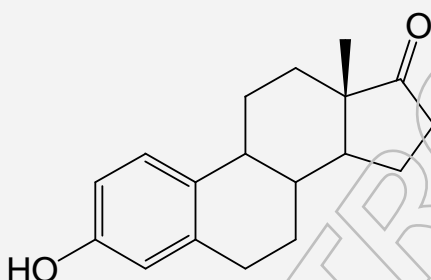


Certificate of Analysis

Reference Substance

Estrone

Catalogue Number:	LGCFOR0282.00	Long-term Storage:	2 to 8 °C, dark
Lot Number:	6671	Appearance:	white solid
Molecular Formula:	C ₁₈ H ₂₂ O ₂	Melting Point:	260 °C
Molecular Weight:	270.37	Assay 'as is':	99.2 %
CAS Number:	[53-16-7]		



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: 2011-03-10

LGC GmbH



Dr. Sabine Schröder
Product Release

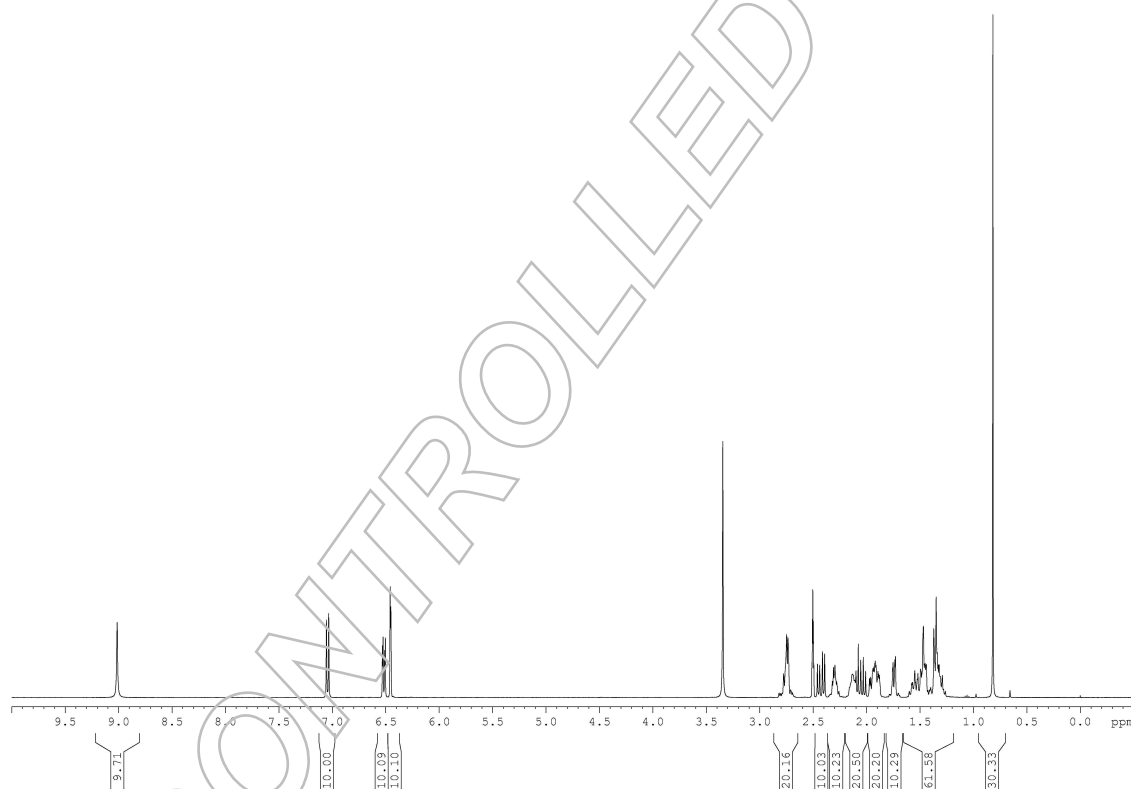
I. Identity

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

Ia. ¹H-NMR Spectrum

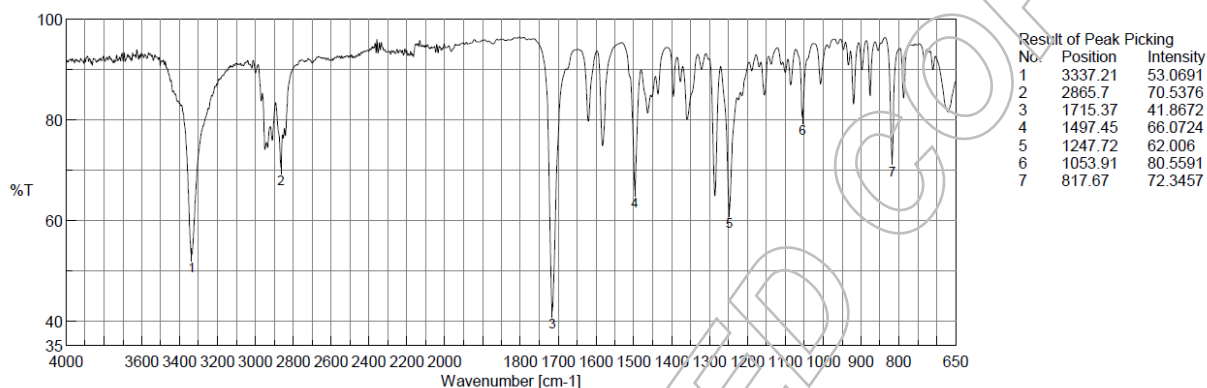
Conditions: 400 MHz, DMSO-d₆

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



Ib. 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:

RP 60 Select B
5 µm, 125 x 4 mm

Conditions:

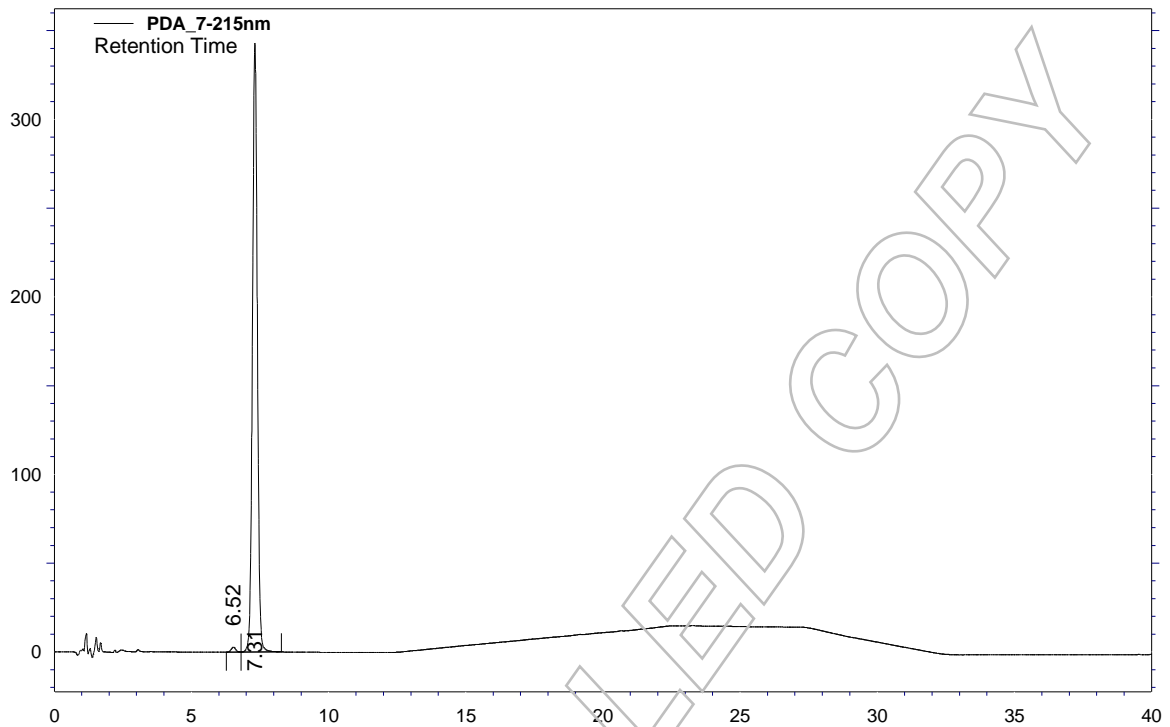
1.0 ml/min, 40 °C
0 – 10 min Water/Acetonitrile 60/40
10 – 20 min Water/Acetonitrile to 30/70
20 – 25 min Water/Acetonitrile 30/70
25 – 30 min Water/Acetonitrile to 60/40
30 – 40 min Water/Acetonitrile 60/40 (v/v);
0.1 % H₃PO₄

Detector:

DAD
215 nm

Injector:

Auto
5 µl; 0.1072 mg/ml in
Methanol



Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	6.52	30329	0.71
2	7.31	4265691	99.29
Totals		4296020	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 99.27 %
Number of results n=6
Standard deviation 0.01 %

III. Water Content

Method: Karl Fischer titration

Results:

Average	0.07 %
Number of results	n=2

IV. Residual Solvents

Method: ¹H-NMR

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

V. Final Result

Total impurities (HPLC)	0.73 %
Water content	0.07 %
Residual solvents	n. d. (not detected)
Assay (100 % method) ¹	99.20 %

The assay is assessed to be 99.2 % 'as is'

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

¹ The calculation of the 100 % method follows the formula:

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

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

LGCFOR0282.00 Lot Number 6671

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