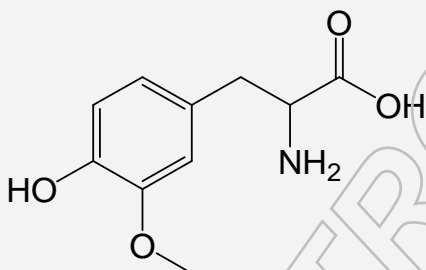


## Certificate of Analysis

### Reference Substance

(DL)-3-O-Methyldopa (3-Methoxy-DL-tyrosine)

Catalogue Number:	LGCFOR0262.05	Long-term Storage:	2 to 8 °C, dark
Lot Number:	22045	Appearance:	off-white solid
Molecular Formula:	C <sub>10</sub> H <sub>13</sub> NO <sub>4</sub>	Melting Point:	245 °C (dec.) hygroscopic
Molecular Weight:	211.21	Assay 'as is':	97.6 %
CAS Number:	[ 7636-26-2 ]		



Date of shipment: **2016-May-20**

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

Release Date: 2013-01-04

LGC GmbH

Dr. Sabine Schröder

Product Release

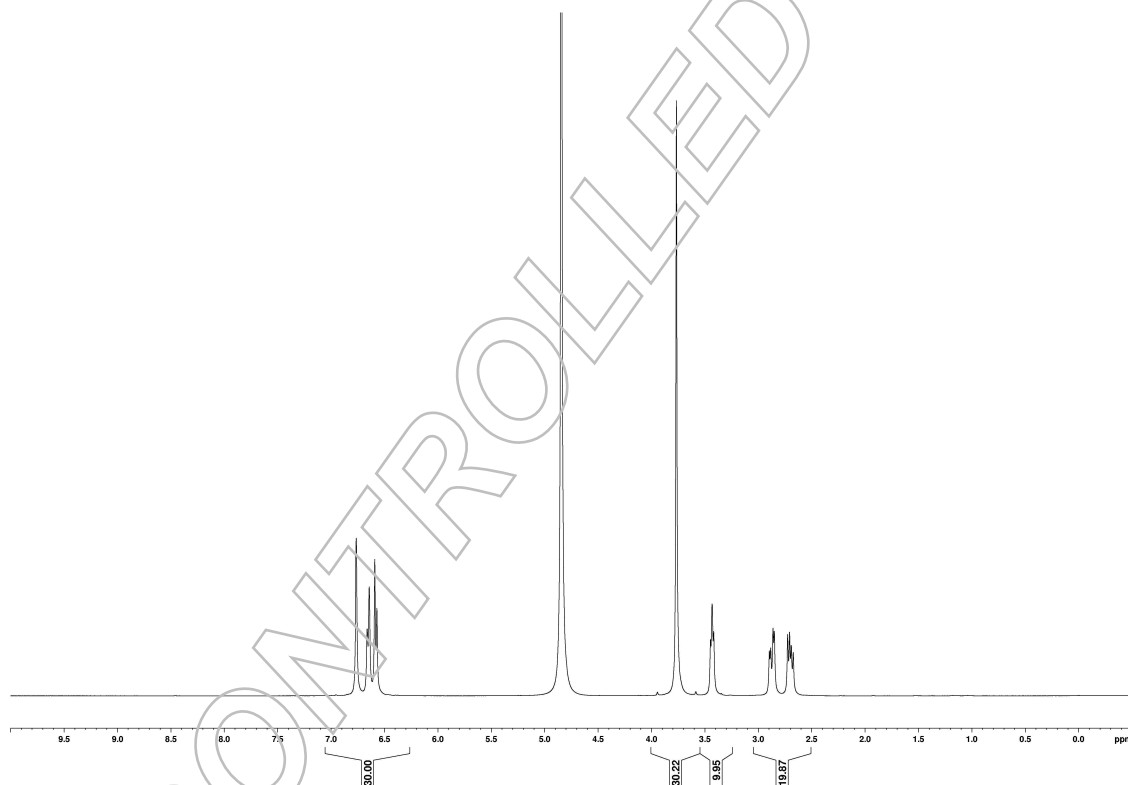
## I. Identity

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

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

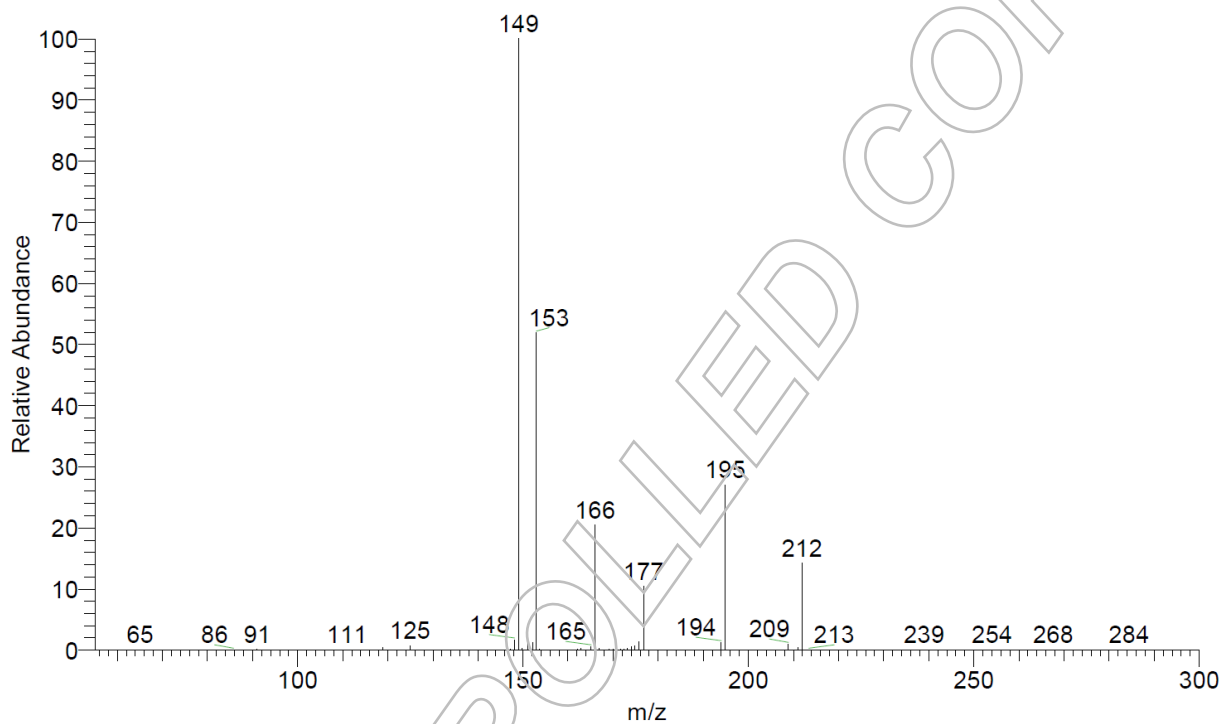
Conditions: 400 MHz, D<sub>2</sub>O/NaOD

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



## 1b. Mass Spectrum

Method: 4.5 kV ESI; vaporization temperature: 200 °C, direct inlet

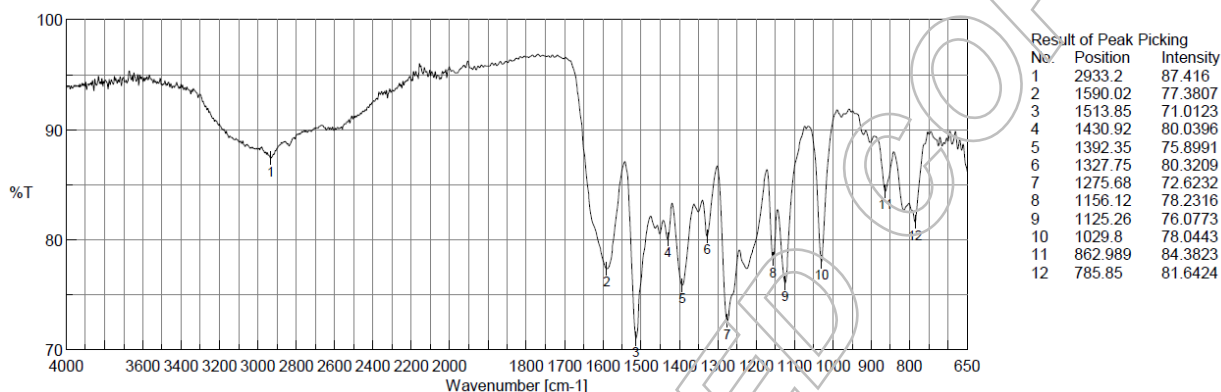


m/z	fragments
212	[ MH ]
195	[ MH – OH ]
166	[ M – COOH ]

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

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

### HPLC Conditions:

**Column:**

ODS-AQ  
5 µm, 250 x 4.6 mm

**Conditions:**

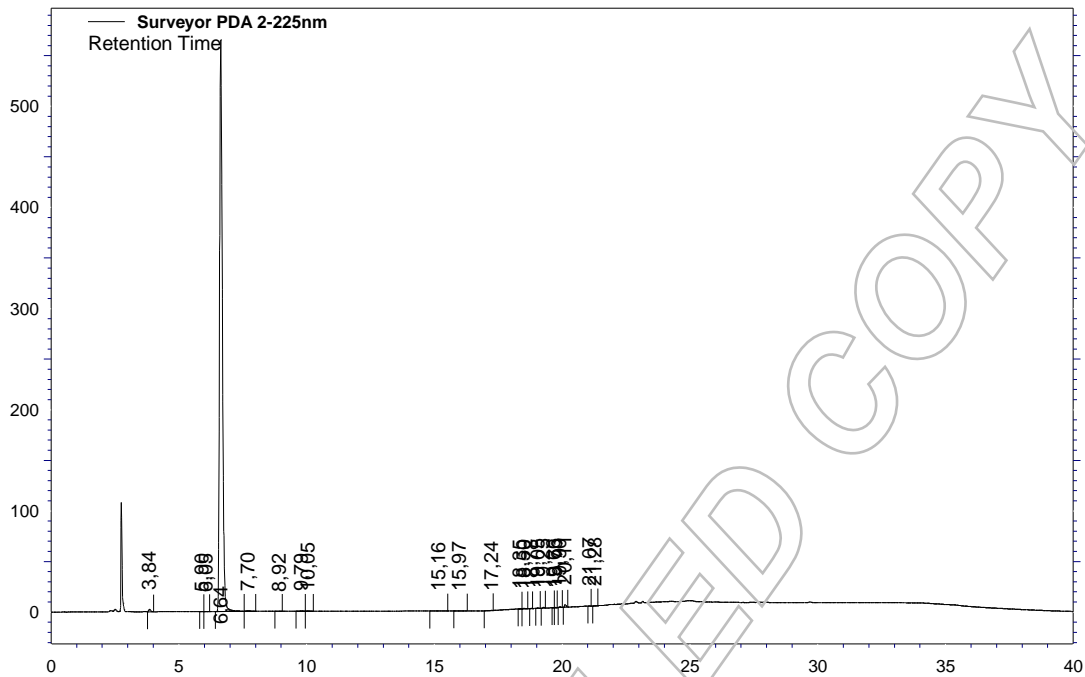
1.0 ml/min, 40 °C  
0 – 12 min Water/Acetonitrile 97/3  
12 – 20 min Water/Acetonitrile to 60/40  
20 – 30 min Water/Acetonitrile 60/40  
30 – 35 min Water/Acetonitrile to 97/3  
35 – 40 min Water/Acetonitrile 97/3 (v/v);  
0.1 % H<sub>3</sub>PO<sub>4</sub>

**Detector:**

DAD  
225 nm

**Injector:**

Auto  
8 µl; 0.724 mg/ml in  
1 M HCl



**Area Percent Report - Sorted by Signal**

Pk #	Retention Time	Area	Area %
1	3.84	11436	0.27
2	5.90	418	0.01
3	6.05	577	0.01
4	6.64	4229651	98.66
5	7.70	6500	0.15
6	8.92	648	0.02
7	9.79	2800	0.07
8	10.05	2196	0.05
9	15.16	1691	0.04
10	15.97	1632	0.04
11	17.24	1388	0.03
12	18.35	1335	0.03
13	18.50	3095	0.07
14	18.78	1143	0.03
15	19.05	3011	0.07
16	19.25	3412	0.08
17	19.66	528	0.01
18	19.73	604	0.01
19	19.90	2970	0.07
20	20.11	10609	0.25
21	21.07	714	0.02
22	21.28	595	0.01
Totals		4286953	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:**

<b>Average</b>	98.66 %
<b>Number of results</b>	n=3
<b>Standard deviation</b>	0.03 %

**III. Water Content**

Method: Karl Fischer titration

**Results:**

<b>Average</b>	1.03 %
<b>Number of results</b>	n=3
<b>Standard deviation</b>	0.01 %

**IV. Residual Solvents**

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

No significant amounts of residual solvents were detected (< 0.05 %).

## V. Final Result

<b>Total impurities (HPLC)</b>	1.34 %
<b>Water content</b>	1.03 %
<b>Residual solvents</b>	n. d. (not detected)
<b>Assay (100 % method)<sup>1</sup></b>	97.64 %

The assay is assessed to be 97.6 % 'as is'

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

---

<sup>1</sup> The calculation of the 100 % method follows the formula:

$$\text{Assay (\%)} = (100 \% - \text{KF} - \text{RES}) \times \frac{\text{Purity HPLC (\%)}}{100 \%}$$

Water (KF) and Residual solvents (RES) are considered as absolute contributions, HPLC purity is considered as relative contribution.

LGCFOR0262.05 Lot Number 22045

LGC GmbH, Im Biotechnologiepark, TGZ II, D-14943 Luckenwalde, Germany

© 2012 LGC limited. All rights reserved. LGC Standards is part of the LGC Group. LoGiCal is a registered trademark of LGC Standards GmbH