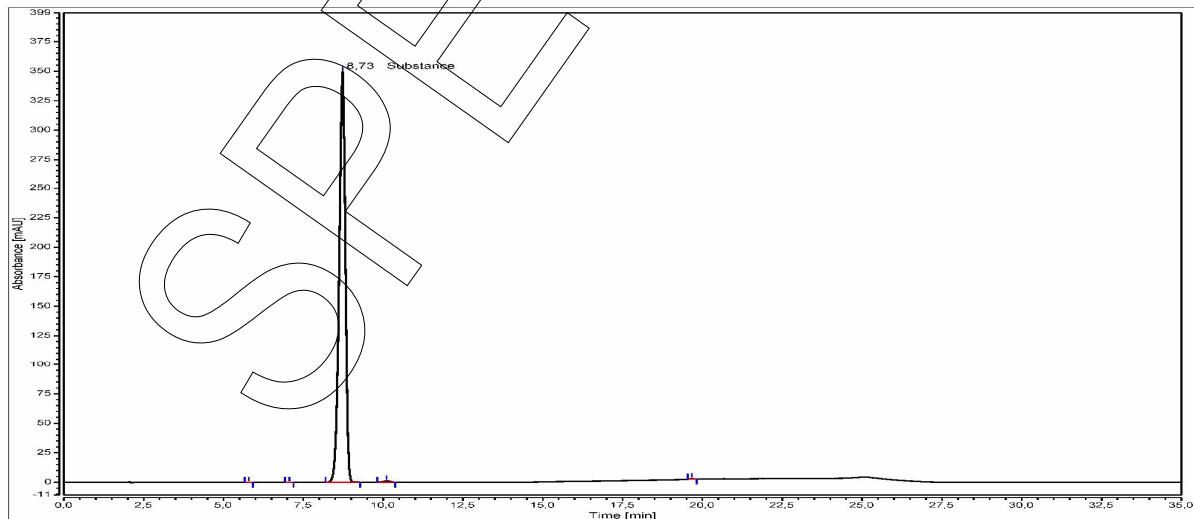


Purity

Purity by high performance liquid chromatography (HPLC)

HPLC Conditions:	
Column	Hypersil Gold C18; 5 μ m, 150 x 4.6 mm
Column temperature	40 °C
Detector	DAD, 300 nm
Injector	Auto 4 μ l; 0.0532 mg/ml in Acetonitrile/Water 50/50 (v/v)
Flow rate	1.0 ml/min
Phase A	Water, 0.1 % H ₃ PO ₄
Phase B	Acetonitrile, 0.1 % H ₃ PO ₄
Gradient program	0-12 min A/B 70/30 12-17 min A/B to 20/80 17-22 min A/B 20/80 22-25 min A/B to 70/30 25-35 min A/B 70/30 (v/v)

HPLC chromatogram and peak table





Area percent report - sorted by signal

Pk #	Retention time	Area	Area %
1	5.795	0.0206	0.03
2	7.063	0.0176	0.02
3	8.728	78.2408	99.55
4	10.110	0.2623	0.33
5	19.670	0.0534	0.07
Totals		78.5947	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) 99.55 %; U = 0.19 %

Volatile content

Water content

Method Karl Fischer titration

Result (n = 3) No significant amounts of water were detected (< 0.05 %).

Residual solvents

Method GC headspace

Result (n = 3) Sum: 0.37 %; U = 0.05 %
0.37 % Acetonitrile

Inorganic residues

Method: Elementary analysis

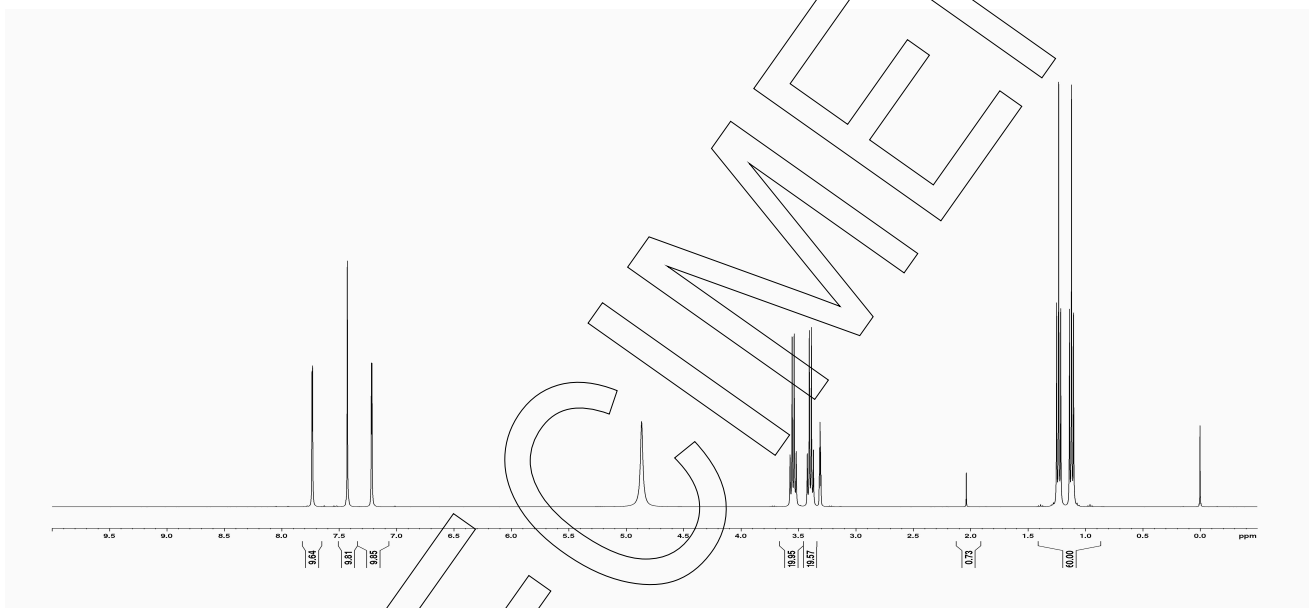
Inorganic residues can be excluded by elementary analysis (CHN).



Identity

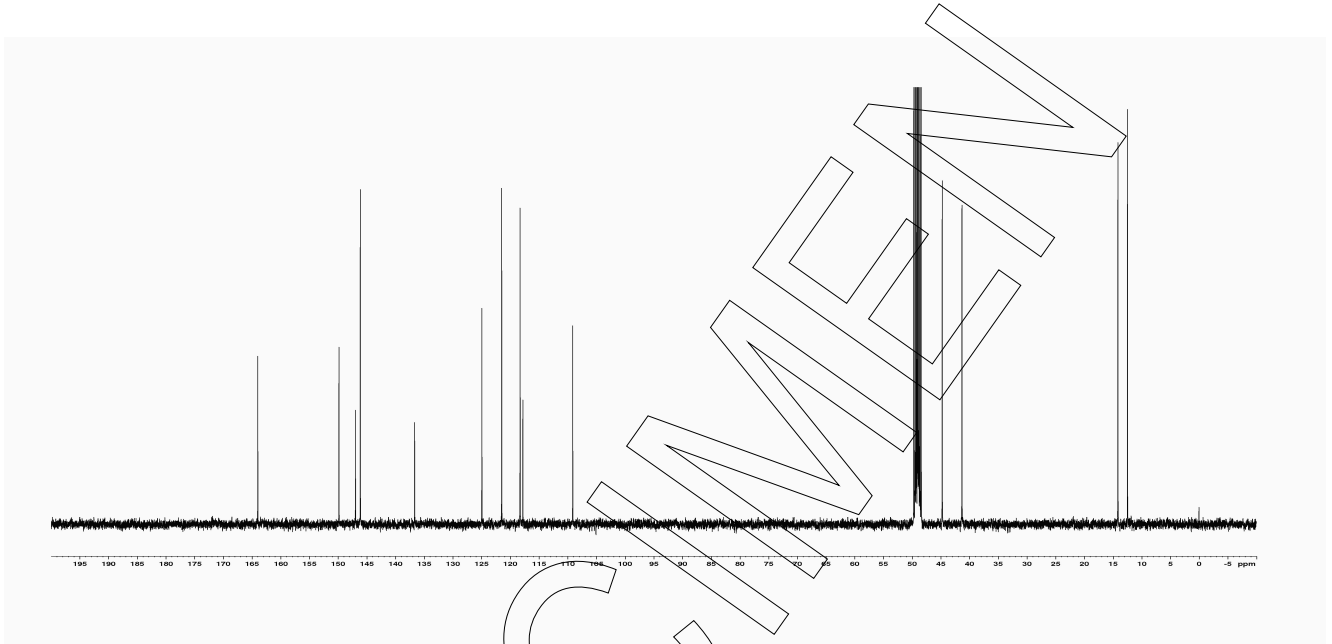
The identity is assessed by ISO/IEC 17025 accredited testing methods.

Method	Conditions	Result
$^1\text{H-NMR}$	400 MHz, CD_3OD	Structure confirmed





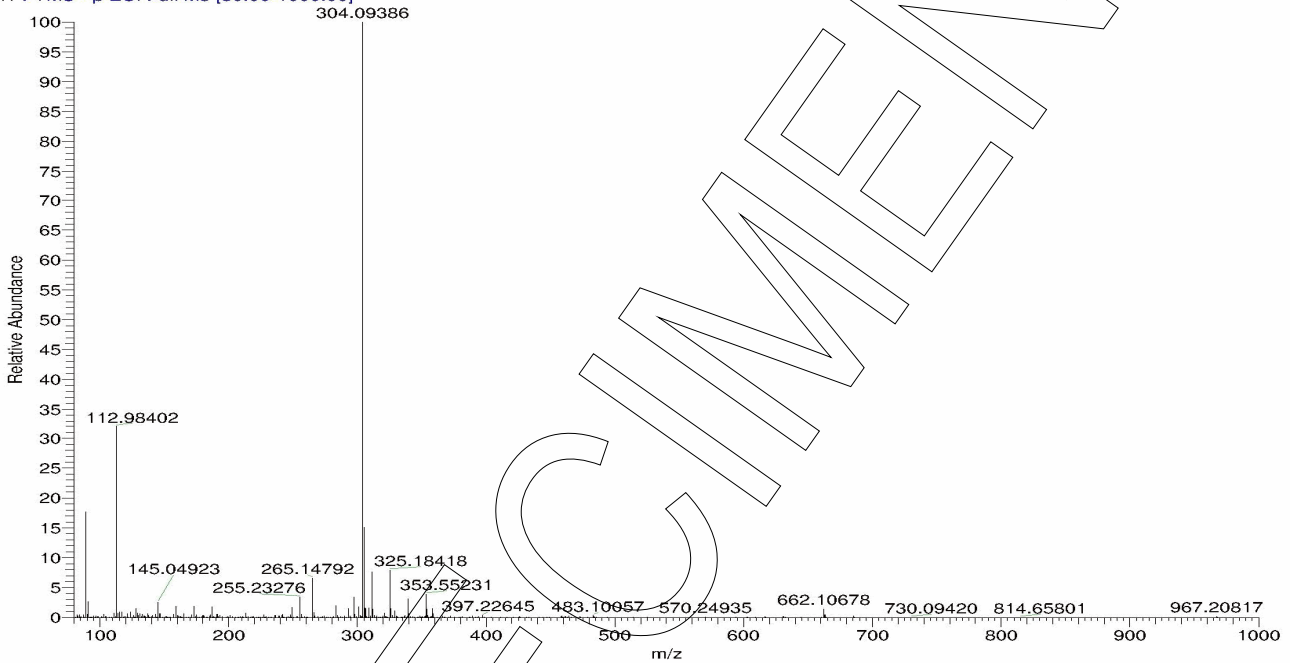
Method	Conditions	Result
¹³ C-NMR	100 MHz, CD ₃ OD	Structure confirmed





Method	Conditions	Result
MS	3.2 kV ESI-; capillary temperature: 269 °C Theoretical value: 304.09389	Structure confirmed

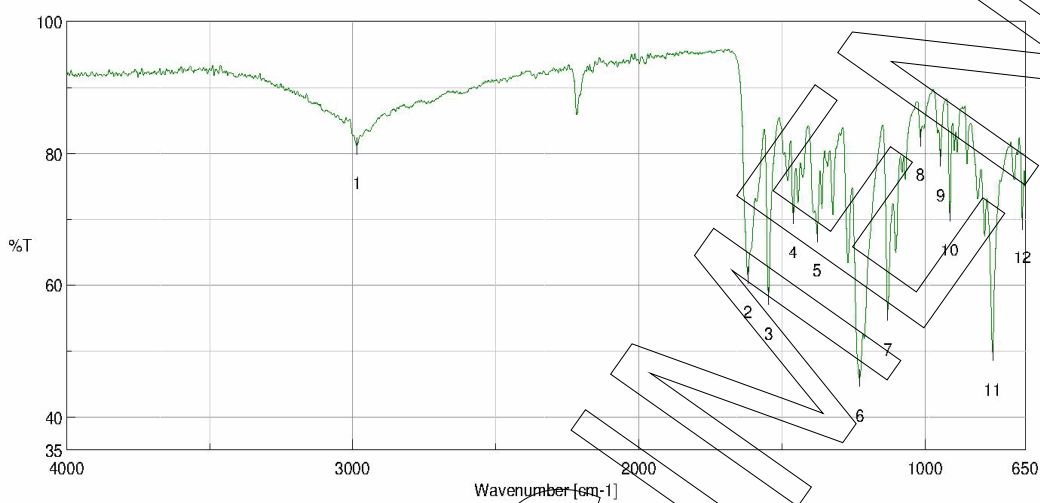
114440 #1-224 RT: 0.00-1.00 AV: 224 NL: 5.03E7
T: FTMS - p ESI Full ms [80.00-1000.00]



SPECIMEN



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



No.	Position	Intensity
1	2985.27	81.1253
2	1618.95	61.4683
3	1546.63	58.2411
4	1460.81	70.5875
5	1376.93	67.8362
6	1228.43	45.8603
7	1130.08	55.8381
8	1016.3	82.3732
9	945.913	79.2779
10	913.129	70.9643
11	763.673	49.7375
12	659.536	69.7634

Revision table

Revision	Date	Reason for revision
00	13 Sep 2021	Release of the Certificate of Analysis - initial version

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