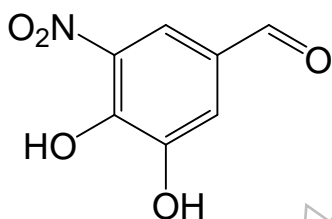




# Certificate of Analysis

## Reference Standard

3,4-Dihydroxy-5-nitrobenzaldehyde



Molecular Formula: C<sub>7</sub>H<sub>5</sub>NO<sub>5</sub>  
Molecular Weight: 183.12  
CAS Number: [ 116313-85-0 ]

Catalogue Number: MM1221.01  
Lot Number: 1425  
Long-term Storage: 2 to 8 °C, dark  
Appearance: brown solid  
Melting Point: > 146 °C (dec.)  
hygroscopic  
Assay 'as is': 99.1 %

Date of shipment: **2015-July-05**

This certificate is valid for two years from the date of shipment provided the substance is stored under the recommended conditions.



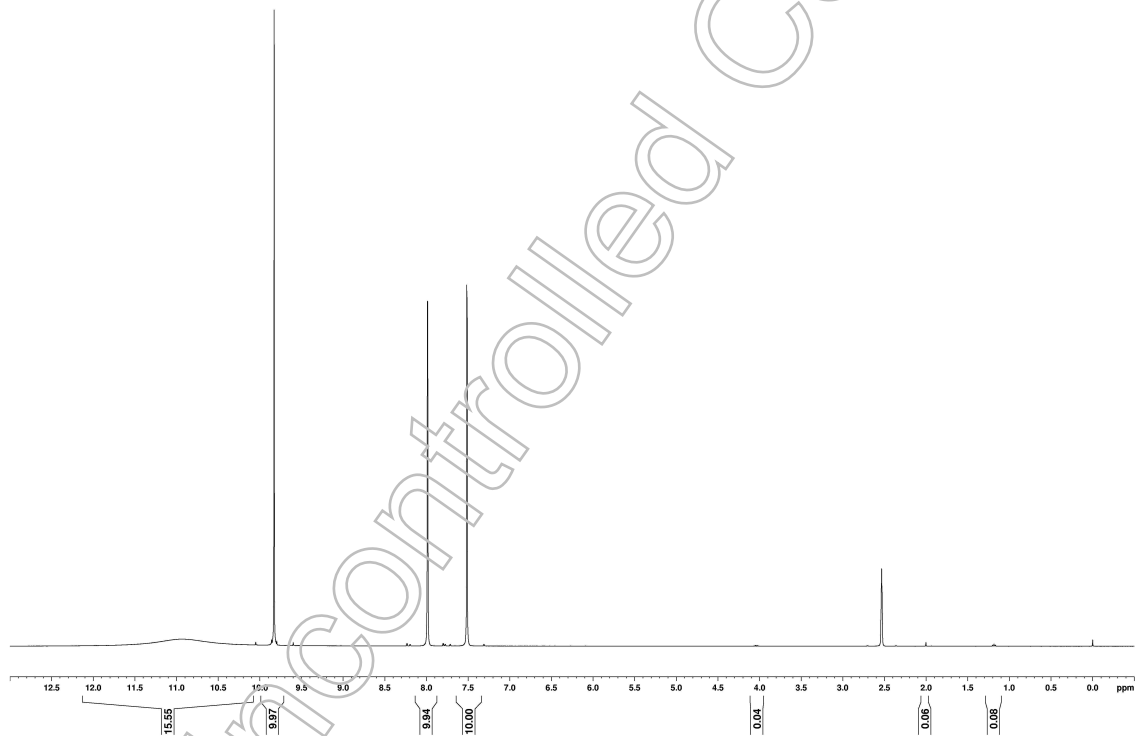
## I. Identity

The identity of the reference substance was established by following analyses.

### Ia. <sup>1</sup>H-NMR Spectrum

Conditions: 400 MHz, DMSO-d<sub>6</sub>

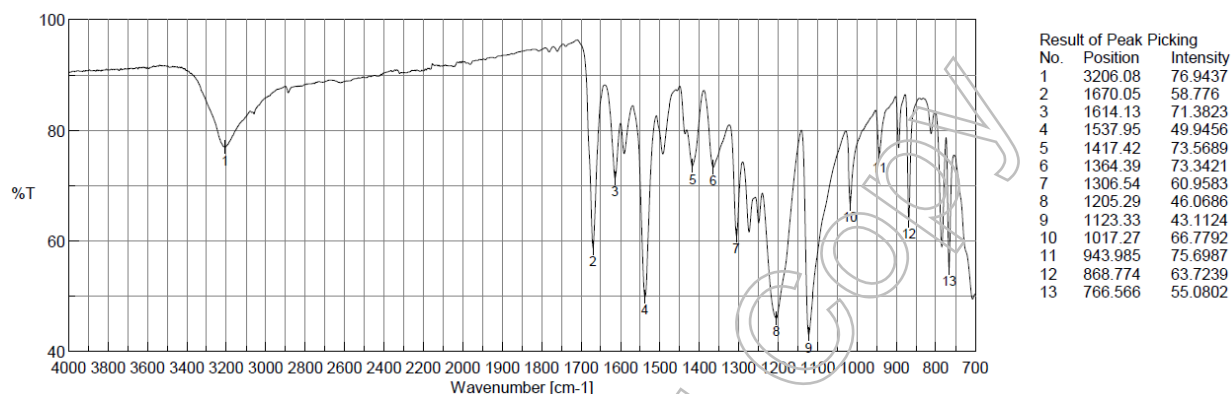
The structure is confirmed with the signals of the spectrum and their interpretation.





## 1b. IR Spectrum

Method: Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy



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

## II. Purity

The purity of the reference substance was analysed by high performance liquid chromatography (HPLC).

### HPLC Conditions:

#### Column:

Hypersil Gold (C18)  
5  $\mu\text{m}$ , 150 x 4.6 mm

#### Conditions:

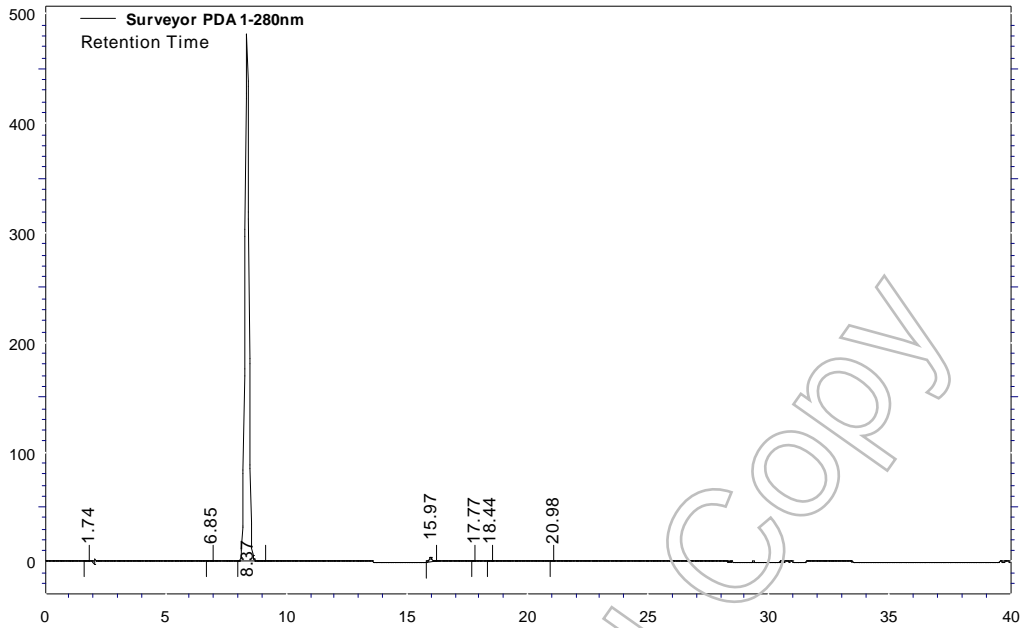
1.0 ml/min, 40 °C  
0 – 10 min Water/Acetonitrile 85/15  
10 – 20 min Water/Acetonitrile to 30/70  
20 – 30 min Water/Acetonitrile to 85/15  
30 – 40 min Water/Acetonitrile 85/15 (v/v);  
0.1 %  $\text{H}_3\text{PO}_4$

#### Detector:

DAD  
280 nm

#### Injector:

Auto  
3  $\mu\text{l}$ ; 0.0896 mg/ml in  
Water/Acetonitrile 50/50 (v/v)



**Area Percent Report - Sorted by Signal**

Pk #	Retention Time	Area	Area %
1	1.74	1318	0.02
2	6.85	832	0.02
3	8.37	5428654	99.36
4	15.97	28528	0.52
5	17.77	947	0.02
6	18.44	2213	0.04
7	20.98	1103	0.02
Totals		5463595	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 %.

**Results:**

**Average** 99.36 %  
**Number of results** n=6  
**Standard deviation** 0.01 %



### III. Water Content

Method: Karl Fischer titration

**Results:**

<b>Average</b>	0.17 %
<b>Number of results</b>	n=3
<b>Standard deviation</b>	0.01 %

### IV. Residual Solvents

Method: <sup>1</sup>H-NMR

Result: 0.11 % Ethyl acetate

### V. Final Result

<b>Total impurities (HPLC)</b>	0.64 %
<b>Water content</b>	0.17 %
<b>Residual solvents</b>	0.11 %
<b>Assay (100 % method)</b>	99.08 %

The assay is assessed to be 99.1 % 'as is'

The assay 'as is' is equivalent to the assay based on the not anhydrous and not dried substance respectively.

Release Date: 2010-09-20

LGC GmbH

Dr. Sabine Schröder  
Product Release