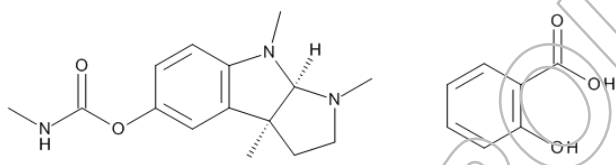




# Certificate of Analysis

## Reference Standard

Physostigmine Salicylate



Molecular Formula:  $C_{15}H_{21}N_3O_2$   $C_7H_6O_3$   
Molecular Weight: 413.47  
CAS Number: 57-64-7

Catalogue Number: MM1707.00  
Lot Number: 62696  
Long-term Storage: 2 to 8 °C, dark  
Appearance: white solid  
Melting Point (DSC): 187 °C  
Assay 'as is': 99.8 %

Date of shipment: **2017-September-05**

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

LGC Quality ISO 9001:2008  
DQS 102448 QM08

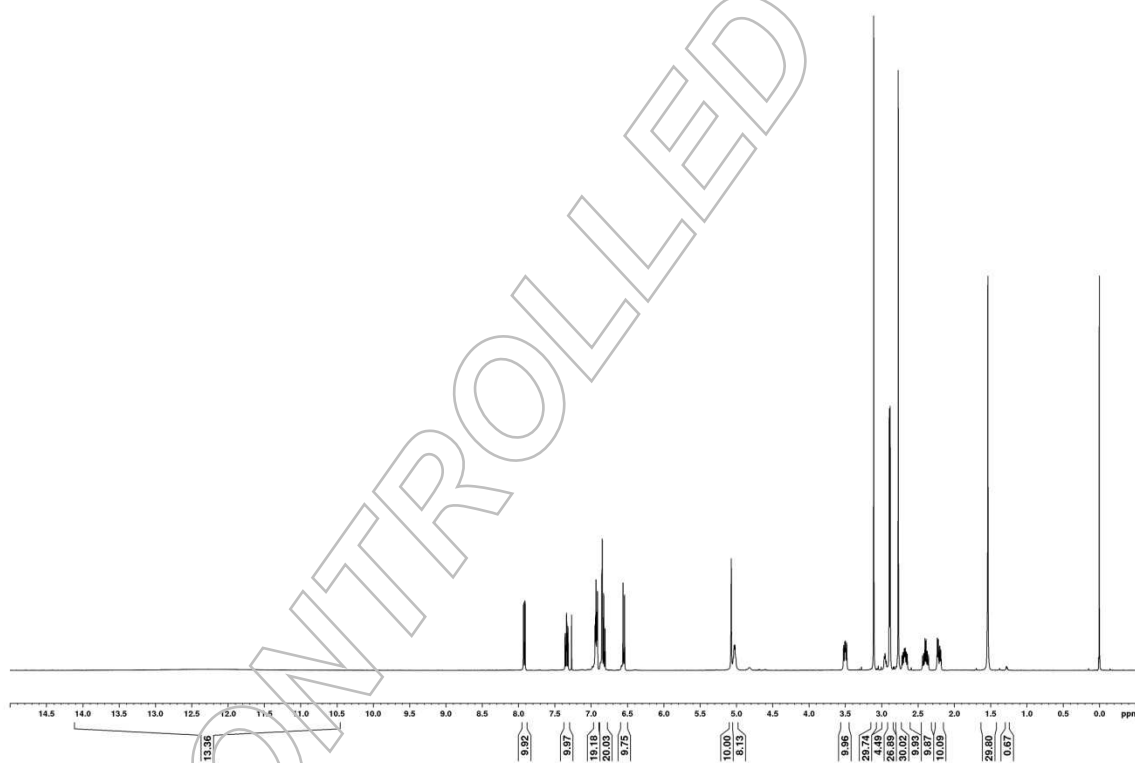


## I. Identity

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

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

Conditions: 400 MHz, CDCl<sub>3</sub>

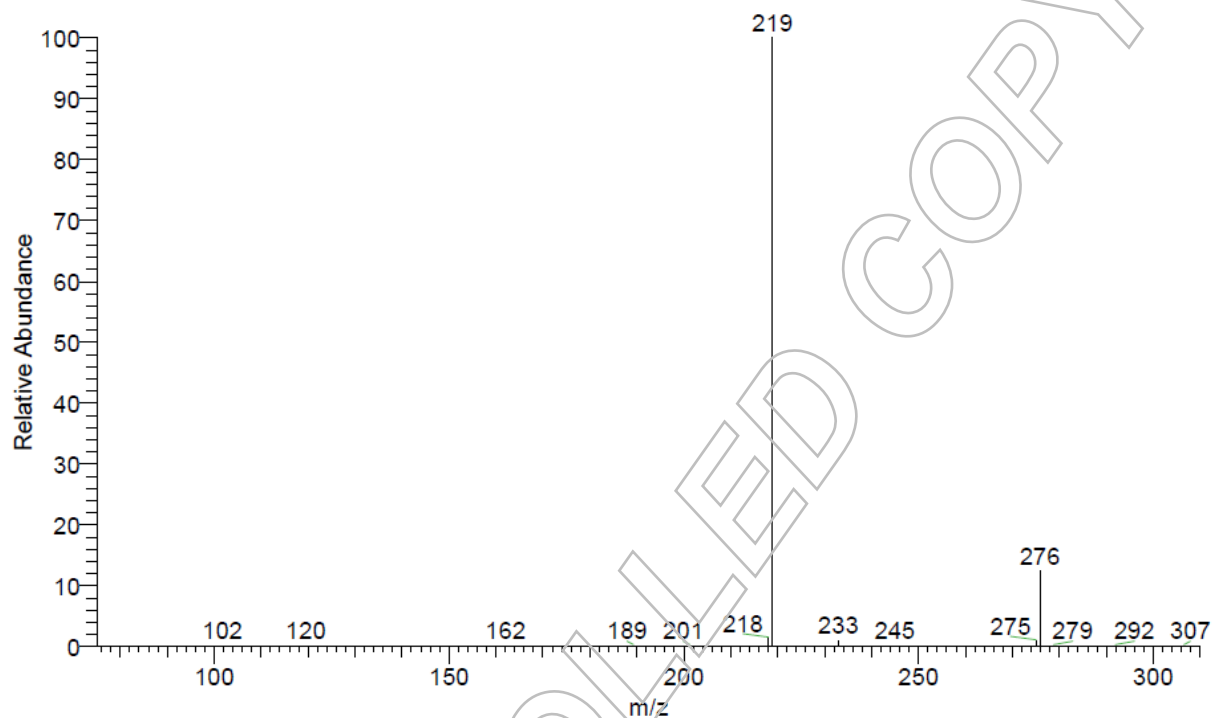


The structure is confirmed by the signals of the spectrum and their interpretation.



### Ib. Mass Spectrum

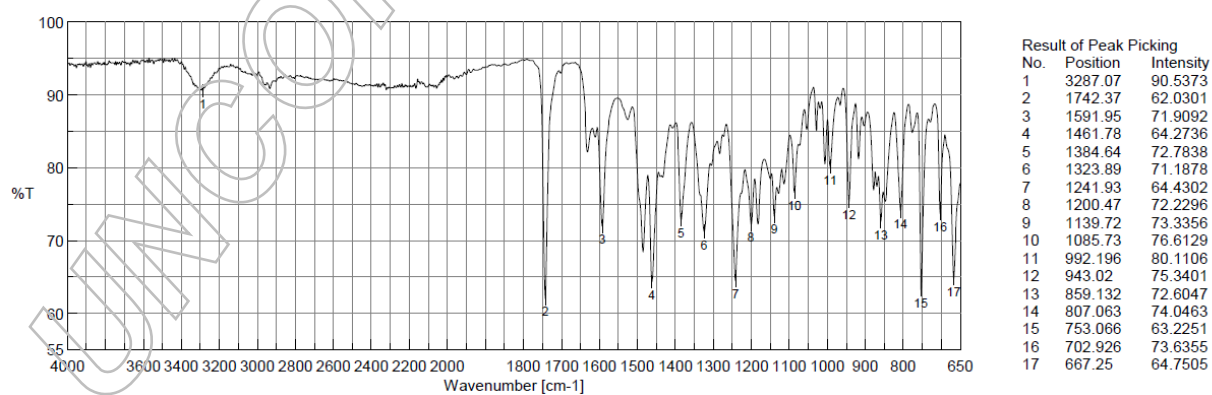
Method: 4.5 kV ESI+; vaporization temperature: 200 °C



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

### Ic. 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

### IIa. High Performance Liquid Chromatography (HPLC)

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

#### HPLC Conditions:

##### Column:

Cortecs UPLC C18+  
1.6 µm, 75 x 2.1 mm

##### Conditions:

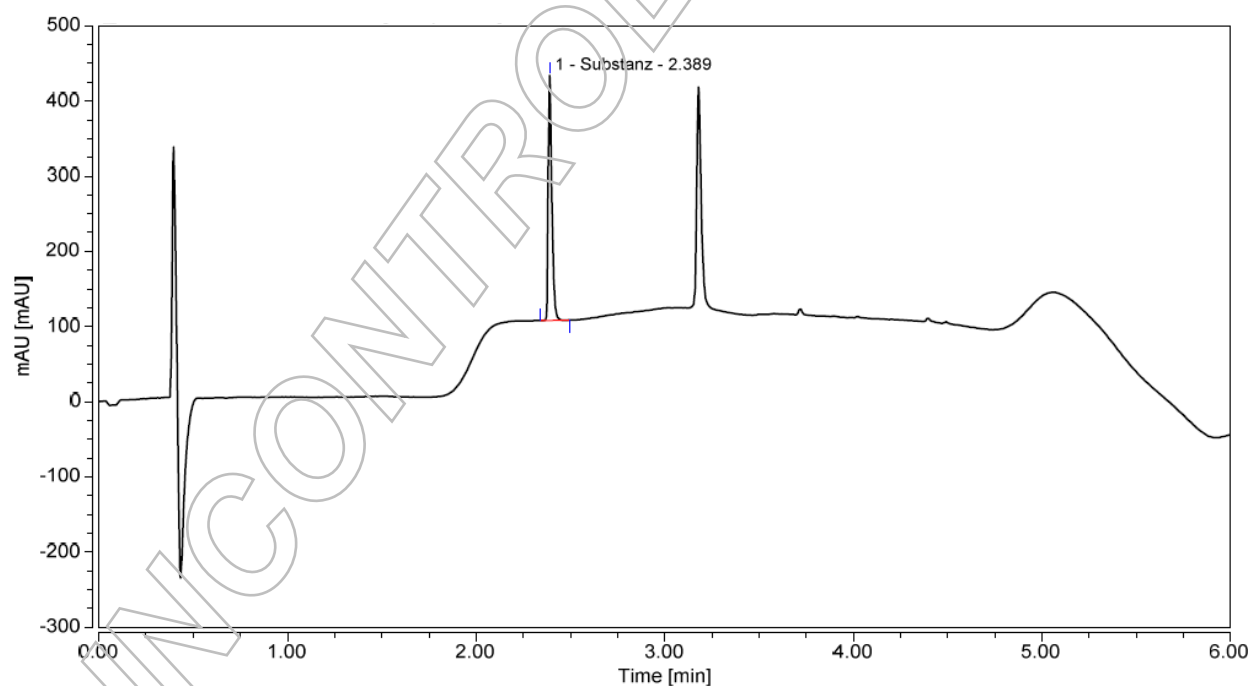
1.0 ml/min, 40 °C  
0-1 min Water/Acetonitrile 98/2  
1-4 min Water/Acetonitrile to 2/98  
4-5 min Water/Acetonitrile to 98/2  
5-6 min Water/Acetonitrile 98/2 (v/v);  
0.1 % HCOOH

##### Detector:

DAD  
210 nm

##### Injector:

Auto  
1 µl; 0.042 mg/ml in  
Water/Acetonitrile 50/50 (v/v)





### Area Percent Report - Sorted by Signal

| Pk #   | Retention Time | Area  | Area % |
|--------|----------------|-------|--------|
| 1      | 2.389          | 7.246 | 100.00 |
| Totals |                | 7.246 | 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 100.00 %  
Number of results n=3  
Standard deviation < 0.01 %

#### IIb. Water Content

Method: Karl Fischer titration

#### Results:

Average 0.13 %  
Number of results n=3  
Standard deviation 0.01 %

#### IIc. Residual Solvents

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

Result: 0.05 % Acetone



### III. Assay by Elementary Analysis (Carbon Titration)

Method: percentage carbon found in relation to percentage carbon as calculated for molecular formula

#### Results:

Arithmetic mean (n=3) 99.97 % (mass fraction)

### IV. Final Result

|                                   |          |
|-----------------------------------|----------|
| Chromatographic purity (HPLC)     | 100.00 % |
| Water content                     | 0.13 %   |
| Residual solvents                 | 0.05 %   |
| Assay (100 % method) <sup>1</sup> | 99.82 %  |

The assay is assessed to be 99.8 % 'as is'

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

Release Date:

Luckenwalde, 2015-08-27

Dr. Sabine Schröder  
Product Release

<sup>1</sup> 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, purity is considered as relative contribution.