

# Certificate of Analysis

**LGC Quality**

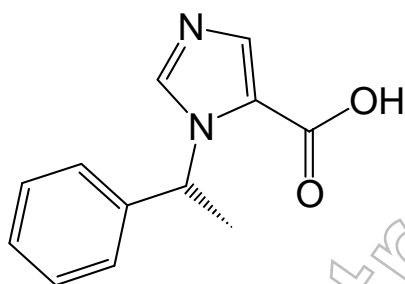
**ISO Guide 34:2009**  
DAkkS D-RM-14176-01-00

**ISO/IEC 17025:2005**  
DAkkS D-PL-14176-01-00

**ISO 9001:2008**  
DQS 102448 QM08

## Primary Reference Standard

1-[(1R)-1-Phenylethyl]-1H-imidazole-5-carboxylic Acid (Etomidate Acid)



Molecular Formula:  $C_{12}H_{12}N_2O_2$   
Molecular Weight: 216.24  
CAS Number: [ 56649-48-0 ]

Catalogue Number: LGCQUANT1264.04

Lot Number: 16805

Long-term Storage: 2 to 8 °C, dark

Appearance: white solid

Melting Point: 165 °C

Assay<sup>1</sup> 'as is': 99.9 %

Uncertainty<sup>2</sup> U: 0.4 %

Date of shipment: **2014-May-26**

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

<sup>1</sup> The value is based on the results of analytical techniques. Calibration and verification were carried out with standards traceable to SI-units. The value is expressed on an "as is" basis. The identity is verified by data from international scientific literature.

<sup>2</sup> The uncertainty "U" is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM). It is corresponding to a level of confidence of about 95 %. Coverage factor  $k = 2$ .

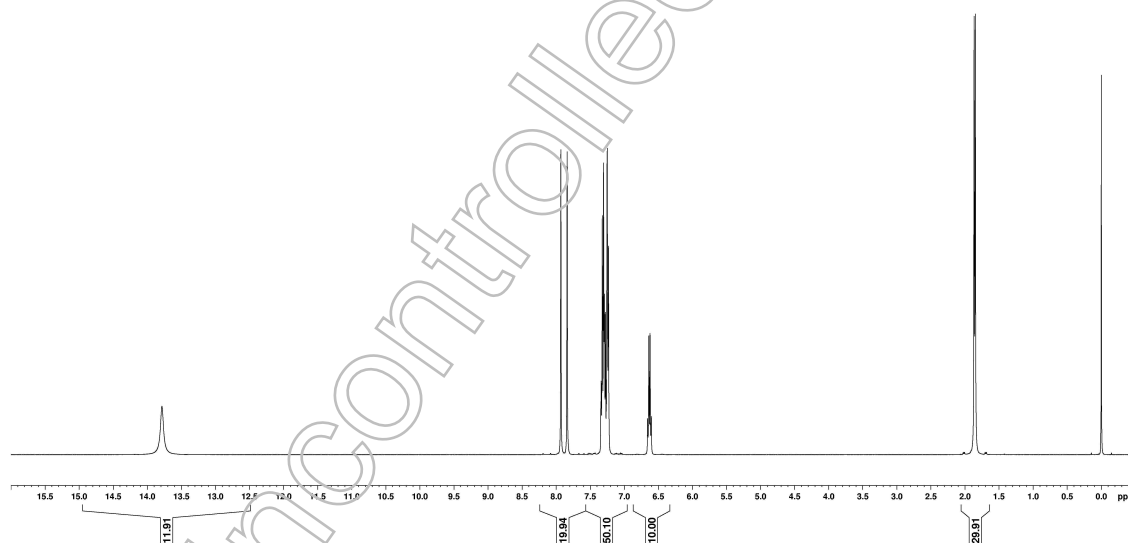
## I. Identity

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

### Ia. $^1\text{H-NMR}$ Spectrum

Conditions: 400 MHz,  $\text{CDCl}_3$

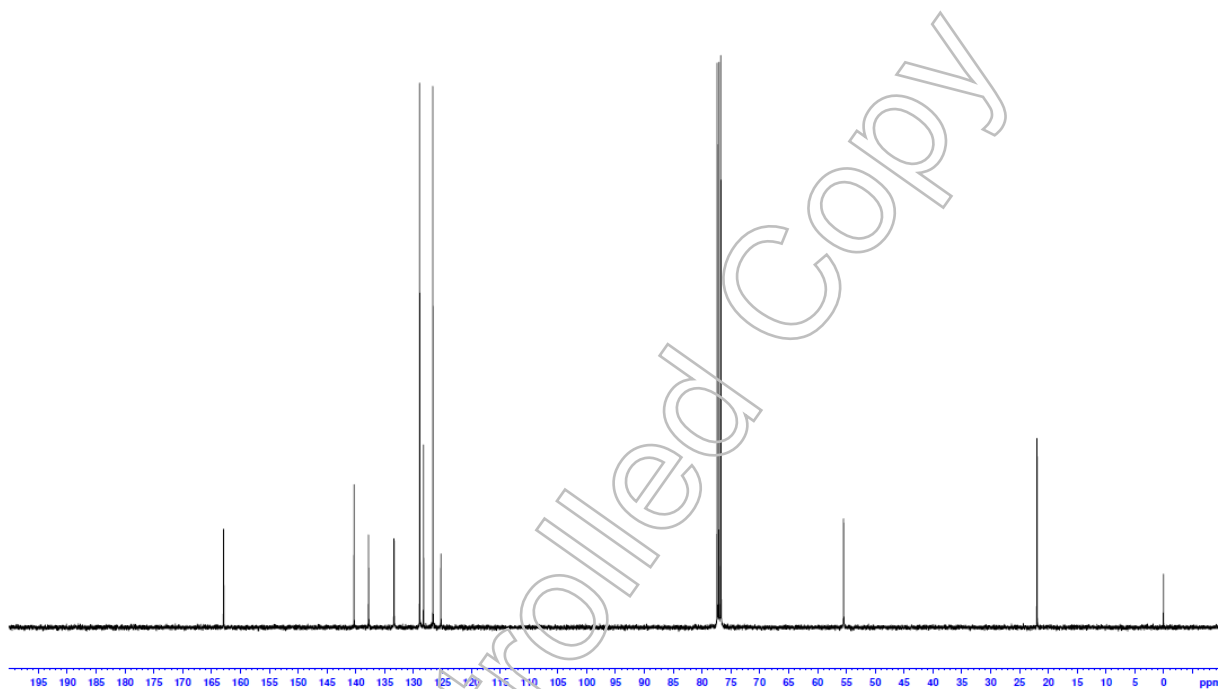
The structure is confirmed by the signals of the spectrum and their interpretation.



**Ib.  $^{13}\text{C}$ -NMR Spectrum**

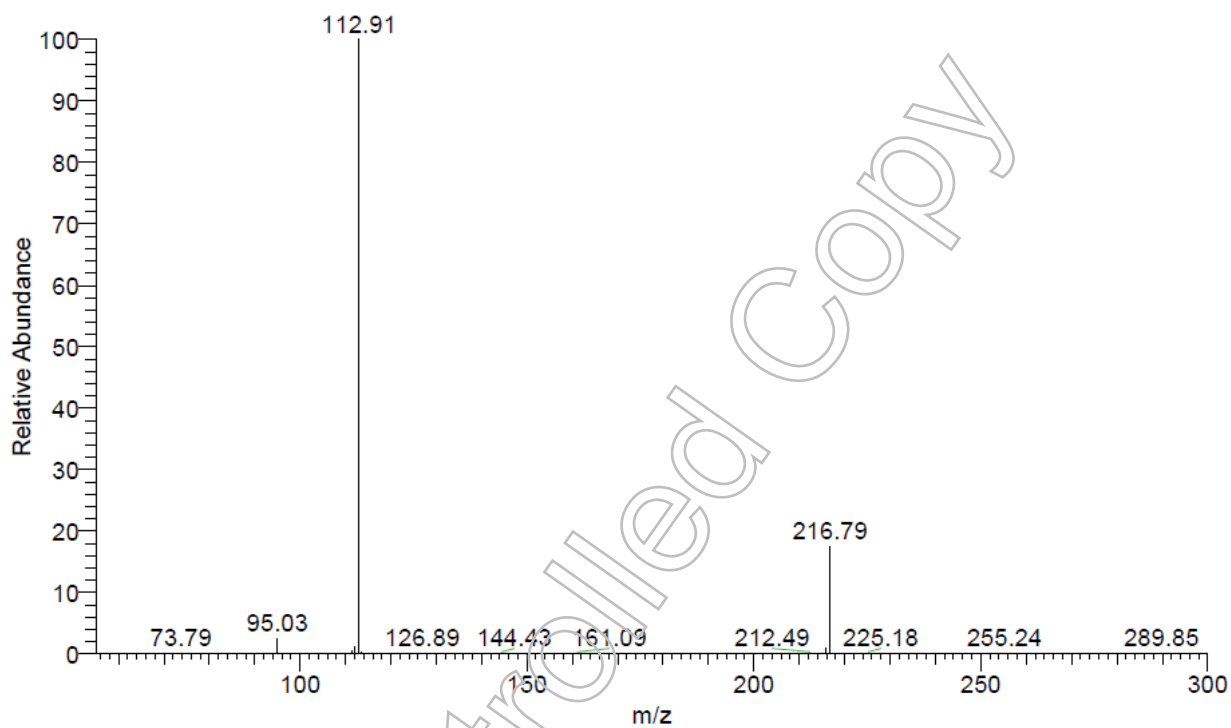
Conditions: 100 MHz,  $\text{CDCl}_3$

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



### lc. Mass Spectrum

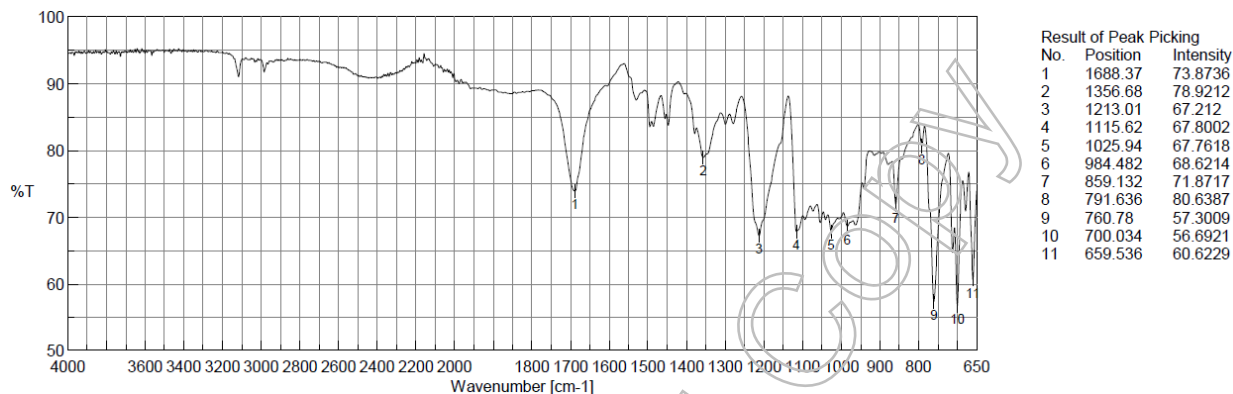
Method: 4.5 kV ESI; vaporization temperature: 200 °C, direct inlet



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

## Id. IR Spectrum

Method: Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy



The signals of the IR spectrum and their interpretation are consistent with scientific literature.

## Ie. Melting Point

Method: Capillary Method

Arithmetic mean (n=3): 165 °C

## II. Purity

The purity of the reference material was determined by high performance liquid chromatography (HPLC).

### HPLC Conditions\*:

**Column:**

Hypersil Gold (C18)  
5 µm, 150 x 4.6 mm

**Conditions:**

1.0 ml/min, 40 °C  
0 – 11 min Water/Acetonitrile 92/8  
11 – 17 min Water/Acetonitrile to 60/40  
17 – 20 min Water/Acetonitrile to 30/70  
20 – 23 min Water/Acetonitrile to 92/8  
23 – 31 min Water/Acetonitrile 92/8 (v/v);  
0.1 % H<sub>3</sub>PO<sub>4</sub>

**Detector:**

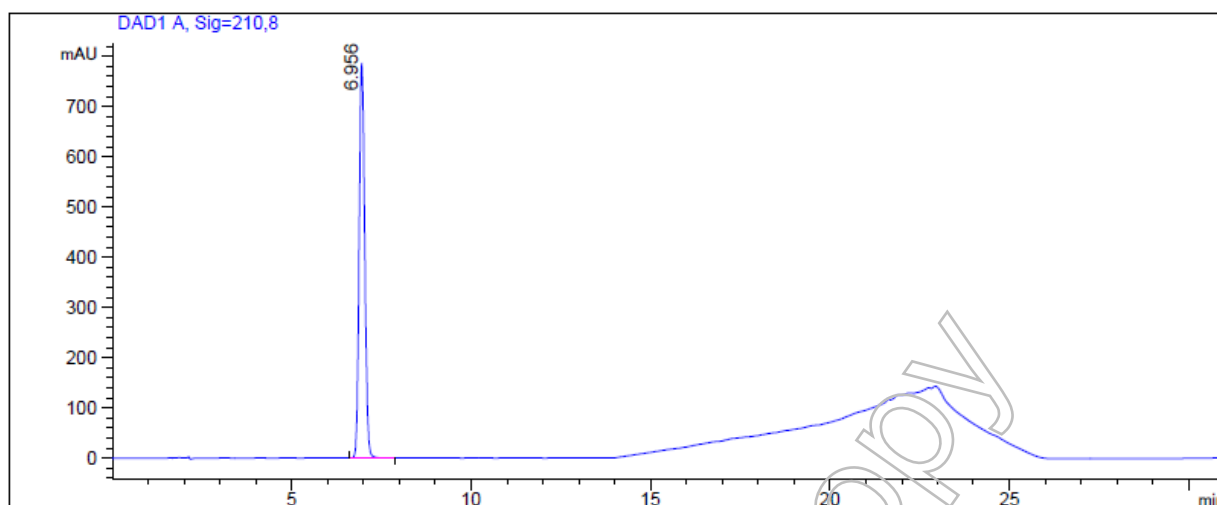
DAD  
210 nm

**Injector:**

Auto  
4 µl; 0.09254 mg/ml in  
Water/Acetonitrile 50/50 (v/v)

\*not accredited testing method





### Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	6.96	8535.67	100.00
Totals		8535.67	100.00

For the calculation the system peaks were ignored. The content of the analyte was determined as the ratio of the peak area of the analyte and the cumulative areas of the purities, added up to 100 %.

#### Results:

Average 100 %  
 Number of results n=6  
 Standard deviation < 0.01 %

### III. Loss on Drying

Conditions: 105 °C for 4 h

No significant amounts of volatile contents were detected (< 0.05 %).

#### IV. Sulphated Ash

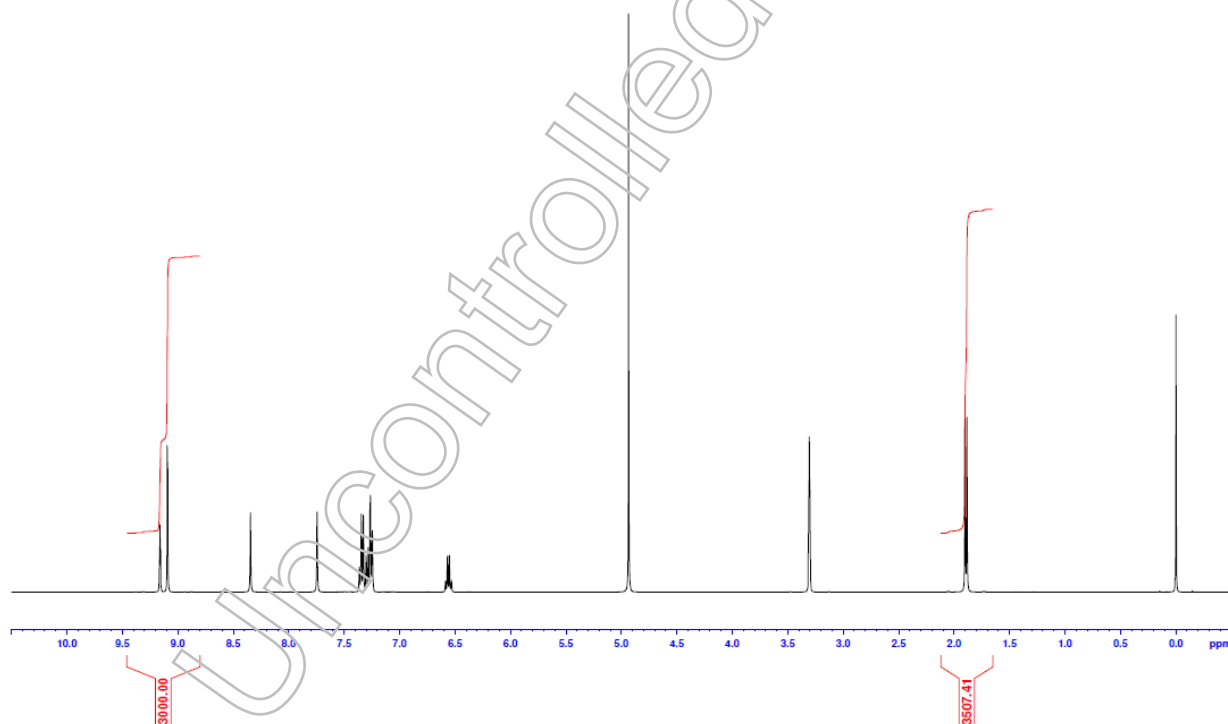
Method: EP 6.0 (2.4.14)

##### Results:

Average	0.16 %
Number of results	n=3
Standard deviation	< 0.01 %

#### V. Assay by quantitative NMR spectroscopy

The assay of the reference substance was established by quantitative NMR spectroscopy in CD<sub>3</sub>OD as the solvent and with 3,5-Dinitrobenzoic acid (certified reference material, signal 8.80 – 9.46 ppm, 3 H) as internal standard.



##### Results:

Average	99.85 %
Number of results	n=6
Uncertainty U (expanded)	0.41 %

## VI. Final Result

<b>Chromatographic purity (HPLC)</b>	100 %
<b>Loss on drying</b>	n.d. (not detected)
<b>Sulphated ash</b>	0.16 %

### Assay:

**quantitative NMR spectroscopy** 99.85 %

The assay is assessed to be 99.9 % 'as is'

The assay 'as is' is equivalent to the assay based on the not anhydrous and not dried substance respectively. It is determined by quantitative NMR spectroscopy and its expanded uncertainty ( $k = 2$ ) is 0.4 %.

The assay is verified by the 100 % method (HPLC): 100 % which is inside the expanded uncertainty of the assay by quantitative NMR spectroscopy.

## VII. Stability and Homogeneity

Accelerated stability studies indicate no significant instability. The given validity period is based on this data. This is backed up by additional stability testing and historical data over the range of several years. Homogeneity assured by qualified process of preparation, verified by homogeneity testing.

## VIII. Further Information

### General

For laboratory use only. Not suitable for human or animal consumption.

This material conforms to the characteristics of a primary standard as described within ISO Guide 30 (Terms and definitions used in connection with reference materials).

The values quoted in this certificate are LGC's best estimate of the true values within the stated uncertainties and based on the techniques described in this certificate.

The production of this reference material was realized taking into account the principles of ISO Guide 34.





## Intended Use

Use for identification and quantification.

## Handling of the RM

Before usage of the reference material, it should be allowed to warm to room temperature.

## Assay and uncertainty

The identity and the assay are assessed by an ISO/IEC 17025 accredited testing method.

The calculation of the 100 % method (HPLC) follows the formula:

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

Volatile contents are considered as absolute contributions, HPLC purity is considered as relative contribution.

Uncertainty of the assay is expressed as an expanded uncertainty in accordance with ISO/IEC 17025 at the about 95 % level of confidence.


For quantitative applications use the assay as a calculation value on the as is basis. The uncertainty of the assay can be used for estimation/calculation of your own measurement uncertainty.

## Quality Control Assessment

The product quality is controlled by regularly performed quality control tests (retests).

Release Date: 2012-12-10

LGC GmbH



Dr. Sabine Schröder  
Product Release

Revision	Date	Reason for Revision
00	2012-12-10	Release of the Lot – initial version
01	2013-10-29	Accreditation Number added

