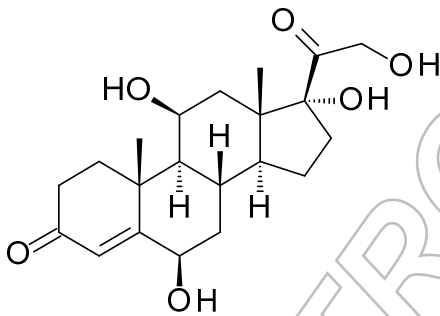




# Certificate of Analysis

## Reference Standard

6 $\beta$ ,11 $\beta$ ,17,21-Tetrahydroypregn-4-ene-3,20-dione (6 $\beta$ -Hydroxycortisone)



Molecular Formula: C<sub>21</sub>H<sub>30</sub>O<sub>6</sub>  
Molecular Weight: 378.46  
CAS Number: [53-35-0]

Catalogue Number: MM0185.06  
Lot Number: 57945  
Long-term Storage: 2 to 8 °C, dark  
Appearance: white solid  
Melting Point (DSC): 233 °C  
hygroscopic  
Assay 'as is': 99.0 %

Date of shipment: **2017-January-25**

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.



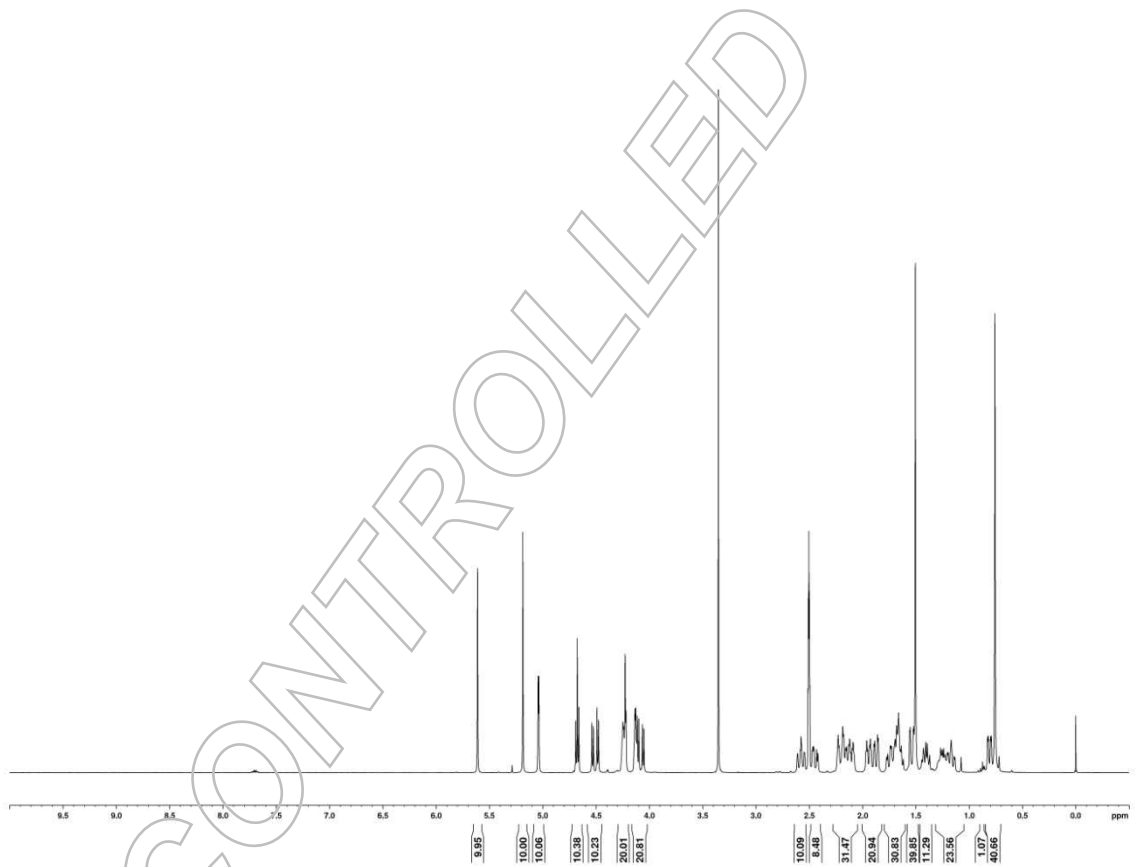
## I. Identity

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

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

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

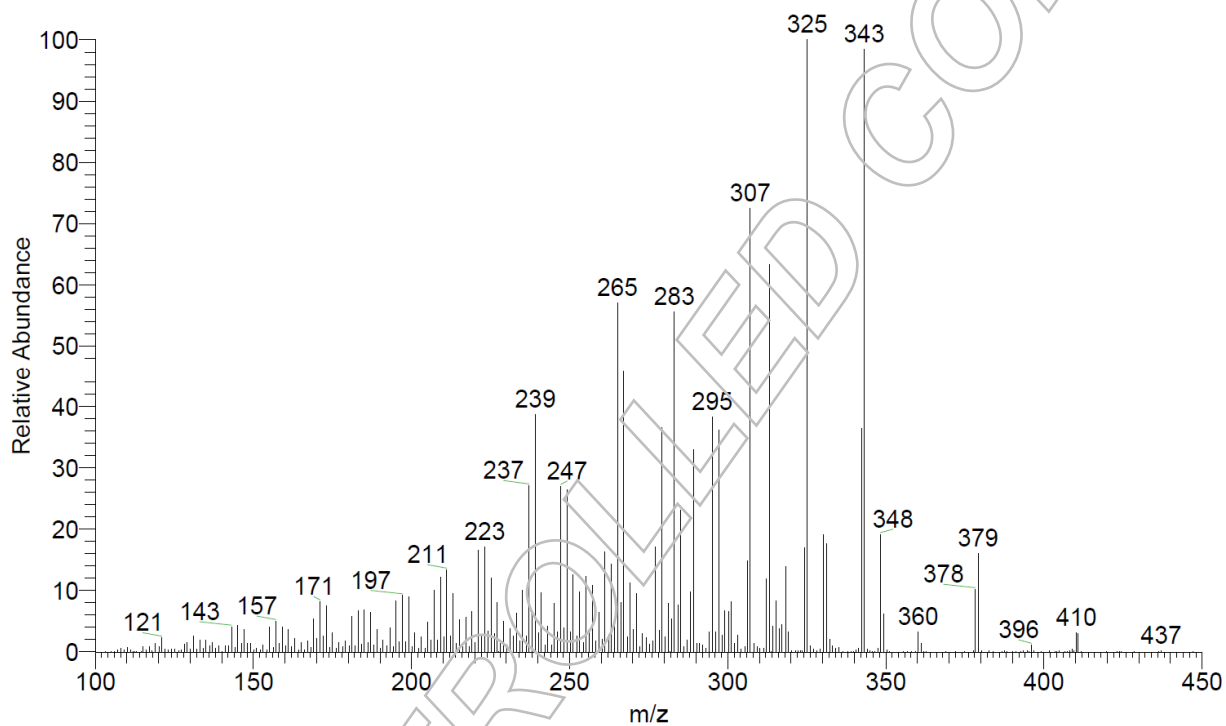
The structure is confirmed with the signals of the spectrum and their interpretation.





## Ib. Mass Spectrum

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



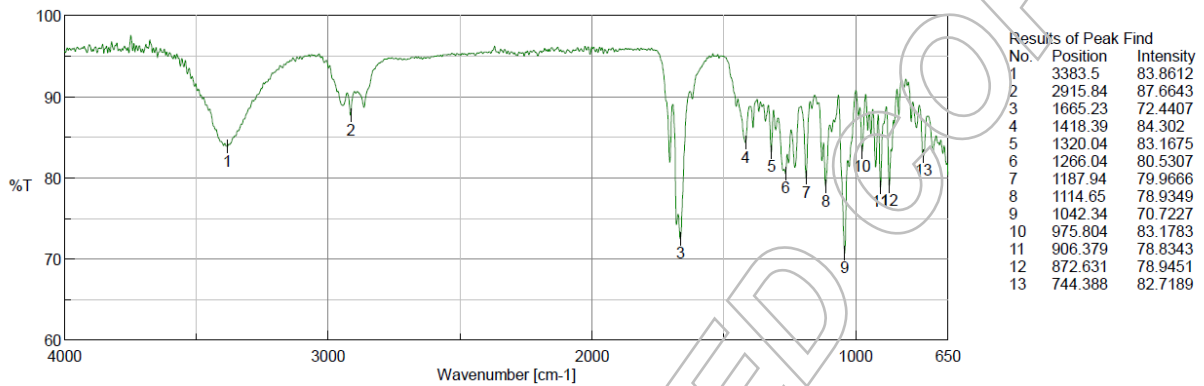
| m/z | fragments                  |
|-----|----------------------------|
| 379 | [ MH ]                     |
| 343 | [ MH - 2H <sub>2</sub> O ] |
| 325 | [ 343 - H <sub>2</sub> O ] |
| 307 | [ 325 - H <sub>2</sub> O ] |

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:

RP 60 Select B  
5 µm, 125 x 4 mm

#### Conditions:

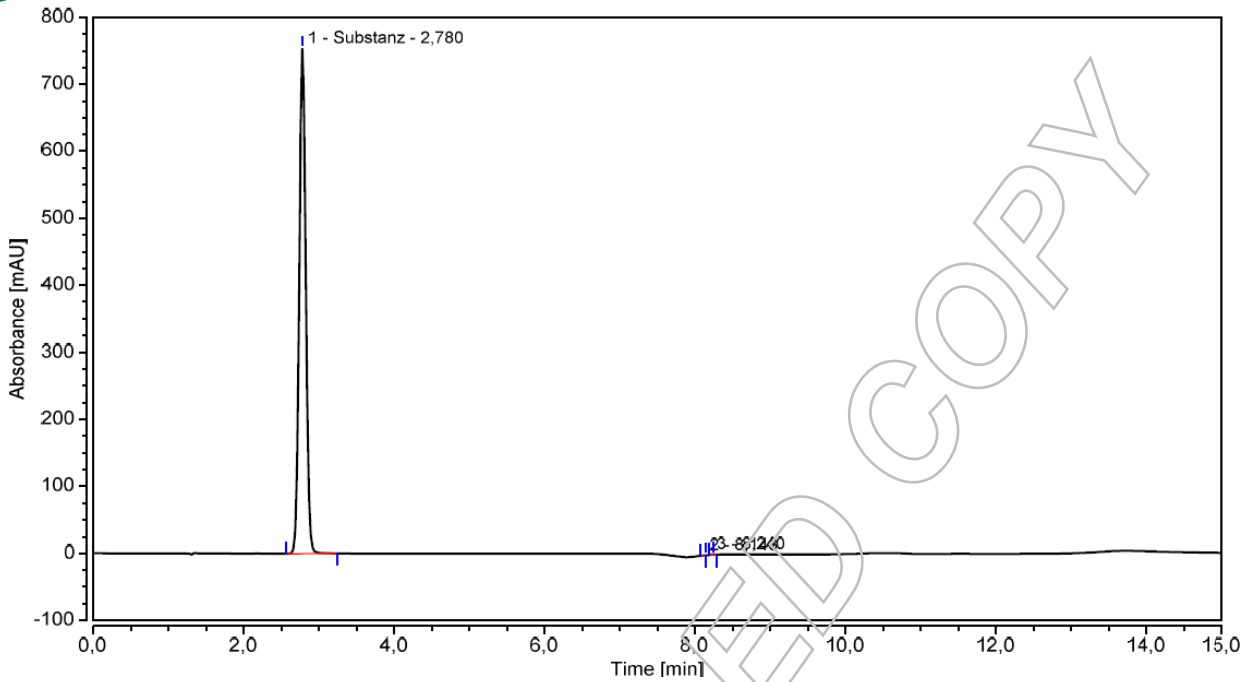
1.0 ml/min, 40 °C  
0-5 min Water/Acetonitrile 85/15  
5-6 min Water/Acetonitrile to 50/50  
6-10 min Water/Acetonitrile 50/50  
10-11 min Water/Acetonitrile to 85/15  
11-15 min Water/Acetonitrile 85/15 (v/v);  
0.1 % H<sub>3</sub>PO<sub>4</sub>

#### Detector:

DAD  
240 nm

#### Injector:

Auto  
2 µl; 0.176 mg/ml in  
Water/Acetonitrile 50/50 (v/v)



**Area Percent Report - Sorted by Signal**

| Pk #   | Retention Time | Area   | Area % |
|--------|----------------|--------|--------|
| 1      | 2.780          | 76.777 | 99.94  |
| 2      | 8.143          | 0.010  | 0.01   |
| 3      | 8.240          | 0.037  | 0.05   |
| Totals |                | 76.824 | 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** 99.94 %  
**Number of results** n=3  
**Standard deviation** < 0.01 %



### III. Water Content

Method: Karl Fischer titration

#### Results:

|                    |        |
|--------------------|--------|
| Average            | 0.52 % |
| Number of results  | n=3    |
| Standard deviation | 0.02 % |

### IV. Residual Solvents

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

Result: 0.40 % *n*-Hexane

### V. Final Result

|                                   |         |
|-----------------------------------|---------|
| Total impurities (HPLC)           | 0.06 %  |
| Water content                     | 0.52 %  |
| Residual solvents                 | 0.40 %  |
| Assay (100 % method) <sup>1</sup> | 99.02 % |

The assay is assessed to be 99.0 % 'as is'

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

Release Date: 2014-08-20

LGC GmbH

Dr. Sabine Schröder  
Product Release

<sup>1</sup> 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.