

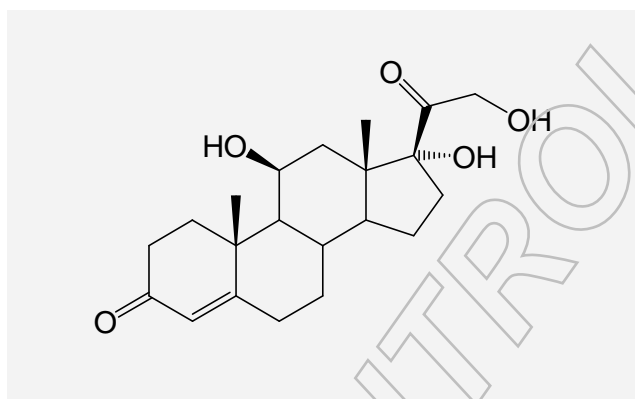
Certificate of Analysis

Reference Substance

Hydrocortisone (Cortisol)

Catalogue Number: LGCFOR0185.00
Lot Number: 6692
Molecular Formula: $C_{21}H_{30}O_5$
Molecular Weight: 362.46
CAS Number: [50-23-7]

Long-term Storage: 2 to 8 °C, dark
Appearance: white solid
Melting Point: 215 °C
Assay 'as is': 98.7 %



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: 2011-03-14

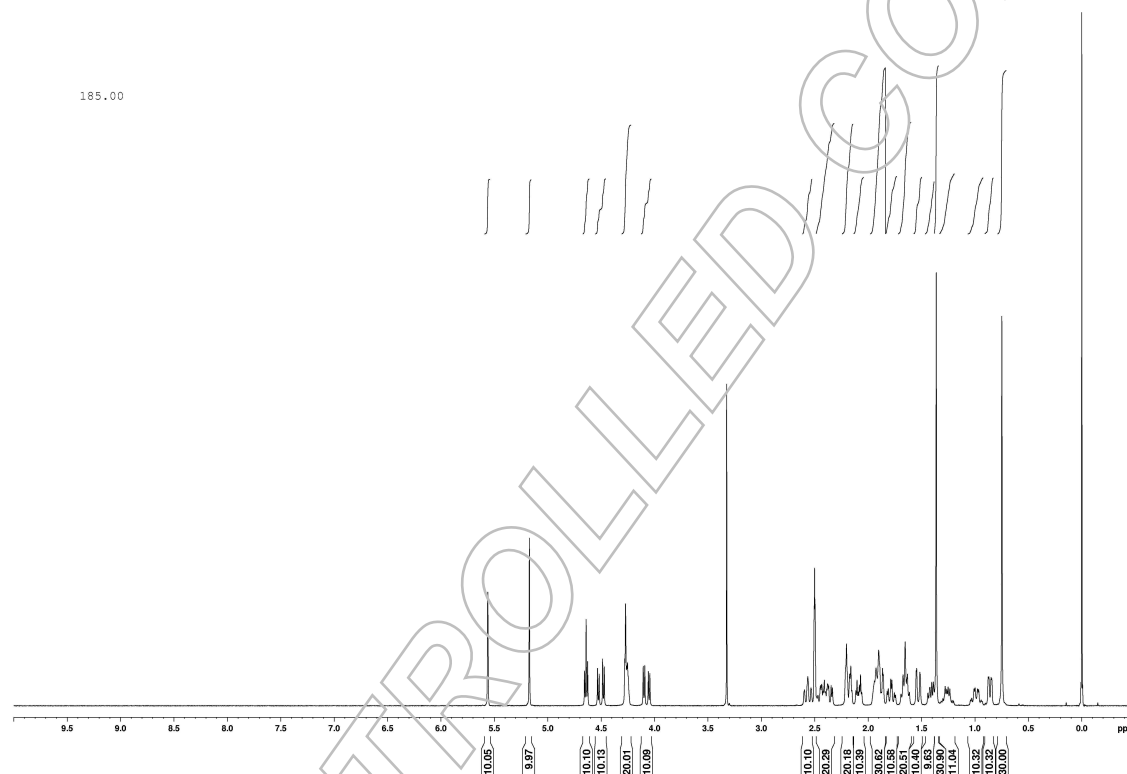
LGC GmbH

Dr. Sabine Schröder
Product Release

I. Identity

The identity of the reference substance was established by ^1H -NMR spectroscopy. The structure is confirmed with the signals of the spectrum and their interpretation.

Conditions: 400 MHz, DMSO-d_6



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
Water/Acetonitrile 75/25 (v/v);
0.1 % H_3PO_4

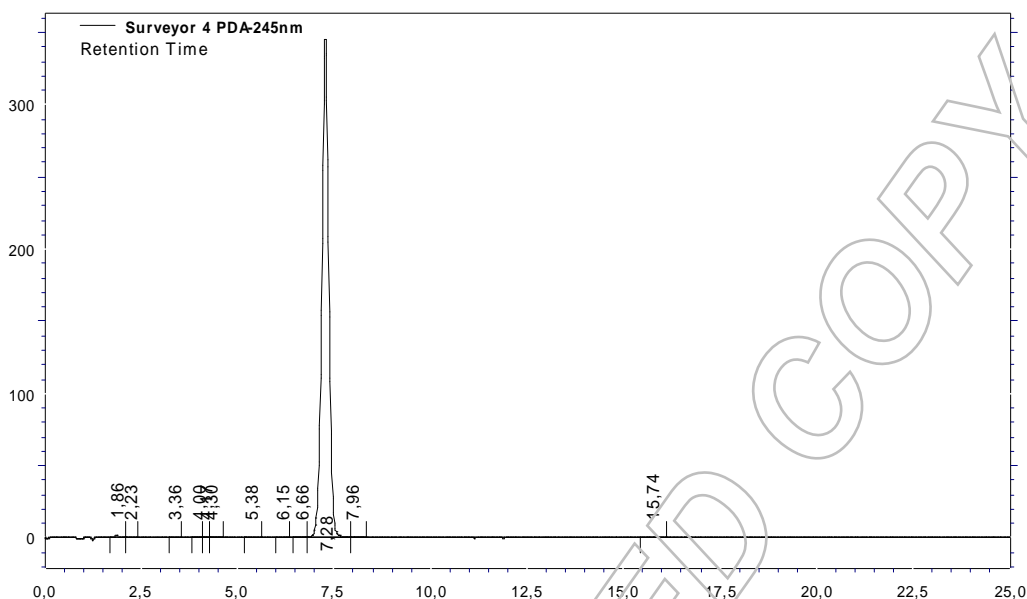
Detector:

DAD
245 nm

Injector:

Auto
5 μl ; 0.0766 mg/ml in
Water/Acetonitrile 50/50 (v/v)

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Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	1.86	9629	0.21
2	2.23	903	0.02
3	3.36	1362	0.03
4	4.00	5714	0.12
5	4.17	6630	0.14
6	4.30	6697	0.15
7	5.38	10350	0.23
8	6.15	1366	0.03
9	6.66	2078	0.05
10	7.28	4543395	98.84
11	7.96	4108	0.09
12	15.74	4327	0.09
Totals		4596559	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 98.84 %
Number of results n=6
Standard deviation 0.05 %

III. Water Content

Method: Karl Fischer titration

Results:

Average	0.10 %
Number of results	n=3
Standard deviation	0.01 %

IV. Residual Solvents

Method: ¹H-NMR

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

V. Final Result

Total impurities (HPLC)	1.16 %
Water content	0.10 %
Residual solvents	n. d. (not detected)
Assay (100 % method) ¹	98.74 %

The assay is assessed to be 98.7 % '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:

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

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

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