

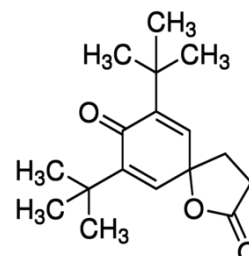
## Certified Reference Material

## Reference material certificate

## 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione

TraceCERT®  
Traceable Certified Reference Materials

**Product no.:** CRM78478  
**Lot no.:** BCCH5366  
**Description of CRM:** Solid neat material  
**Expiry date:** FEB 2025  
**Storage:** 2-8°C; storage under Argon  
**Chemical formula:** C<sub>17</sub>H<sub>24</sub>O<sub>3</sub>  
**Molecular mass:** 276.4 g/mol  
**CAS No.:** 82304-66-3



## Sample

Certified value ± Expanded uncertainty,  
 $U = k \cdot u$  ( $k = 2.8$ )<sup>[1][2]</sup>  
as mass fraction

7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione

0.990 g/g ± 0.015 g/g (99.0 % ± 1.5 %)

**Metrological traceability:** NIST PS1 (Benzoic acid)<sup>[3]</sup>  
Details see "Certification process details" on page 2.

**Measurement method:** The certified value is established by high-resolution quantitative NMR measurements (qNMR) in accordance with ISO/IEC 17025.<sup>[4]</sup>

**Intended use:** Use this certified reference material (CRM) as calibrant for chromatography or any other analytical technique.

**Minimum sample size:** The sample is solid at room-temperature. 10 mg is recommended as the minimum sample size. If less material is used, it is recommended to increase the certified uncertainty by a factor of two for half of sample and a factor of four for a quarter of sample.

**Instructions for handling and correct use:** This material does not require drying before use. The CRM should be stored in the original bottle. Warm to room temperature before opening. After use the bottle should be tightly closed and protected from excessive moisture and light.

**Accreditation:** Sigma-Aldrich Production GmbH is accredited by the Swiss Accreditation Service SAS as reference material producer under no. SRMS 0001 in accordance with international standard ISO 17034.<sup>[5]</sup>

**Certificate issue date:** 09 MAR 2023



ISO 17034  
SRMS 0001

Dr. A. Rück – CRM Operations

Dr. P. Zell – Approving Officer



**Health and safety information:**

Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

**Packaging:**

Brown glass bottle

**Starting material details:**

Potential raw materials are checked for suitability in terms of purity. Only materials of highest available purity are accepted. 2D-NMR (H-H COSY) measurements are applied to guarantee that no impurities underlie the peak of interest. Detection limit usually is below 0.1 %.

Compatibility of candidate substance with solvent and internal qNMR reference is checked (t=0 and t=24 h comparison).

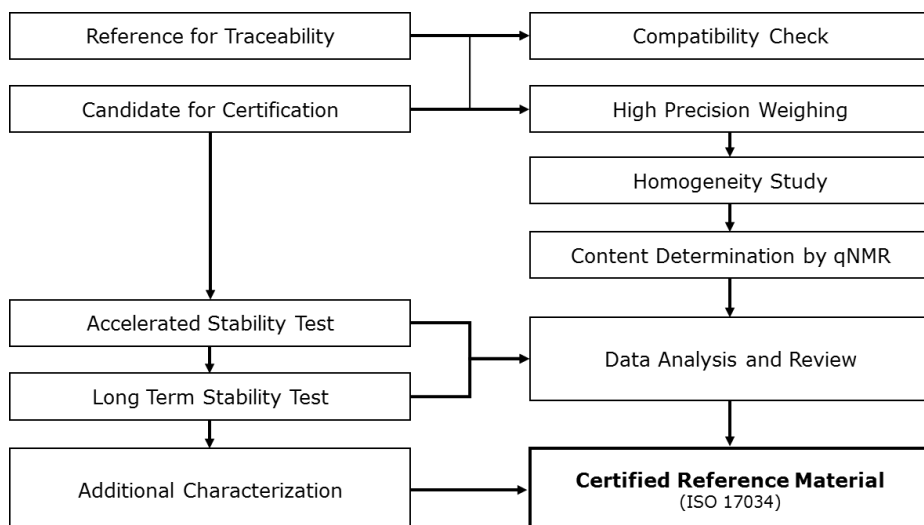
**Certification process details:**

In order to guarantee highest reliability of this *TraceCERT* CRM a multi-component approach was applied whereby the certified value is determined by high-resolution quantitative NMR measurements (qNMR on a 600 MHz NMR spectrometer).<sup>[4][6]</sup> The whole workflow follows the ISO 17034 guidelines. The certificate is designed in accordance with ISO Guide 31.<sup>[7]</sup>

The certified values are confirmed by extended analytical data. These data are not covered by the scope of accreditation but determined following best practices in analytical measurements.

The high precision weighing steps are performed in accordance with ISO/IEC 17025<sup>[4]</sup> using ultra-micro balances certified by DKD and calibrated with OIML Class E2 weights.

Absolute content determination by qNMR is performed using 4-5 separate samples of the candidate substance which are each spiked with an adequate amount of internal reference and then immediately dissolved in deuterated solvent. In most cases 16-32 scans are recorded for every sample with a <sup>1</sup>H relaxation time of d1= 60 seconds. Quantification of the candidate content is directly calculated from the <sup>1</sup>H-NMR peak areas and the initial weights of the candidate and reference substance. After ANOVA the resulting standard deviation is included into the uncertainty calculation of the certified value. Extensive stability and homogeneity tests are considered for certification.<sup>[8]</sup>

**Homogeneity assessment:**

Homogeneity of the material is tested by qNMR measurements using 4-5 subsamples which are taken from different positions in the entire bulk material. The recommended minimal sample size is taken for all the homogeneity test samples. Analysis of variance (ANOVA) results are included into the calculation of content uncertainty of this CRM.

**Stability assessment:**

An accelerated stability test is performed with samples which are stored above the recommended storage temperature. The material is tested by qNMR double determination at appropriate time intervals, e.g. 3 months.

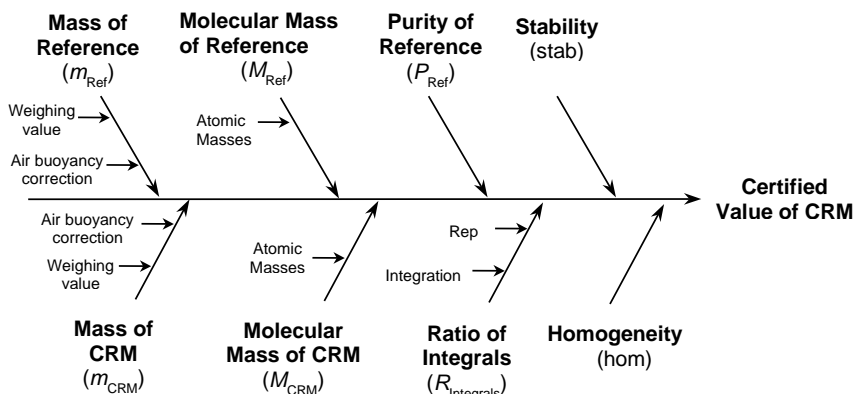
The long-term stability test is performed with samples which are stored at the recommended storage temperature and applying qNMR double determination at appropriate time intervals, e.g. 24 months.

## Uncertainty evaluation:

The uncertainty contributions are illustrated by the following cause-effect diagram.

Typical relative contributions are:

$u(P_{\text{Ref}})$	< 0.10 %
$u(m_{\text{Ref}})$	< 0.05 %
$u(m_{\text{CRM}})$	< 0.05 %
$u(M_{\text{Ref}})$	< 0.003 %
$u(M_{\text{CRM}})$	< 0.003 %
$u(R_{\text{Integrals}})$	< 0.15 %
$u_{\text{hom}}$	< 0.05 %
$u_{\text{stab}}$	< 0.55 %

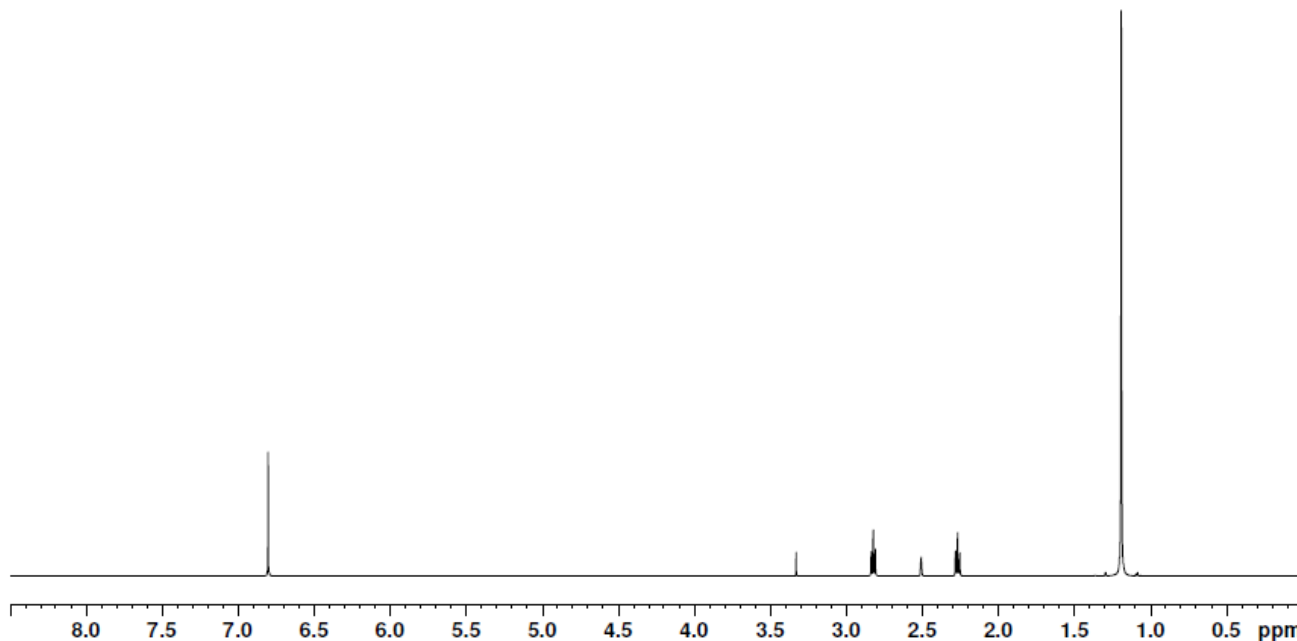


The combined standard uncertainty is calculated by combination of the standard uncertainties of the input estimates according to Eurachem/CITAC Guide "Quantifying Uncertainty in Analytical Measurement" and ISO 17034.<sup>[2][5]</sup>

Expanded uncertainty is then calculated to a confidence level of 95 %, typically by multiplying with a confidence level factor of  $k=2.8$ .

## Indicative values:

**$^1\text{H}$ -NMR Spectrum** (600 MHz, 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione in  $\text{DMSO-d}_6$ )



## References:

- [1] ISO Guide 35:2017, "Reference materials - Guidance for characterization and assessment of homogeneity and stability"
- [2] Eurachem/CITAC Guide, 3<sup>rd</sup> Ed. (2012), "Quantifying uncertainty in analytical measurement"
- [3] Eurachem/CITAC Guide, 2<sup>nd</sup> Ed. (2019), "Metrological traceability in chemical measurement"
- [4] The accredited testing laboratory STS 0490 performs the measurements and weighing steps for the certification of this CRM under ISO/IEC 17025:2017, "General requirements for the competence of testing and calibration laboratories"
- [5] ISO 17034:2016, "General requirements for the competence of reference material producers"
- [6] Weber M, Hellriegel C, Rueck A, Sauermoser R, Wuethrich J, Accred. Qual. Assur. 18 (2013) 91-98
- [7] ISO Guide 31:2015, "Reference materials - Contents of certificates, labels and accompanying documentation"
- [8] Weber M, Hellriegel C, Rueck A, Wuethrich J, Jenks P, JPBA 93 (2014) 102-110

## Certificate of analysis revision history:

Certificate version	Date	Reason for version
01	09 MAR 2023	Initial version

## Disclaimer:

The purchaser must determine the suitability of this product for its particular use. Sigma-Aldrich Production GmbH makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by Sigma-Aldrich Production GmbH. We do not guarantee that the product can be used for a special application.

