TraceCERT®
Traceable Certified Reference Materials

Fluka Analytical

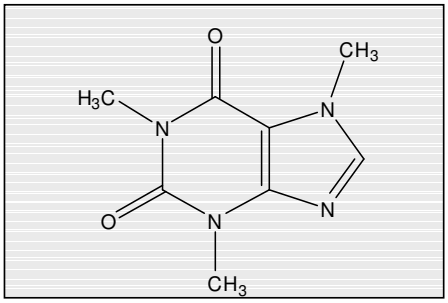
Certificate

Produced in double accredited laboratory fulfilling ISO/IEC 17025 and ISO Guide 34

This certificate is designed in accordance with ISO Guide 31 [1].

Substance: **Caffeine**

Product no.: **56396**
Lot no.: BCBF1317V
Formula: $C_8H_{10}N_4O_2$
Molecular mass: 194.19
Traceability [2]: NIST SRM 84l (KHP) and
NIST SRM 350b (Benzoic acid)
Certificate issue date: June 20, 2012 (Version 2)
Expiry: FEB 2013



Certified value and uncertainty according to ISO Guide 35 [3] and Eurachem/CITAC Guide [4]		
Substance	Certified value (mass fraction)	Expanded uncertainty, $U = k \cdot u_c$ ($k = 2$) (mass fraction)
Caffeine	**99.9 %**	**0.2 %**

Minimum sample: 20 mg is recommended as the minimal sample amount. 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.

Drying instruction: This material does not require drying before use.

Intended use: Use this CRM as calibrant for chromatography or any other analytical technique.

Storage and handling: The CRM should be stored in the original bottle at room-temperature (20-25°C). After use the bottle should be tightly closed and protected from excessive moisture and light.

CRM operations: A. Rück
A. Rück, Ph.D.

Certification body: K.-D. Schmidt
K.-D. Schmidt, Ph.D.

SWISS CRM-PRODUCTION SRMS 001 **ISO Guide 34**

SWISS TESTING STS 490 **ISO/IEC 17025**

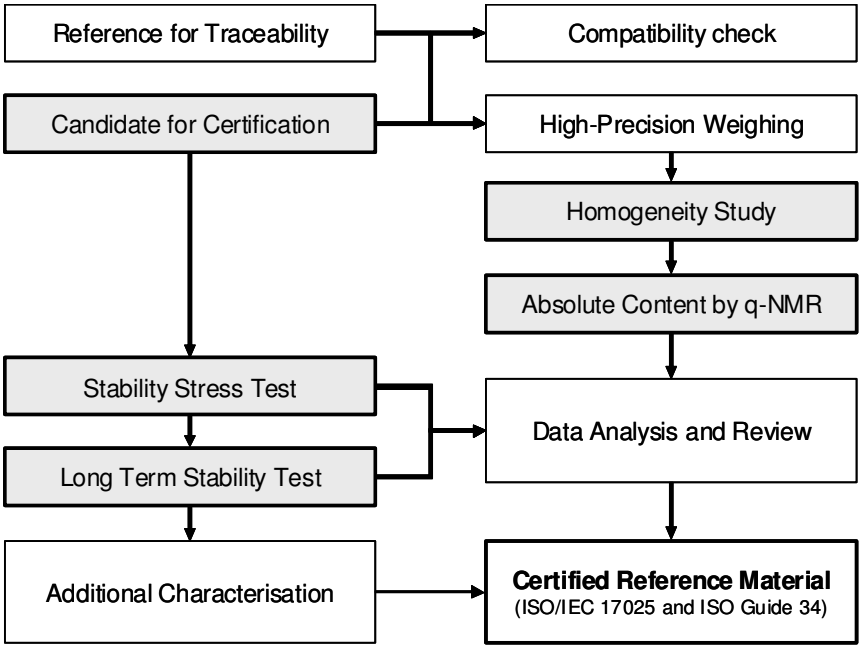
Certified Management System SQS ISO 9001:2000 16368-02 **ISO 9001**

Sigma-Aldrich Production GmbH, Industriestrasse 25, 9471 Buchs/ Switzerland

CERTIFICATION

In order to guarantee highest reliability of this *Trace***CERT** CRM a multi-component approach was applied whereby the certified value is determined by high-resolution quantitative NMR measurements (q-NMR on a Bruker 600 MHz Avance III NMR spectrometer). This certified value is determined under **double-accreditation** in accordance with **ISO/IEC 17025** [5] and also **ISO Guide 34** [6]. Extensive stability and homogeneity tests are considered for certification.

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



Candidate substances 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 to a peak of interest. Detection limit usually is below 0.1%.

Compatibility check guarantees that the candidate substance does not react neither with the solvent nor with the internal q-NMR reference (t=0 and t=24h comparison).

High precision weighing is performed under ISO/IEC 17025 accreditation with ultra-micro balances certified by DKD and calibrated with OIML Class E2 weights.

Stability stress test is performed with samples which are stored above the recommended storing temperature (mostly at 45 °C) and q-NMR double determinations after 1 and 3 months.

Long term stability test is performed with samples which are stored at the recommended storing temperature and q-NMR double determination after 3, 9 and 24 or 36 months.

Homogeneity of the material is tested by q-NMR measurements using 5-10 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.

Absolute content by q-NMR is performed using 5-10 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 32 scans are recorded for every sample with a ^{1}H relaxation time of d1= 60 seconds. Quantification of the candidate content is directly calculated from the ^{1}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.

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UNCERTAINTY CALCULATION

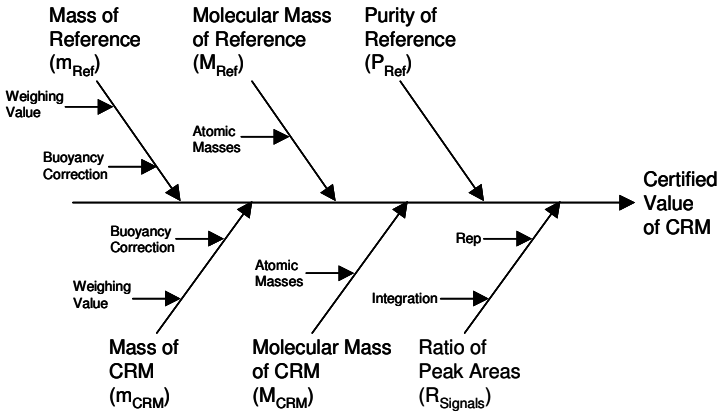
All uncertainties are calculated according to Eurachem/CITAC Guide and reported as combined expanded uncertainties. The uncertainty contributions are illustrated by the following cause-effect diagram.

Typical relative contributions are:

$u(P_{Ref})$ < 0.05 %
$u(m_{Ref})$ < 0.03 %
$u(m_{CRM})$ < 0.03 %
$u(M_{Ref})$ < 0.003 %
$u(M_{CRM})$ < 0.003 %
$u(R_{Signals})$ 0.05-0.15 %

The combined uncertainty is calculated by combination of the squared contribution values.

Expanded uncertainty is then calculated to a confidence level of 95%, typically by multiplying with a confidence level factor of k=2.



EXTENDED ANALYTICAL DATA

mp	233-238 °C (lit.)

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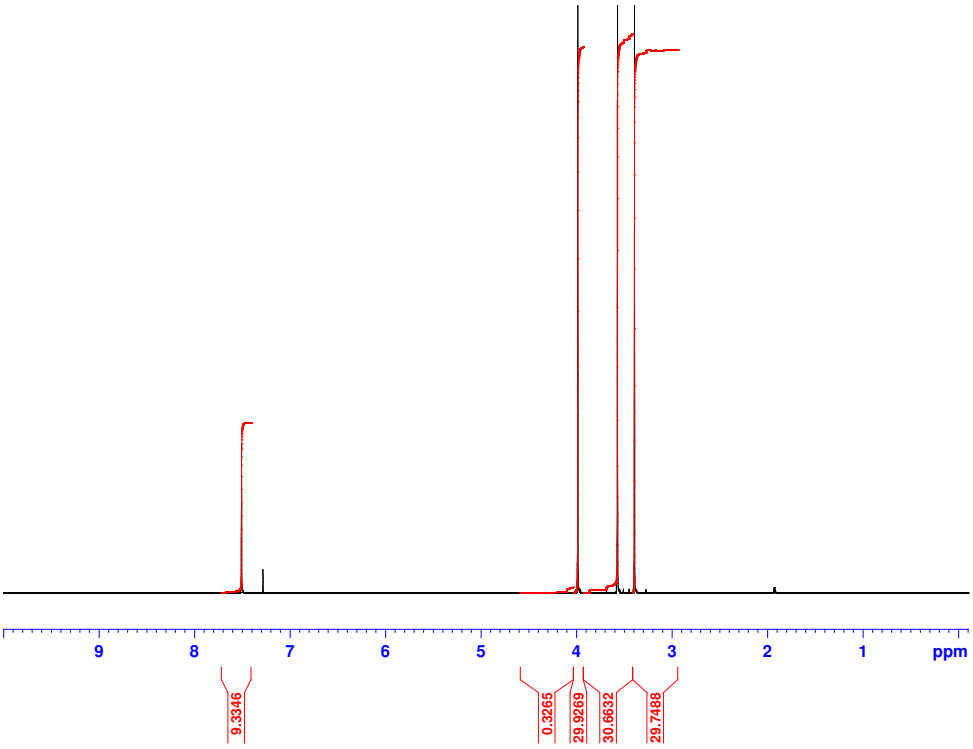
EXTENDED ANALYTICAL DATA (CONT.)

^{1}H-NMR Spectrum (600 MHz, Caffeine in $CDCl_3$)



REFERENCES

[1] ISO Guide 31, 2nd Ed. (2000), "Reference materials - Contents of certificates and labels"
[2] Eurachem/CITAC Guide, 1st Ed. (2003), "Traceability in chemical measurement"
[3] ISO Guide 35, 3rd Ed. (2006), "Reference materials - General and statistical principles for certification"
[4] Eurachem/CITAC Guide, 2nd Ed. (2000), "Quantifying uncertainty in analytical measurement"
[5] ISO/IEC 17025, 2nd Ed. (2005), "General requirements for the competence of testing and calibration laboratories"
[6] ISO Guide 34, 3rd Ed. (2009), "General requirements for the competence of reference material producers"

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