



## Certificate of Analysis

Characterisation methods are accredited according to

### ISO 17025

### Reference Material

#### Product name

5-Amino-1H-pyrazole-4-carboxamide Hemisulphate

#### Product code

MM0034.01-0025

#### CAS number

27511-79-1

#### Molecular weight

350.31

#### Molecular formula

$2C_4H_6N_4O \cdot H_2SO_4$

#### Lot number

W1006309

#### Appearance

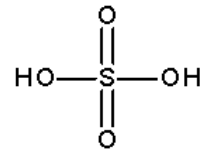
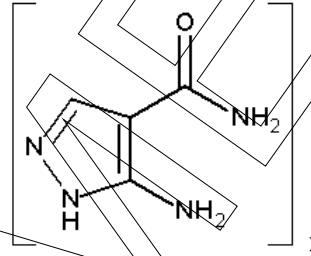
pale yellow solid

#### Melting point

221 °C

#### Long-term storage

2 to 8 °C, dark



Assay<sup>1</sup> "as is"  
**95.1 %**

Uncertainty<sup>2</sup> U  
**0.9 %**

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

Date of shipment: **02 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.

<b>Release by:</b>	<b>Date of Release:</b>		Product Release
Dr. Sabine Schröder	Luckenwalde, 19 Aug 2019		

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

<sup>2</sup> 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": 95.08 %; U = 0.88 %**

The assay "as is" is assessed by carbon titration of elemental analysis 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 - carbon titration of elemental analysis

<b>Method</b>	percentage carbon found in relation to percentage carbon as calculated for molecular formula
<b>Result (mass fraction, n = 3)</b>	95.08 %; U = 0.88 %

### Method 2: Value verifying technique - 100% method

<b>100% method (mass balance) with chromatographic purity by HPLC</b>	
<b>Result</b>	94.09 %

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.

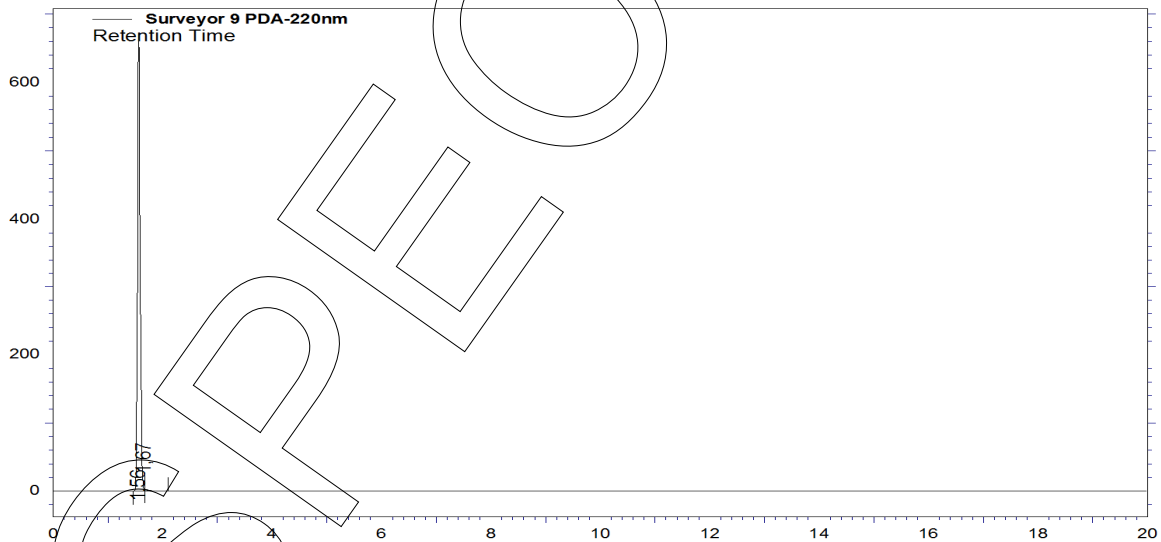


## Purity

### Purity by High Performance Liquid Chromatography (HPLC)

HPLC Conditions:	
Column	LiChrospher 60 RP-select B; 5 µm, 125 x 4.0 mm
Column temperature	40 °C
Detector	DAD, 220 nm
Injector	Auto 3.00 µl; 6.076 mg/ml in Water
Flow rate	1.0 ml/min
Phase A	Water, 0.1 % H <sub>3</sub> PO <sub>4</sub>
Phase B	Acetonitrile, 0.1 % H <sub>3</sub> PO <sub>4</sub>
Gradient program	A/B 98/2 (v/v)

HPLC chromatogram and peak table





## Area percent report - sorted by signal

Pk #	Retention time	Area	Area %
1	1.56	2256192	99.45
2	1.67	12463	0.55
<b>Totals</b>		<b>2268655</b>	<b>100.00</b>

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)** 99.44 %; U = 0.20 %

### Volatile content

#### Water content

**Method** Karl Fischer titration  
**Result (n = 3)** 5.38 %\*; SD = 0.04 %

\*not accredited testing method

#### Residual solvents

**Method** <sup>1</sup>H-NMR  
**Result (n = 1)** No significant amounts of residual solvents were detected (< 0.05 %).\*

\*not accredited testing method

### Inorganic residues

**Method:** Elementary analysis

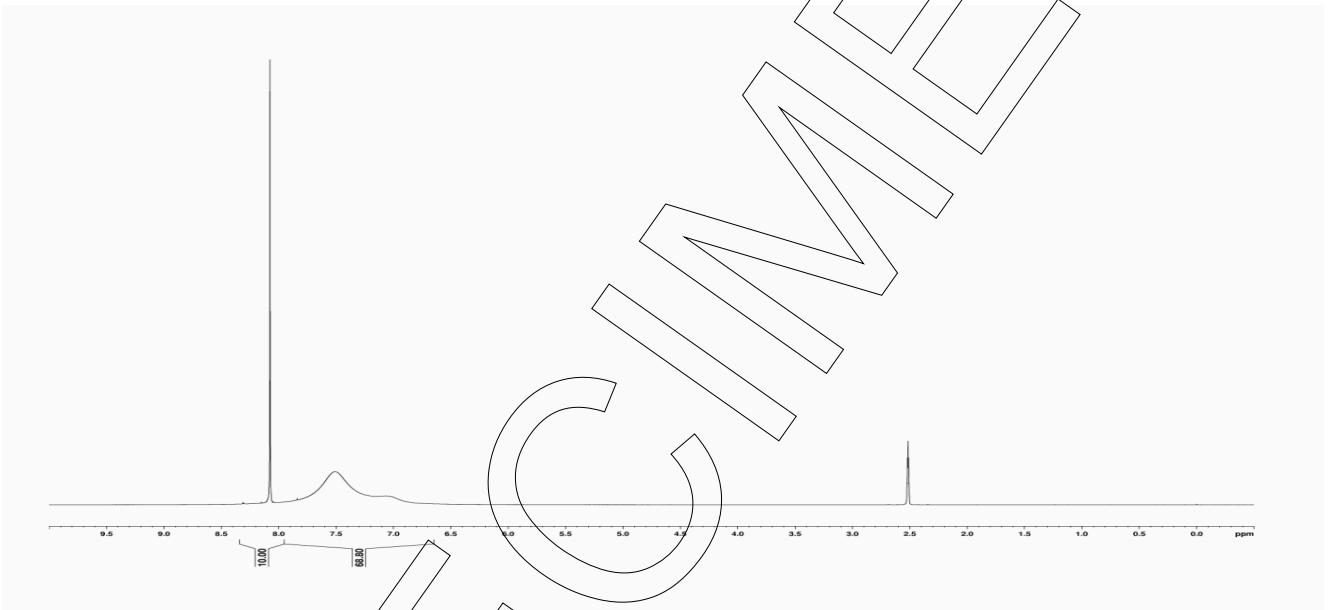
Inorganic residues can be excluded by elementary analysis (CHN).



### Identity

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

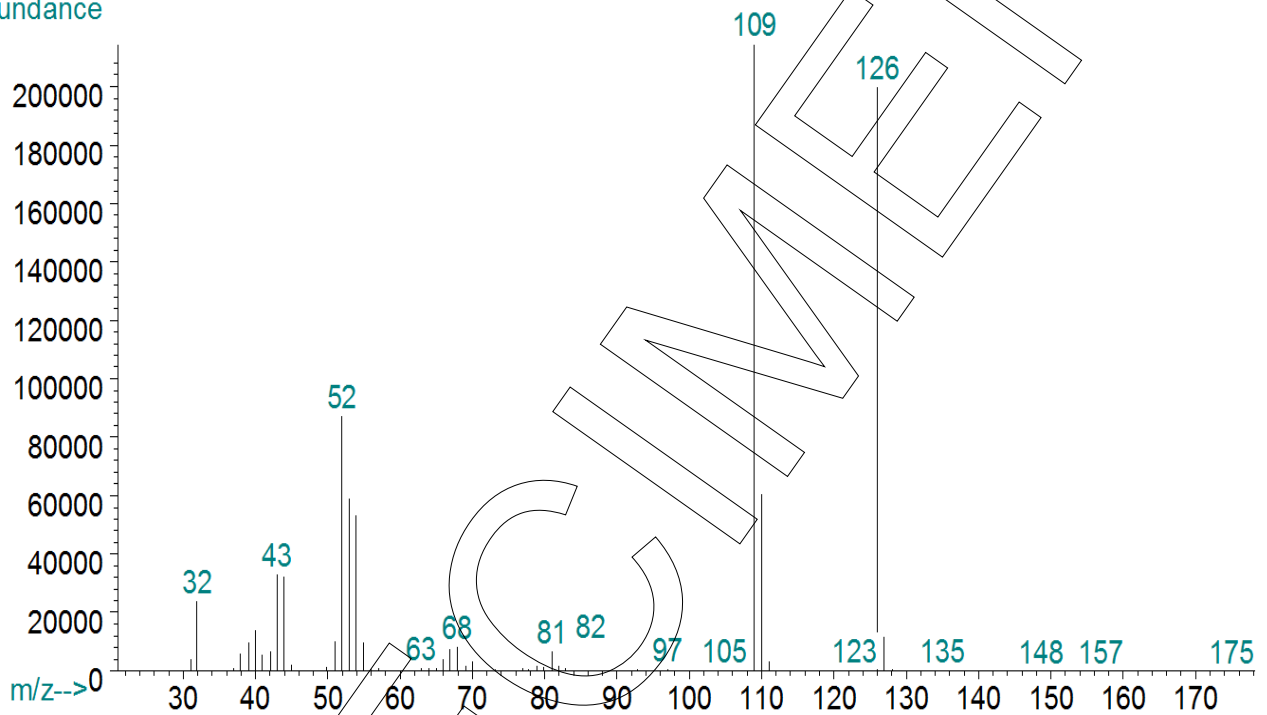
Method	Conditions	Result
<sup>1</sup> H-NMR	400 MHz, DMSO-d <sub>6</sub>	Structure confirmed





Method	Conditions	Result
MS	EI, 70eV, detector temperature: 280 °C	Structure confirmed

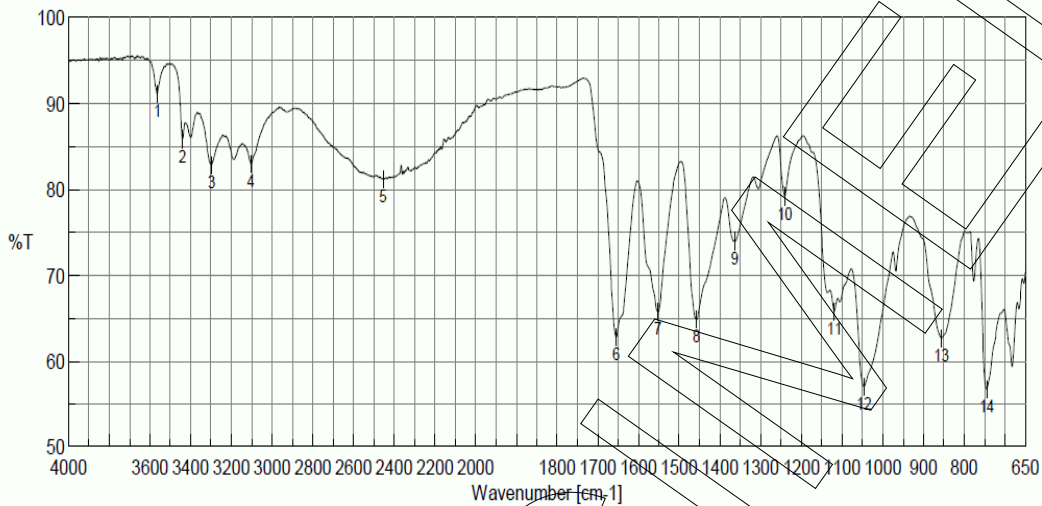
Abundance



SAMPLE



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



Result of Peak Picking		
No.	Position	Intensity
1	3564.77	91.0795
2	3440.39	85.7955
3	3297.68	82.8255
4	3102.9	82.9286
5	2453.97	81.1167
6	1654.62	62.7218
7	1552.42	65.6792
8	1457.92	64.8005
9	1364.39	73.9075
10	1240.97	79.1086
11	1119.48	65.7444
12	1046.19	56.994
13	856.239	62.6512
14	744.388	56.7179

## Revision table

Revision	Date	Reason for revision
00	19 Aug 2019	Release of the Certificate of Analysis - initial version

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