



Certification of the Deuterium-to-Hydrogen (D/H) ratio in a 1,1,3,3-tetramethylurea master batch IRMM-425

R. Zeleny, H. Emteborg, F. Ulberth



EUR 23031 EN - 2007

The mission of the IRMM is to promote a common and reliable European measurement system in support of EU policies.

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information

Address: R. Zeleny
E-mail: Reinhard.zeleny@ec.europa.eu
Tel.: 014/571 615
Fax: 014/571 548

<http://www.irmm.jrc.be/html/homepage.htm>
<http://www.jrc.ec.europa.eu>

Legal Notice

Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of this publication.

A great deal of additional information on the European Union is available on the Internet. It can be accessed through the Europa server
<http://europa.eu/>

EUR 23031 EN
ISBN 978-92-79-07550-6
ISSN 1018-5593
DOI 10.2787/52761

Luxembourg: Office for Official Publications of the European Communities

© European Communities, 2007

Reproduction is authorised provided the source is acknowledged

Printed in Belgium

**Certification of the
Deuterium-to-Hydrogen (D/H) ratio in a
1,1,3,3-tetramethylurea master batch**

IRMM-425

R. Zeleny, H. Emteborg, F. Ulberth

European Commission, Joint Research Centre
Institute for Reference Materials and Measurements (IRMM)
2440 Geel, Belgium

Summary

This report describes the production of a tetramethylurea reference material (IRMM-425), certified for its deuterium-to-hydrogen (D/H) ratio. The material is to be used as an internal standard in site-specific natural isotope fractionation – nuclear magnetic resonance (SNIF-NMR) spectroscopy measurements for determining the D/H ratios of ethanol distilled from wines, an important measure in wine authenticity testing (Commission Regulation 2676/90, [1]).

Commercially obtained TMU with a sufficiently high D/H ratio ($>120 \times 10^{-6}$) was purified by removing most of the residual water and filled into amber glass ampoules in 7.8 mL portions. Homogeneity was tested and no heterogeneity observed. Stability studies indicated no material degradation for 4 weeks at 60 °C and for 18 months at –20 °C. Batch characterisation was accomplished in an inter-laboratory comparison using the SNIF-NMR technique exclusively.

The certified value was obtained as the unweighted mean of the laboratory means of the accepted sets of results. The expanded uncertainty associated ($k = 2$) includes contributions from (potential) heterogeneity, potential instability, characterisation, as well as the carried-over uncertainty from BCR-656 (96% wine ethanol) that was used as the internal standard in the measurements. Due to its negligible contribution, the uncertainty associated with the purity of the material did not need to be included.

The certified value and its associated uncertainty are:

	Amount-of-substance fraction	
	Certified value ^{1,2,4)}	Uncertainty ³⁾
Deuterium-to-hydrogen (D/H) ratio	141.9×10^{-6}	0.7×10^{-6}
1) Traceable to V-SMOV (Vienna Standard Mean Ocean Water) by strictly adhering to the Community reference method (SNIF-NMR, site-specific natural isotope fractionation – nuclear magnetic resonance spectroscopy) as defined in Annex 8, Commission Regulation 2676/90. For V-SMOV, a value of 155.76 ppm was used (Tellus (1970) 22: 712-715).		
2) This value was obtained by an inter-laboratory comparison (11 laboratories) employing SNIF-NMR at 46.1 (300), 61.4 (400), and 76.7 (500) MHz for ² H (¹ H). The value has been corrected for the material impurities (including water).		
3) Expanded uncertainty with a coverage factor of $k = 2$, according to the Guide to the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.		
4) The certified value has a measurement unit of one; it is commonly expressed in parts per million (ppm).		

This CRM constitutes a master batch and will be used for the certification of secondary batches; the material is not for sale.

Table of Contents

Summary	1
Table of contents	3
Glossary	4
1 Introduction	5
2 Time table of the project	9
3 Participants	10
4 Processing	11
5 Methodology used	13
6 Homogeneity	16
6.1 Homogeneity	16
6.2 Minimum sample intake	17
7 Stability studies	18
7.1 Short-term stability	18
7.2 Long-term stability	19
8 Characterisation	21
8.1 Discussion of results	21
8.2 Certified value and uncertainty budget	24
9 Metrological traceability	26
10 Instructions for use	26
10.1 Intended use	26
10.2 Storage	26
10.3 Minimum sample intake	26
10.4 Use of the certified values	26
10.5 Safety precautions	26
11 Acknowledgements	27
12 Annexes	27
13 References	28
Annex A – homogeneity data	30
Annex B – short-term stability data	32
Annex C – long-term stability data	34
Annex D – characterisation data	36
Annex E – characterisation – method-related information	37

Glossary

ANOVA	Analysis Of Variance
<i>b</i>	Slope of the linear regression
BCR	Community Bureau of Reference
BEVABS	European Office for Wine, Alcohol and Spirit Drinks
CAP	Certification Advisory Panel
CI	Confidence interval
CRM	Certified reference material
D	Deuterium (² H)
D/H	Deuterium-to-hydrogen ratio
δ	Chemical shift
<i>df</i>	Degrees of freedom
EA-P	Elemental analysis – pyrolysis
EU	European Union
FID	Flame ionization detection
GC	Gas chromatography
IHCP	Institute for Health and Consumer Protection
IRMM	Institute for Reference Materials and Measurements
IRMS	Isotope ratio mass spectrometry
ISO	International Organisation for Standardization
JRC	Joint Research Centre
<i>k</i>	Coverage factor
KFT	Karl Fischer titration
m/m	Mass/mass
MHz	Mega hertz
MS	Mass spectrometry
<i>MS_{between}</i>	Mean of squares between groups (ANOVA)
<i>MS_{within}</i>	Mean of squares within groups (ANOVA)
NIST	National Institute of Standards and Technology
ppm	Parts per million
QC	Quality Control
<i>RSD</i>	Relative standard deviation
<i>s</i>	Standard deviation
<i>S_{bb}</i>	Between-bottle (in)homogeneity standard deviation
SNIF-NMR	Site-specific natural isotope fractionation – nuclear magnetic resonance
<i>S_{wb}</i>	Within-bottle standard deviation
<i>t</i>	<i>t</i> value
<i>t_m^D</i>	Alcoholic grade of BCR-656, expressed in mass %
TMU	Tetramethylurea
<i>u[*]_{bb}</i>	Standard uncertainty due to the inhomogeneity that can be hidden by the method repeatability
<i>u_{BCR-656a}</i>	Uncertainty of D/H _I value of BCR-656
<i>u_{BCR-656b}</i>	Uncertainty of the <i>t_m^D</i> value of BCR-656
<i>u_{char}</i>	Standard uncertainty from characterisation
<i>U_{CRM}</i>	Expanded uncertainty associated to the CRM
<i>u_{lts}</i>	Standard uncertainty associated to long-term (in)stability
V-SMOW	Vienna Sea Mean Ocean Water

1 Introduction

The isotopic ratio of deuterium to hydrogen (D/H) is regularly measured in ethanol distilled from wine to detect wine adulterations, such as mixing high with low quality wines, or the addition of sugar before fermentation (chaptalisation) outside the allowed limits. The Community reference method to determine the wine ethanol D/H ratio, specified in Commission Regulation 2676/90 [1], is Site-specific Natural Isotope Fractionation measured by Nuclear Magnetic Resonance (SNIF-NMR[®]) [2]. To monitor wine authenticity, the measured values are compared with those from the respective authentic wines listed in the European Union (EU) Wine Databank [3], operated by the European Office for Wine, Alcohol and Spirit Drinks (BEVABS) at the Joint Research Centre (JRC) of the European Commission.

The reference method specifies the internal standard in the SNIF-NMR measurements to be tetramethylurea (TMU, figure 1) with a known D/H ratio, available as a certified reference material (CRM) from the Institute for Reference Materials and Measurements (IRMM) – current batch "STA-003". This compound possesses appropriate physico-chemical properties, such as a low volatility, and excellent miscibility with wine ethanol. Furthermore, it exhibits a convenient chemical shift in NMR analysis to avoid interference with ethanol peaks and it yields a signal width similar to that of the ethanol methyl signal.

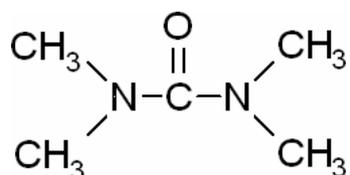


Figure 1. 1,1,3,3 – Tetramethylurea, CAS Number: 632-33-4

The first TMU reference material was certified measuring mixtures of TMU with V-SMOW (Vienna - Standard Mean Ocean Water) by SNIF-NMR [4]; all subsequent TMU batches, however, were certified using reference ethanols, which themselves were calibrated against the penultimate TMU batch. This procedure has some drawbacks, such as the dependence of the certified value on a former material, resulting in an increasing uncertainty and an elongated traceability chain from batch to batch. In addition, value assignment was accomplished using only three dedicated NMR spectrometers, which is reflected in the high coverage factor (4.3) of the expanded uncertainty.

IRMM strived for revising the certification strategy by producing a TMU master batch, to which all future secondary batches shall be linked. An independent property value assignment was envisaged for shortening the traceability chain and lowering the uncertainty of the certified value. Isotope Ratio Mass Spectrometry (IRMS) was intended to be used, which allows a direct link to internationally recognised standards such as V-SMOW, and presumably reveals results with a lower overall uncertainty due to highly precise measurements obtainable by IRMS.

Prior to application of this concept, key prerequisites had to be verified and confirmed: IRMS results need to be in agreement with SNIF-NMR results, because value consistency is indispensable to maintain validity of the wine databank. Furthermore, the between-lab variation has to be reasonably small (in the range of that from SNIF-NMR data) to keep the uncertainty of the certified value at an acceptable level. Therefore, an inter-comparison was conducted to evaluate the currently obtainable method performance of the EA-P-IRMS (Elemental Analysis–Pyrolysis–IRMS) methodology and to evaluate its applicability for characterisation measurements of TMU. The results reveal that expert laboratories are able to produce highly precise and comparable data, which demonstrates the potential of IRMS in food authenticity control. However, a systematic bias between IRMS and NMR results was observed, which rules out the IRMS technique for characterisation measurements of the TMU master batch [5]. Although further measurements are needed to identify possible reasons for this finding, it seems that the observed bias cannot be attributed solely to differences in the measurement principles, but is also compound-dependent [6].

The finally chosen strategy for the certification of the TMU master batch is the following: TMU-ethanol mixtures are analysed by SNIF-NMR. As the ethanol, BCR-656 with a certified D/H_I and D/H_{II} value is used [7]. Taking the peak heights and the masses of TMU and ethanol into account, the D/H ratio of TMU can then be calculated using the formula stipulated in Commission Regulation 2676/90 [1] and depicted in chapter 5. The originally envisaged independence of the certified value from previous CRMs is thus not achieved, and the uncertainty of the D/H_I value of BCR-656 has to be taken up in the uncertainty budget; however, this was deemed a necessary and suitable compromise due to the unavailability of IRMS for this application, and the fact that not data accuracy (true value of the D/H ratio), but data consistency to previous TMU batches has to be guaranteed to maintain and safeguard the validity of the European Wine Databank. Furthermore, the master batch represents a new anchor point, so neither the length of the traceability chain nor the uncertainty will increase for future secondary batches. Finally, a considerably larger number of laboratories (11) were foreseen in this certification campaign for performing the characterisation measurements, which substantially reduces the coverage factor and thus the overall

uncertainty. It shall be noted that the certified value and its uncertainty are expressed as a ratio ($141.9 \pm 0.7 \times 10^{-6}$) with the measurement unit of one; commonly SNIF-NMR results are expressed in parts per million (ppm).

2 Time table of the project

Activity	Finalised by
Procurement of base material	May 2005
Processing	September 2005
Homogeneity testing	January 2006
Short-term stability testing	January 2006
Long-term stability testing	March 2007
Characterisation	April 2007

3 Participants

Processing

Eurofins Scientific Analytics, Nantes	FR
European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel (ISO Guide 34 accreditation; BELAC 268-TEST)	BE

Homogeneity and stability studies

Bundesinstitut für Risikobewertung, Berlin (ISO/IEC 17025 accreditation; AKS-P22001-EU)	DE
--	----

Characterisation*

Bundesinstitut für Risikobewertung, Berlin (ISO/IEC 17025 accreditation; AKS-P22001-EU)	DE
Central Science Laboratory, York (ISO/IEC 17025 accreditation; UKAS 1642)	GB
Eurofins Scientific Analytics, Nantes (ISO/IEC 17025 accreditation; COFRAC N° 1-0287)	FR
European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra (ISO/IEC 17025 accreditation, SINAL 0387)	IT
Instituto Nacional de Engenharia, Tecnologia e Inovação, Lisboa (ISO/IEC 17025 accreditation; IPAC L-0324)	PT
Laboratoire Interrégional des Douanes de Paris	FR
Landesuntersuchungsamt Rheinland-Pfalz, Speyer (ISO/IEC 17025 accreditation; SAL-RLP-L 19.04.03)	DE
Ministry of Health, State General Laboratory, Nicosia (ISO/IEC 17025 accreditation; ESYD 290)	CY
National Institute of Chemistry, Ljubljana	SI

*In alphabetical order

Project Management & Data evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel (ISO Guide 34 accreditation; BELAC 268-TEST)	BE
---	----

4 Processing of the material

A batch of 100 litres of TMU was acquired in China with a sufficiently high deuterium-to-hydrogen (D/H) ratio (> 120 ppm). This material, which contained about 0.5 % m/m of water, was further processed at Eurofins Scientific Analytics to remove most of the water from the product, as water initiates hydrolysis of TMU to trimethylurea and other degradation products. Effective removal of most of the water was accomplished by adding molecular sieve to the TMU.

The identity of TMU was investigated at Eurofins performing GC-MS measurements using an ion-trap mass spectrometer, and comparing the obtained spectrum with the NIST library spectrum of TMU. For all 3 independently sampled aliquots, identity could be confirmed.

Purity assessment was accomplished at Eurofins employing GC-FID, ^1H -NMR, and ^{13}C -NMR. GC-FID gave an average value of 99.89 ± 0.01 % ($n = 3$), calculated as TMU area to total peak area in the chromatogram. ^1H NMR ($n = 3$) and ^{13}C NMR ($n = 3$) agreed with literature data and did not show any significant impurity.

The final product exhibited a water content of 0.08 ± 0.01 % m/m ($n = 5$) as determined by Karl Fischer titration (KFT; shown in Table 1), resulting in a corresponding to a purity of 99.92 % m/m. The sum of impurities detected by GC-FID and KFT was calculated, and the corresponding purity of TMU yielded 99.81 %. The uncertainty of the combined impurities detected by GC-FID and KFT was calculated as the square root of the sum of the squares of the 2 contributions, and yielded 0.01 %.

5 L of the purified TMU, aliquoted in 10 bottles (brown glass, screw cap, additional wax stopper; see figure 2) were shipped to IRMM and stored at -20 °C until ampouling. Three small feasibility studies were carried out to find the best possible conditions of ampouling the slightly volatile and hygroscopic TMU. Details can be found in [8].

Ampouling was finally accomplished as follows: 555 amber 10 mL ampoules were opened and stored in desiccators one day prior to filling. The ten 500 mL TMU bottles (depicted in Fig. 2) were taken out of the refrigerator the evening before filling to equilibrate at 20 °C in the dark. A dispenser and a 10 L borosilicate bottle were set up for operation. The inlet (suction-side) of the dispenser was constantly flushed with argon. The 10 L flask was first rinsed with 300 mL of TMU from one of the Eurofins bottles, and another 100 mL of TMU was used to rinse the dispenser. Thereafter, the content of the ten TMU bottles was pooled

in the 10 L flask. Finally, ampoules were filled with 7.6 g (7.8 mL) of TMU and transferred to an ampouling machine for flame-sealing (ROTA R 910/PA, Wehr, Germany). The first 18 ampoules were discarded, and 531 ampoules were labelled (order of filling). Finally, the samples were stored at -20 °C

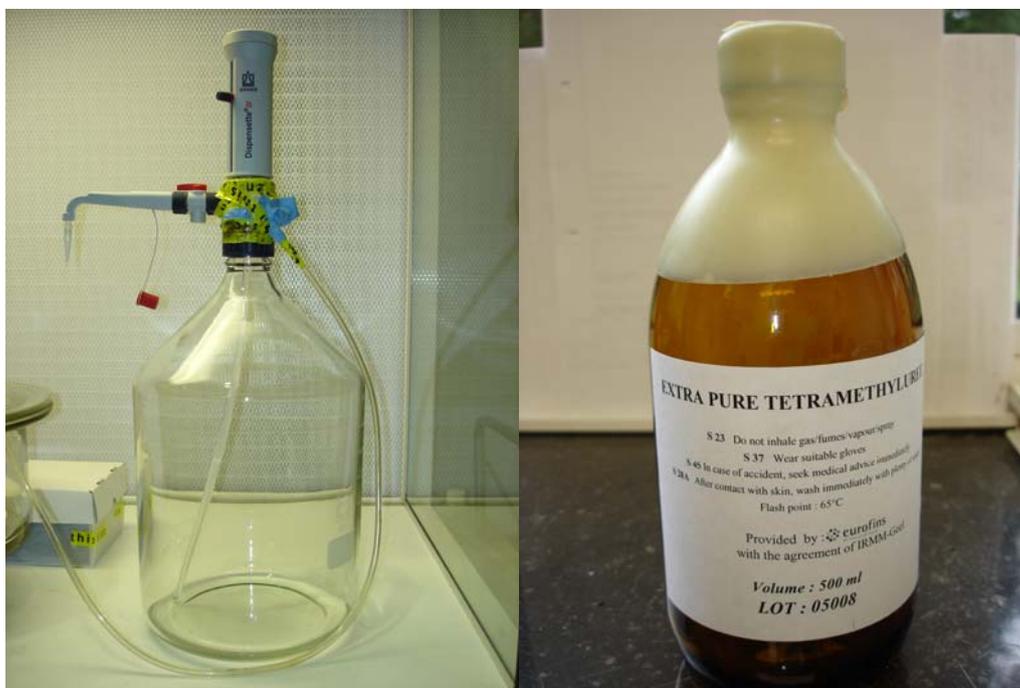


Figure 2. Dispenser and 10 L flask (left), TMU as provided by Eurofins (right)

Quality control after processing

After processing, five samples were picked using a random-stratified sample picking scheme for water analysis by coulometric Karl Fischer titration. Table 1 shows the results, confirming the expected low water levels in the final TMU candidate reference material.

Table 1. Water content in IRMM-425 as measured by Karl Fischer titration

Bottle n.	Water content*, [g/100 g]
67	0.078
122	0.078
242	0.077
363	0.078
509	0.079

* Mean of two replicates

5 Analytical methodology used

All measurements were accomplished by employing site-specific nuclear isotope fractionation-nuclear magnetic resonance (SNIF-NMR) spectroscopy in accordance with Commission Regulation 2676/90 [1]. BCR-656 (96 vol% ethanol) was provided by IRMM and used as the internal standard. This material is certified for two site-specific ratios of deuterium to hydrogen in the ethanol molecule: D/H_I refers to the peak to the right of the TMU peak (methyl group, CH_2DCH_2OH), whereas D/H_{II} refers to the peak to the left of the TMU peak (methylene group, CH_3CHDOH), [1]. The OH-group signal (CH_3CH_2OD) was not evaluated (figure 3).

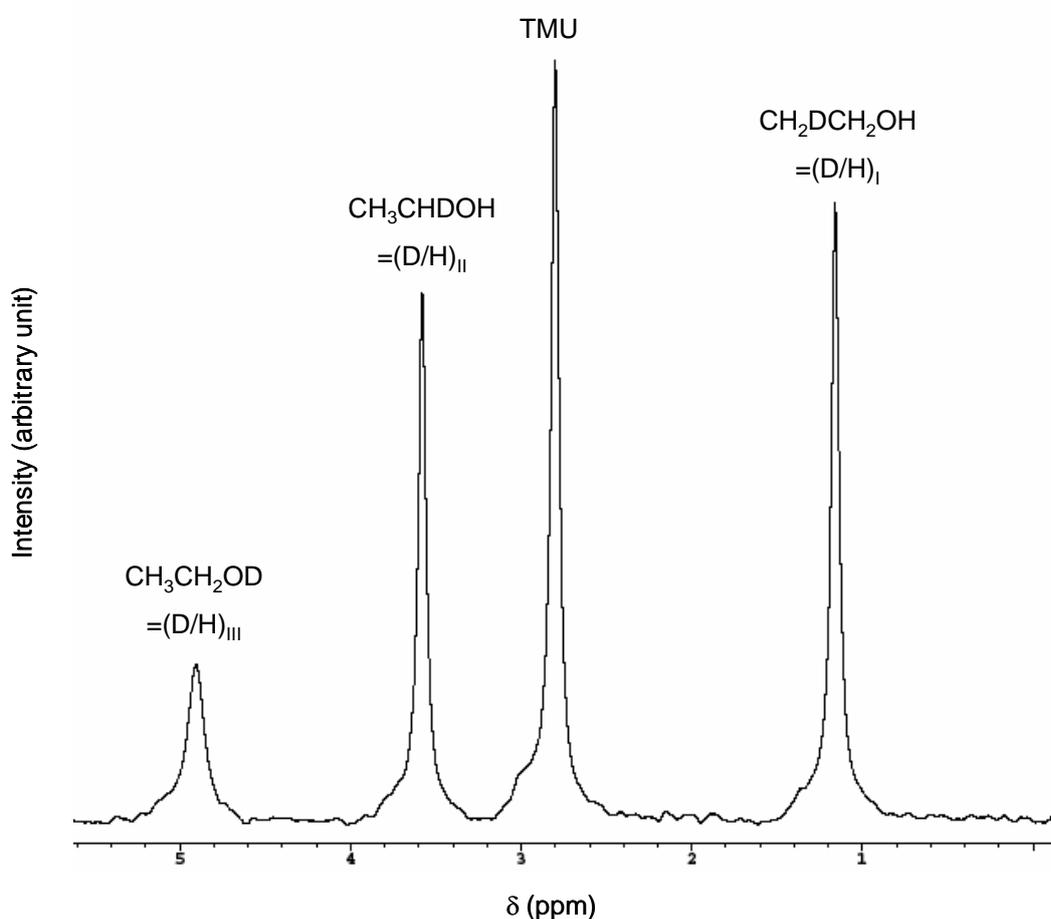


Figure 3. Representative 2H spectrum of ethanol/TMU mixture. Ethanol reveals three peaks, reflecting the position of the deuterium in the molecule

Measurements were acquired on 300, 400 or 500 - MHz NMR appliances. 10 mm diameter NMR tubes were used.

For sample preparation, the TMU ampoules were opened and two NMR tubes were prepared immediately per ampoule (duplicate analysis). Sample preparation was done by weighing in

ca. 3.2 mL of reference alcohol BCR-656 (ca. 2.5 g) and ca. 1.3 mL of TMU (ca. 1.3 g); finally, some 100 - 200 μL (ca. 0.2 – 0.3 g) of the lock substance hexafluorobenzene was added. All exact weights and sample numbers were recorded and reported. In addition, date and time of measurements were documented.

SNIF-NMR instrument performance was verified by analysing laboratory QC samples; most frequently, BCR-123 (3 different ethanols from beet, grape, and cane origin) was used [9]; some laboratories also used in-house ethanols distilled from wine with an assigned D/H value.

One analysis consisted of 10 recorded spectra. Finally, data processing was accomplished by Fourier transformation, line broadening and automated baseline correction. Peak heights were determined by the respective software used (details are given in Annex D).

The formulae depicted below and stipulated in [1] are used to calculate the ethanol D/H values using the certified TMU D/H value.

$$(D/H)_I = 1.5866 \times T_I \times \frac{m_{st}}{m_A} \times \frac{(D/H)_{St}}{t_m^D} \quad (D/H)_{II} = 2.3799 \times T_{II} \times \frac{m_{st}}{m_A} \times \frac{(D/H)_{St}}{t_m^D}$$

with

T_I , height of signal I ($\text{CH}_2\text{DCH}_2\text{OH}$) divided by height of signal of TMU

T_{II} , height of signal II (CH_3CHDOH) divided by height of signal of TMU

m_{st} , mass of internal standard (TMU) to nearest 0.1 mg

m_A , mass of distilled wine ethanol (BCR-656) to nearest 0.1 mg

$(D/H)_{St}$, certified value of TMU

t_m^D , strength by mass of the wine ethanol in % (m/m), whereas

$$t_m^D = \frac{p - p'}{p} \times 100, \text{ whereas}$$

p, mass of wine ethanol

p', mass of water content in the wine ethanol determined by Karl Fischer titration.

The factors of 1.5866 and 2.3799, respectively, derive from:

$$1.5866 = \frac{M_A}{M_{St}} \times \frac{P_{St}}{P_I} \quad 2.3799 = \frac{M_A}{M_{St}} \times \frac{P_{St}}{P_{II}}$$

with

M_A , molecular mass of ethanol

M_{St} , molecular mass of internal standard (TMU)

P_{St} , number of hydrogen atoms in TMU molecule

P_I , number of hydrogen atoms at site I of the ethanol molecule

P_{II} , number of hydrogen atoms at site II of the ethanol molecule

In this exercise, the formula was inverted to calculate the TMU D/H value using either the certified D/H_I or D/H_{II} value of BCR-656.

The values and their corresponding uncertainties of BCR-656 [6] used in this study are listed in Table 2:

Table 2. Relevant certified values and uncertainties of BCR-656

	Certified value	Uncertainty
D/H _I in ppm	102.84	0.20
t_m^D in % (m/m)	94.61	0.05

Preferentially, the D/H_I value of BCR-656 is used for the calculation of the TMU value, due to the favourable stoichiometric factors - 3 H in the case of D/H_I (CH₂D-CH₂-OH) signal, to 12 H in TMU - compared to 2 H in the case of D/H_{II} (CH₃-CHD-OH) signal to 12 H in TMU. An investigation comparing different methods of data evaluation revealed that the official method [1] tends to slightly overestimate the D/H_{II} ratio due to field distortion effects [10], which also underpins our decision to use solely the certified value of D/H_I of BCR-656 for calculating the D/H ratio of the TMU master batch and the uncertainty contributions.

Consequently, only the values calculated using the D/H_I of BCR-656 were further on taken into account (homogeneity, stability, and characterisation measurements).

6 Homogeneity and minimum sample intake

6.1 Homogeneity

Eight samples (about 1.5 % of the batch) were selected from the produced batch using a random stratified sample picking scheme. Samples were analysed in duplicate in a randomised order to allow detection of possible trends in the analytical sequence or the filling order, using SNIF-NMR as described above (400 MHz instrument). Care was taken that not more than 7 measurements were performed in between two replicate measurements of one given sample. This was a technical requirement as the two replicates from one sample (ampoule) are prepared at the same time, and due to the volatility of TMU evaporation might occur in the non-sealed NMR tubes (C. Fauhl, Berlin, personal communication). Measurements were performed under repeatability conditions.

Results were evaluated using SoftCRM [11] to detect possible trends regarding the filling sequence or analytical sequence and to estimate the uncertainty contribution of potential heterogeneity. Data were scrutinised for outliers and normal distribution using normal probability plots. No outliers were detected, and values were found to be normally distributed. The slopes of the regression lines were tested for their significance at 95 % and 99 % level of confidence: no significant trend was observed for neither filling order nor analytical sequence (Table 3).

Table 3. Linear regression and statistical parameters associated to the homogeneity evaluation of IRMM-425

Statistical parameters	Filling sequence	Analytical sequence
Slope (b)	-0.00027	-0.00621
Standard error of slope (s_b)	0.000156	0.00787
Degrees of freedom (df)	6	14
$ b /s_b$	1.74	0.79
$t_{(0.05;6)}$	2.45	-
$t_{(0.05;14)}$	-	2.14
Statistical significance (95% confidence interval)	No	No

Possible heterogeneity is hidden by the method repeatability (ANOVA; $MS_{\text{between}} < MS_{\text{within}}$), which made it necessary to calculate u_{bb}^* to estimate the uncertainty contribution for possible in-homogeneity using the following formula [12]:

$$u^*_{bb} = \frac{RSD_{method}}{\sqrt{n}} \sqrt{\frac{2}{df}}$$

RSD_{method} represents the method repeatability in %, n the number of replicates per sample, and df the degrees of freedom taken from the ANOVA results table. RSD_{method} was calculated using the following formula:

$$RSD_{method} = \frac{\sqrt{MS_{within}}}{\bar{y}} \times 100$$

whereas MS_{within} represents the mean of squares within groups from the ANOVA table, and \bar{y} the overall mean of the homogeneity measurements.

The value obtained was $u^*_{bb} = 0.06$ % using the certified D/H₁ value of BCR-656. The calculated u^*_{bb} holds for the sample intake laid down in Commission Regulation 2676/90, that is about 2.5 g of ethanol and about 1.3 g of TMU, using a 10 mm diameter NMR tube (standard dimension).

Table 4. Summary of the homogeneity test

	Average	S_{bb}	S_{bb} %	S_{wb}	S_{wb} %	u[*]_{bb}	u[*]_{bb} %
D/H ratio	141.828	n.c.	n.c.	0.169	0.119	0.084	0.060

n.c. cannot be calculated as $MS_{between} < MS_{within}$

The estimated value for u^*_{bb} is close to zero, as can be expected for a solution. It can thus be concluded that IRMM-425 is a homogeneous material.

6.2 Minimum sample intake

This parameter was not assessed, as the method predefines the sample intake (Commission Regulation 2676/90): for 10 mm diameter NMR tubes, 1.3 g have to be used.

7 Stability studies

7.1 Short-term stability

A four weeks isochronous study [13] was performed to evaluate stability of the TMU master batch during transport. Samples were selected from the produced batch using a random stratified sample picking scheme.

Samples were stored at +18 °C and +60 °C and at a reference temperature of -20 °C. Three ampoules were stored at each temperature for 0, 1, 2, and 4 weeks. After the indicated storage periods, the samples were transferred to storage at -20 °C until analysis. Samples were analysed in duplicate in the order predefined at IRMM (randomised sample order) using SNIF-NMR as described above (400 MHz instrument). Again, care was taken that not more than 7 measurements were performed in between two replicates of one given sample (see chapter homogeneity). Measurements were performed under repeatability conditions.

SoftCRM [11] was used to evaluate the measurement results for possible significant trends (degradation, enrichment) due to the storage conditions. One outlier was detected using the Grubbs test at 18 °C (95 % level of confidence), whereas no outlier was detected at 60 °C. The outlier was not excluded as data scrutiny revealed no technical reason to do so.

The measurement results and resulting graphs are presented in Annex B. The observed slopes were tested for significance using a t-test, with $t_{\alpha,df}$ being the critical t-value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % confidence interval). The slope was considered as statistically significant when $b/s_b > t_{\alpha,df}$. No significant slope at 95 % level of confidence was detected for both temperatures tested (Table 5).

Table 5. Linear regression and statistical parameters associated to the short term stability evaluation of IRMM-425

Storage Temperature	18 °C	60 °C
Statistical parameters		
Slope (b)	-0.015	0.016
Standard error of slope (s_b)	0.038	0.048
Degrees of freedom (df)	22	22
$ b /s_b$	0.39	0.33
$t_{(0.05;22)}$	2.07	2.07
Statistical significance (95% confidence interval)	No	No

Conclusion: no detectable degradation was observed, even at storage conditions of 60 °C for 4 weeks. Therefore no special precautions are required during transport; the material can be dispatched under ambient conditions.

7.2 Long-term stability

Another isochronous study was performed to evaluate long-term stability of the TMU master batch. Samples were selected from the produced batch using a random stratified sample picking scheme.

Samples were stored at -20 °C and at a reference temperature of -70 °C. The reasoning was that the final material is envisaged to be stored at -20 °C, and therefore it was not evaluated whether long-term stability can be proven also for higher temperatures.

6 ampoules were stored at each temperature for 0, 12, and 18 months. After the indicated storage periods, the samples were transferred to storage at -70 °C until analysis. Samples were analysed in duplicate in the order predefined at IRMM (randomised sample order) using SNIF-NMR as described above (400 MHz instrument). Again, care was taken that not more than 7 measurements were performed in between two replicates of one given sample (see above). Measurements were performed under repeatability conditions.

Data evaluation using SoftCRM [11] was done in the same way as for the short-term stability study. No outliers were detected. Table 6 summarizes the results.

Table 6. Evaluation of long-term stability study, 18 months

Storage Temperature	-20 °C
Statistical parameters	
Slope (<i>b</i>)	0.0030
Std error slope (<i>s_b</i>)	0.0043
<i>df</i>	34
<i>b</i> / <i>s_b</i>	0.71
<i>t</i> _(0.05;34)	2.03
Statistical significance (95 % confidence interval)	No

Finally, the uncertainty contribution u_{lts} due to (in)stability was estimated for a shelf-life of 5 years and found to be 0.18 % (temperature: -20 °C) using the following formula [14]:

$$u_{lts} = \frac{RSD}{\sqrt{\sum (x_i - \bar{x})^2}} * x$$

where

x_i are the time points for each replicate

x is the shelf life (in months)

RSD is the relative standard deviation of all data in this series

The relationship between u_{Its} and shelf-life is illustrated Annex C.

Conclusion:

The TMU master batch did not exhibit any significant instability at a temperature of -20 °C for a period of 18 months. Stability data available from previous TMU batches support these findings. Additional studies to assess stability over a longer period of time are ongoing. Those results will be used for extension of the material's shelf life. In conclusion, the material has shown to be stable when stored at -20 °C.

8 Characterisation

Batch characterisation was accomplished in an inter-laboratory comparison. Laboratories needed to have proven experience in the isotopic analysis of wine ethanol samples by SNIF-NMR and were selected according to the quality criteria required by IRMM. In several cases, laboratories were accredited to ISO 17025; otherwise, the required measurement performance was demonstrated by successful participation in PT schemes, respective quality control charts, and scientific publications in the field.

8.1 Discussion of results

Laboratories had to comply strictly with 2676/90 [1] and thus used the methodology outlined above. Small method variations were mainly due to different analytical appliances, software versions used, the exact amounts of TMU, BCR-656 and lock substance, and differences in calculations as outlined below (correction of water content or not, etc.). Methodical details such as NMR spectrometer type, software and QC measures are listed in table 1 (Annex E). Each laboratory received three units of IRMM-425 randomly selected from the batch. Laboratories were asked to analyse the samples in duplicate under intermediate precision conditions, i.e. 2 days à 3 measurements, whereby replicates of a given sample had to be analysed on different days (day 1: sample 1A, 2A, 3A; day 2: samples 1B, 2B, 3B). Appropriate performance of instrument and method had to be indicated by providing data on QC samples before and after the series. Laboratories were asked to provide all raw data and calculation of the D/H values, a representative spectrum and methodical details.

Data were scrutinized at IRMM and eventually re-calculated, whereby the following considerations applied:

- The value for one sample (replicate), being the mean of 10 acquired spectra, can be calculated in two ways: either the average of the peak heights (areas) for each signal is taken, and the respective values are then used in the formula to calculate the D/H value (approach 1), or the obtained ethanol and TMU peak heights (areas) in each spectra are taken and used in the formula, and then the average of the 10 calculated values is taken as the result for one sample (approach 2); the differences were found to be very minor in all cases (< 0.01 ppm). Approach 2 was used to re-calculate data (when necessary) at IRMM.

- Minor rounding differences (< 0.02 ppm) were sometimes observed between the values calculated and submitted by the laboratory, and re-calculations of the provided raw data performed at IRMM.

Laboratory-specific remarks:

Lab 3: The result of one spectrum in each of two of the samples was indicated to be an outlier (in-house laboratory statistical analysis). However, those values were retained and used for the calculation of the sample values at IRMM.

Lab 5: Due to a lack of sufficient amount of BCR-656, only 1.7 mL of ethanol could be used for preparation of the last sample (usual amount is ca. 3.2 mL per sample); however, the result was retained in the laboratory data set.

Lab 6: In one sample, considerably worse repeatability was obtained among peak heights within the acquired 10 spectra. The laboratory reported no outliers applying their in-house statistics, and the overall mean of the sample complied well with mean values of the other samples. The value was thus retained. Furthermore, this laboratory was the only one where a considerable difference for the TMU value was obtained when using either the D/H_I value of BCR-656 or its D/H_{II} value. No reasonable explanation could be given for this finding. However, as explained earlier, only the values calculated using the D/H_I value of BCR-656 are taken into account for establishing the certified value.

Lab 7: One of the ampoules provided was also checked for the water content, which was found to be < 0.11 % m/m. Due to a lack of sufficient amount of BCR-656, only 1.9 mL of ethanol could be used for preparation of the last sample (usual amount is ca. 3.2 mL per sample); however, the result was retained in the laboratory data set.

Lab 8: One of the ampoules provided was also checked for the water content, which was found to be < 0.11 % m/m. Due to a lack of sufficient amount of BCR-656, a laboratory in-house ethanol (established D/H_I value of 103.00 ppm) was used instead for the preparation of the last sample; the result was retained in the laboratory data set.

Lab 9: One of the ampoules provided was also checked for the water content, which was found to be < 0.11 % m/m.

Lab 10: The D/H value for TMU was calculated by correcting for residual water (0.16 % m/m as determined by Karl Fischer titration at the laboratory); furthermore, rounded molecular masses for TMU and ethanol of 116 and 46 g/mol were used, respectively. Therefore, the TMU D/H values were recalculated at IRMM, using the coefficients in 2676/90 and leaving out the correction for the residual water.

Lab 11: The laboratory re-examined the alcoholic grade of the 2 bottles of BCR-656 provided and reported values of 94.38 and 94.45 (% mass, w/w), respectively, which were then used in the calculation of the TMU D/H value; therefore, the TMU D/H values were re-calculated at IRMM. The laboratory repeated the analysis of the 3rd ampoule due to instrumental problems in the first series for this sample. Analysis was done on 2 different days.

Finally, all data sets were subjected to visual and statistical analysis, employing SoftCRM [11]. These analyses included Dixon test, Nalimov t-test and Grubbs test to detect possible outliers, Cochran and Bartlett test to verify homogeneity of variances, and skewness and kurtosis test to check for normal distribution of laboratory means.

Outlier testing revealed one outlier (laboratory 11) with the Nalimov t-test, whereas the other two tests did not indicate any outliers. After data inspection (laboratory data including the QC samples), it was decided to retain this value for the calculation of the overall mean. Both Cochran and Barlett test indicate the homogeneity of variances, and skewness and kurtosis test revealed normal distribution of laboratory mean values. In conclusion, no data was rejected, and u_{char} was calculated as

$$u_{char} = \frac{s}{\sqrt{n}}$$

s being the standard deviation of the laboratory means, and n the number of accepted data sets (laboratories).

No Pooling - Lab Means and their StDev for D/H I in TMU

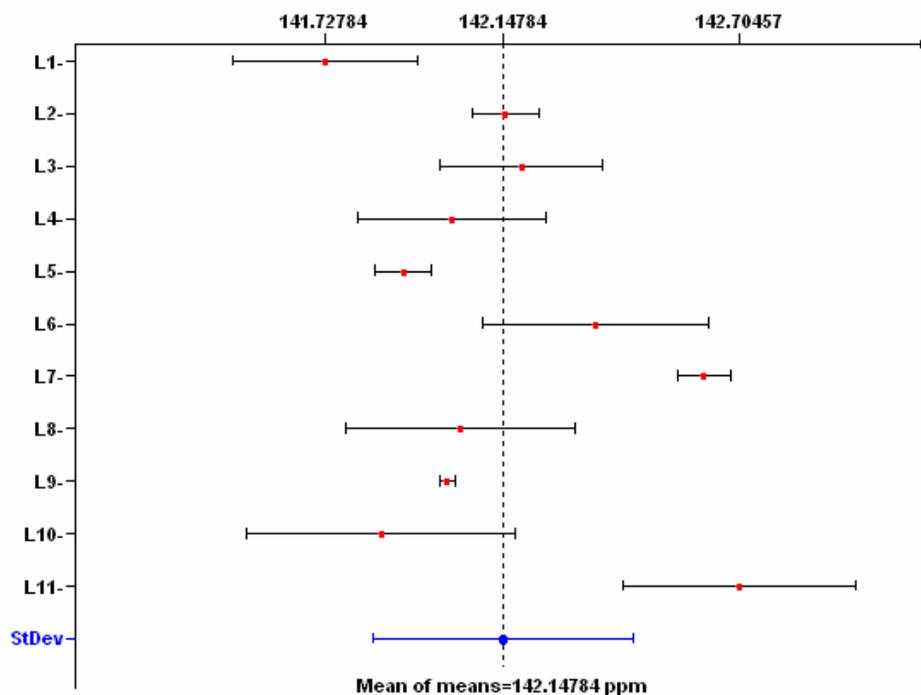


Figure 4. Mean of the laboratory means and its standard deviation, and means from all accepted data sets \pm standard deviations ($n=6$) from the characterisation of IRMM-425

Table 7. Mean of the means of the accepted data sets of results, standard uncertainty from characterisation (u_{char}) and relative standard uncertainty from characterisation ($u_{\text{char}}\%$)

Property	Mean of means ¹⁾	Accepted data sets (p)	u_{char} ¹⁾	$u_{\text{char}} [\%]$ ²⁾
D/H ratio	142.147×10^{-6}	11	0.092×10^{-6}	0.065

¹⁾ Mean value is dimensionless; commonly it is expressed in parts per million (ppm)

²⁾ Relative u_{char} (in percent of the certified value)

The overall mean value finally was corrected for the purity of the material (99.81 % m/m, page 9), thus yielding a certified value of 141.877×10^{-6} (141.877 ppm).

8.2 Certified value and uncertainty budget

Uncertainties of the certified value of an individual unit (U_{CRM}) were calculated using the formula depicted below. In addition to the usually included contributors for homogeneity, long-term stability, and characterisation, also the uncertainties carried over from BCR-656

(D/H_i value, alcoholic strength value) were taken into account. The calculated uncertainty due to purity, however, was not included due to its negligible contribution to the total uncertainty.

$$U_{CRM} = k \cdot \sqrt{u_{bb}^{*2} + u_{lts}^2 + u_{char}^2 + u_{BCR-656a}^2 + u_{BCR-656b}^2}$$

where U_{CRM} is the expanded uncertainty of the certified value

k is the coverage factor; a factor of 2 is applied to give approximately 95 % confidence

u_{bb} is the uncertainty associated to the between bottle variation

u_{lts} is the uncertainty contribution from the estimation of the long-term stability study

u_{char} is the uncertainty associated to the characterisation of the material

$u_{BCR656a}$ is the uncertainty of the D/H_i value of BCR-656

$u_{BCR-656b}$ is the uncertainty of the t_m^D value of BCR-656

Table 8. Certified value and uncertainty budget for D/H ratio of IRMM-425

	IRMM-425
$u_{bb}^* [\%]^{1)}$	0.060
$u_{lts} [\%]^{1)}$	0.179
$u_{char} [\%]^{1)}$	0.065
$u_{BCR-656 a} [\%]^{1)}$	0.089
$u_{BCR-656 b} [\%]^{1)}$	0.024
$u_{pur} [\%]^{1)}$	negligible
$U_{CRM} (k=2) [\%]^{1)}$	0.439
Average ²⁾	141.877×10^{-6}
$U_{CRM} (k=2) ^{2)}$	0.623×10^{-6}

¹⁾ The relative uncertainties are listed (percent of the certified value)

²⁾ Values are dimensionless; commonly they are expressed in parts per million (ppm)

9 Metrological Traceability

The certified value is traceable to V-SMOV by strictly adhering to the Community reference method (SNIF-NMR) as defined in Commission Regulation 2676/90 [1]. For V-SMOW, a value of 155.76 ppm was used (Tellus (1970) 22: 712-715). The certified value is commonly expressed in parts per million (ppm).

10 Instructions for use

10.1 Intended use

IRMM-425 constitutes a master batch to which future secondary batches will be linked. The material is not for sale, and shall be used for the value assignment of the D/H ratio in future secondary TMU batches.

10.2 Storage

The ampoules shall be stored at a temperature of -20 °C.

10.3 Minimum sample intake

The minimum samples intake is 1.3 g of TMU when using 10 mm diameter NMR tubes (Commission Regulation 2676/90, [1]).

10.4 Use of the certified value

The material shall be used as internal standard for the determination of the D/H ratios of wine ethanol according to Commission Regulation 2676/90.

10.5 Safety precautions

The following health and safety clauses apply:

R 20/22	Harmful by inhalation, and if swallowed
R 63	Possible risk of harm to the unborn child

11 Acknowledgments

The authors would like to thank M. Lees (Eurofins) and C. Guillou (JRC/IHCP/BEVABS) for fruitful discussions on a suitable strategy for the certification of this master batch, T. Linsinger and G. Roebben (IRMM) for reviewing certification report and certificate, and P. Gowik (Federal Office of Consumer Protection and Food Safety, DE), E. Nordkvist (National Veterinary Institute, SVA, SE), and J. de Boer (Institute for Environmental Studies, Vrije Universiteit Amsterdam, NL) as Certification Advisory Panel members, for their constructive comments.

12 Annexes

Annex A: Results of the homogeneity study

Annex B: Results of the short-term stability study

Annex C: Results of the long-term stability study

Annex D: Characterisation – method-related information

Annex E: Results of the Characterisation study

13 References

- [1] Commission Regulation (EEC) No. 2676/90 of 17 September 1990 determining Community methods for the analysis of wine. Off. J. Eur. Communities 1990; L272: 1.
- [2] Martin, G.J., Martin, M.L. (1981) Deuterium labelling at the natural abundance level as studied by high field quantitative ^2H NMR. Tetrahedron Lett. 22, 3525.
- [3] Commission Regulation (EC) No. 2729/2000 of 14 December 2000 laying down detailed implementing rules on controls in the wine sector. Off. J. Eur. Communities 2000; L316: 16.
- [4] Guillou, C., Martin G.J. (1993) Characterisation of reference tetramethylurea (TMU) for the determination of H/D ratio in alcohols by SNIF-NMR. EUR 14396 EN.
- [5] Breas, O., Thomas, F., Zeleny, R., Calderone, G., Jamin, E., Guillou, C. (2007) Performance evaluation of elemental analysis/isotope ratio mass spectrometry methods for the determination of the D/H ratio in tetramethylurea and other compounds – results of a laboratory inter-comparison. Rapid Commun. Mass Spectrom. 21, 1555.
- [6] Martin, G.J., Naulet N. (1988) Precision, accuracy, and referencing of isotope ratios determined by nuclear magnetic resonance. Fresenius Z. Anal. Chem. 332, 648.
- [7] Guillou, C., Remaud, G., Lees, M. (2001) The certification of the carbon-13 and deuterium isotopic ratio content and the alcoholic grade of ethanol from wine (BCR-656, 96 % vol), the carbon-13 isotopic ratio content of sugar (BCR-657), the oxygen-18 isotopic ratio content of water from wine (BCR-658, 7 % vol, and BCR-659, 12 % vol), and the carbon-13 and deuterium isotopic ratio content and the alcoholic grade of ethanol from wine (BCR-660, 12 % vol). EUR 20064 EN.
- [8] Bau, A., Oostra, A., de Vos, P., Charoud-Got, J., Zeleny, R., Emteborg, H. private communication (RM unit internal report "Filling of tetramethylurea (TMU) in ampoules. For a master batch to establish the D/H ratio by NMR", GE/RM Unit/15/2005/October27, IRMM 2005).
- [9] Martin, G.J., Trierweiler, M., Ristow, R., Hermann, A., Belliardo, J.-J. (1994) The certification of the three reference ethanols for SNIF-NMR: BCR certified reference materials. EUR 15347 EN.
- [10] Cremonini, M.A., Tacconi, D., Clementi, V., Luchinat, C. (1998) Accurate Determination of Deuterium/Hydrogen Ratios in Natural Organic Compounds through a Nuclear Magnetic Resonance Time-Domain Reference Convolution Method: Application to Ethanol from Three Botanical Sources and Critical Analysis of Systematic Inaccuracies in Previous Methods. J. Agric. Food Chem. 46, 3943.
- [11] SoftCRM ver. 2.0.10 developed by NHRF and JRC/IRMM. Customized version of SoftCRM <http://www.eie.gr/iopc/softcrm/>

- [12] van der Veen, A.M.H., Linsinger, T.P., Pauwels, J. (2001) Uncertainty calculations in the certification of reference materials, 2. Homogeneity study. *Accred. Qual. Assur.* 6, 26.
- [13] Lamberty, A., Schimmel, H., Pauwels, J. (1998) The study of the stability of reference materials by isochronous measurements, *Fres. J. Anal. Chem.*, 360, 359.
- [14] Linsinger, T., Pauwels, J., Lamberty, A., Schimmel, H., van der Veen, A.M.H., Siekmann, L. (2001) Estimating the uncertainty of stability for matrix CRMs. *Fres. J. Anal. Chem.* 370, 183.

Annex A - Homogeneity data

Results of the homogeneity study of IRMM-425. Data was calculated according to formula on page 12 [1], taking the $(D/H)_i$ value from BCR-656 into account.

Ampoule number	Replicate 1 (ppm)	Replicate 2 (ppm)	Average (ppm)
Series 1, $(D/H)_i$ signal taken			
50	141.94	141.94	141.94
115	141.64	141.95	141.80
166	141.74	142.03	141.89
258	141.75	141.72	141.74
300	141.93	141.82	141.88
354	141.84	141.86	141.85
438	141.98	141.66	141.82
521	141.92	141.52	141.72

Homogeneity IRMM-425- Graph

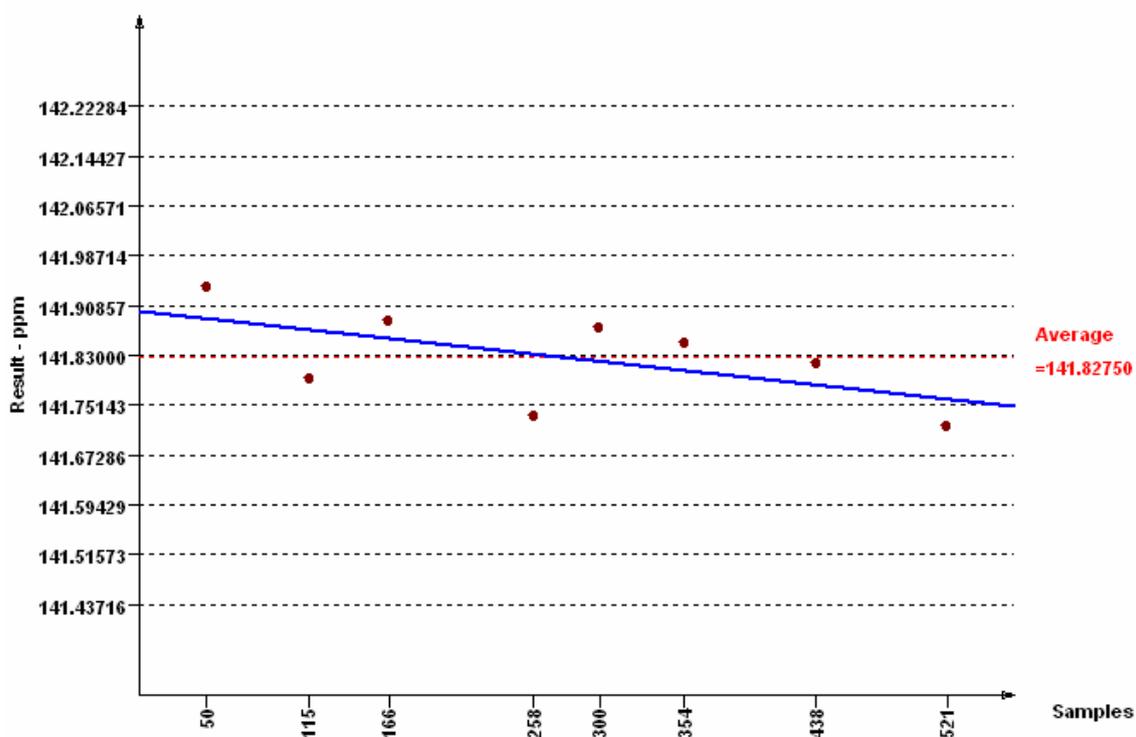


Figure A1. Homogeneity of IRMM-425. The x axis depicts the sample numbers (filling sequence). The indicated points are mean values of duplicate measurements.

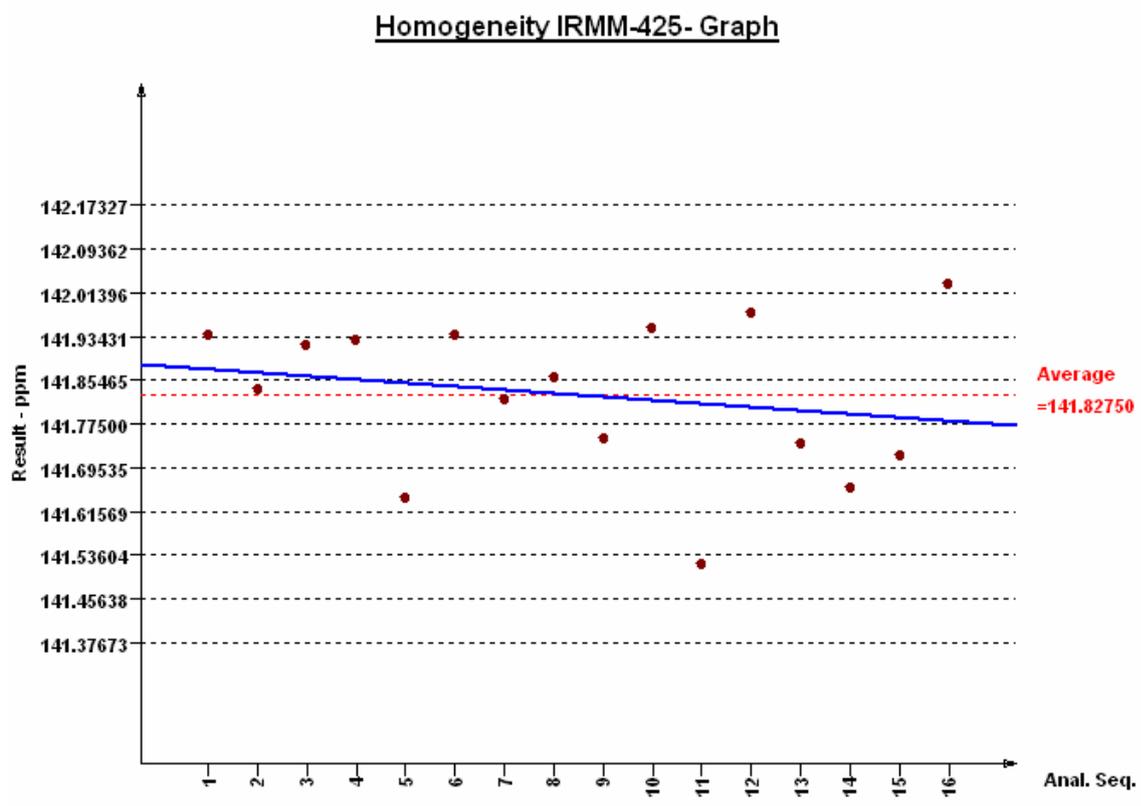


Figure A2. Homogeneity of IRMM-425. The x axis depicts the order of measurements (analytical sequence).

Annex B – Short-term stability

Results of the short-term stability study of IRMM-425. Data was calculated according to formula on page 12 [1], taking the $(D/H)_i$ value from BCR-656 into account.

Time (weeks)	D/H _{TMU} (in ppm)	
	18°C	60°C
0	141.71	141.71
0	141.48	141.48
0	141.59	141.59
0	141.55	141.55
0	140.92	140.92
0	141.45	141.45
1	141.58	140.97
1	141.69	141.46
1	141.49	141.53
1	141.13	141.56
1	141.51	141.88
1	141.42	140.84
2	141.00	141.41
2	141.35	141.61
2	140.61	141.07
2	141.59	141.07
2	141.26	140.91
2	141.52	140.86
4	141.64	141.65
4	141.13	141.07
4	141.46	142.10
4	141.44	141.69
4	141.27	141.37
4	141.61	141.24

Short-term stability IRMM-425 T=18°C

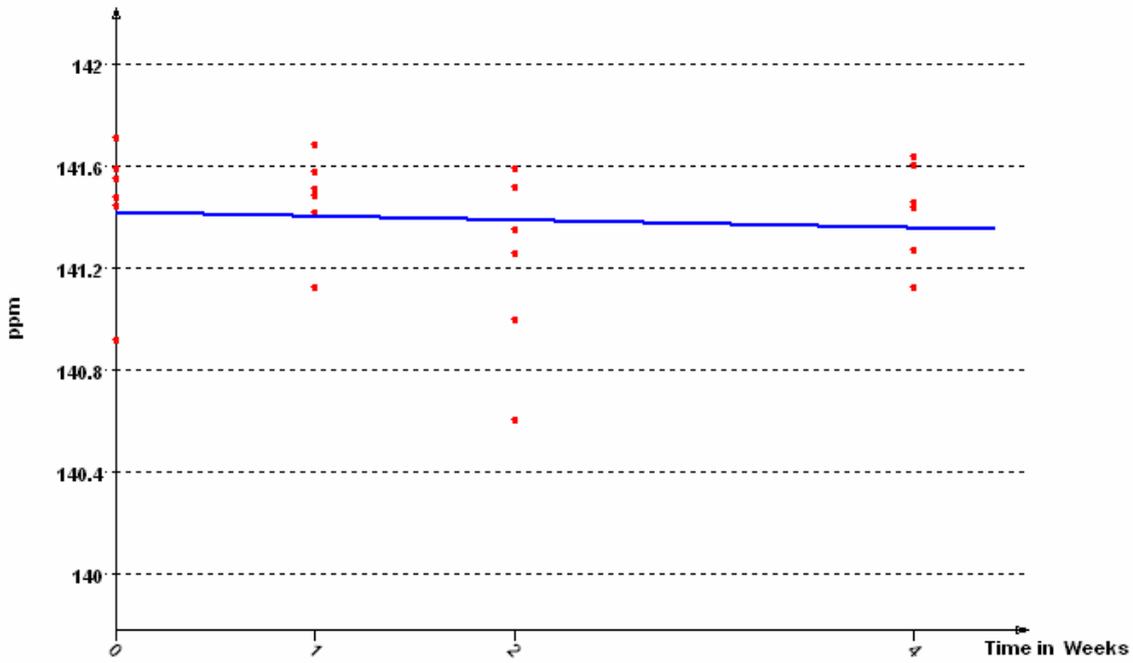


Figure B1. Short-term stability of IRMM-425 at 18 °C.

Short-term stability IRMM-425 T=60°C

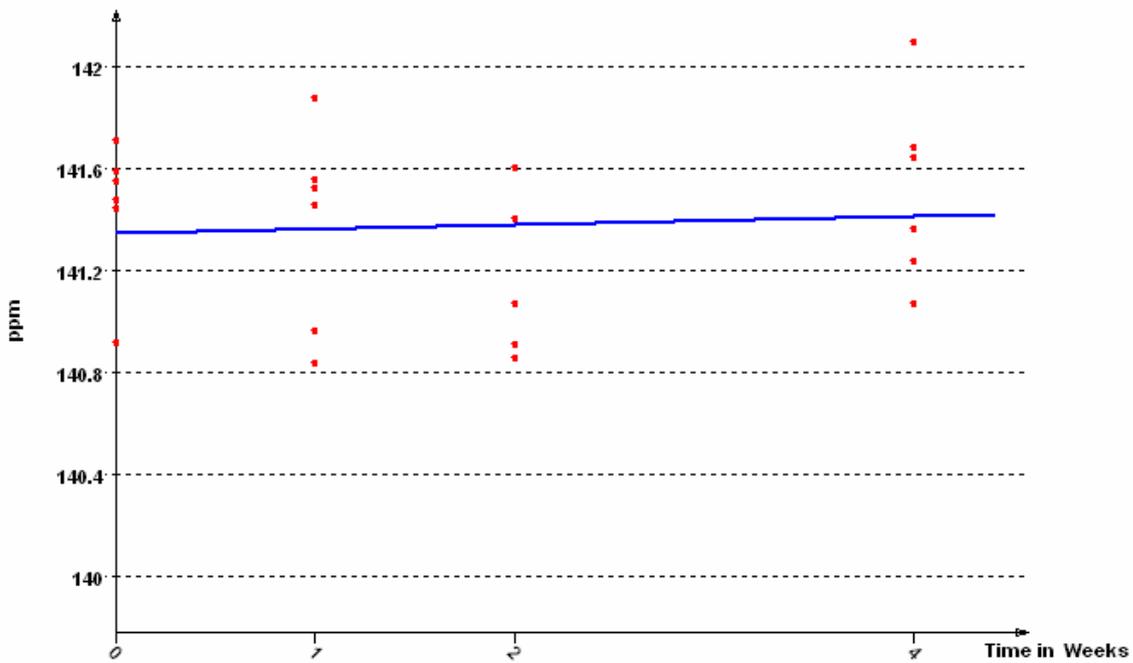


Figure B2. Short-term stability of IRMM-425 at 60 °C.

Annex C – Long-term stability

Results of the long-term stability study of IRMM-425 at -20 °C. Data was calculated according to formula on page 12 [1], taking the $(D/H)_I$ value from BCR-656 into account.

Time (months)	D/H _{TMU} (in ppm)
0	141.55
0	141.47
0	141.34
0	141.14
0	141.34
0	141.68
0	141.52
0	141.82
0	141.52
0	141.61
0	141.26
0	141.24
12	141.37
12	141.77
12	141.71
12	141.44
12	141.09
12	141.18
12	141.23
12	141.82
12	141.57
12	141.55
12	141.78
12	141.45
18	141.61
18	141.66
18	141.29
18	141.55
18	141.58
18	141.40
18	141.41
18	141.57
18	141.56
18	141.41
18	141.58
18	141.51

Shelf Life and Associated UIts, T=-20 °C

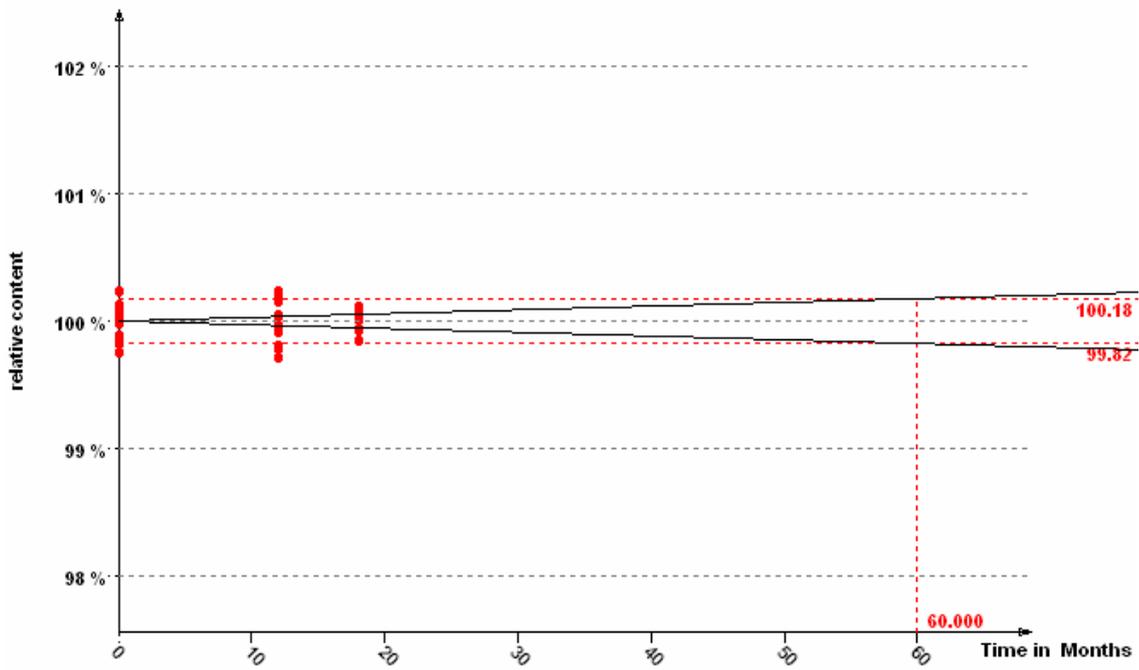


Figure C1. Long-term stability of IRMM-425 at -20 °C. u_{Its} is estimated for a shelf life of 5 years using the formula on page 17 and the measurement data on page 32.

Annex D – Characterisation – method-related information

Laboratory code	Signal property used for calculation	NMR appliance and software	QC measures (instrument verification, QC sample(s) used)
1	Height	Bruker Avance 400, Topspin 1.3	BCR-656, samples of latest TMU batch included in sequence
2	Height	Bruker Avance 400, Topspin 1.3	BCR-123, in-house alcohol
3	Height	Bruker Avance 400, X-Win NMR	BCR-123, TMU calibrated in-house
4	Height	Bruker Avance 400, Icon NMR	BCR-123, in-house wine ethanol sample (95 vol%)
5	Height	Varian Unity Inova 300, VNMRJ rev. 1.1D	BCR-123
6	Area	Bruker ARX 500, X-Win NMR 2.6	BCR-123
7	Height	Bruker AM 500, Aspect X32	BCR-123
8	Height	Bruker AMX 500, X-Win NMR 2.6	BCR-123
9	Height	Bruker DPX 400, Topspin 1.3.6	BCR-123
10	Height	Bruker Avance 400, X-Win NMR 3.1	BCR-123
11	Height	Bruker DXR 500, X-Win NMR 2.3	BCR-123

Annex E – Characterisation data

Characterisation data of IRMM-425. Data (ratio of deuterium to hydrogen) is expressed in ppm and has been acquired from TMU/BCR-656 mixtures strictly adhering to 2676/90 [1]. Values were calculated using the D/H_i value of BCR-656 and the formula depicted on page 12. All 6 individual measurements (2 days, 3 samples in duplicate) per laboratory are presented. Data were eventually re-calculated at IRMM performing a harmonised way of calculation (see page 22).

Laboratory code	Day 1			Day 2		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1	141.42	141.26	141.91	141.93	141.83	142.02
2	142.48	142.06	142.28	141.82	142.10	142.19
3	142.36	142.15	142.36	142.40	141.84	142.24
4	141.72	142.14	142.26	142.00	141.74	142.30
5	142.08	142.30	142.19	141.57	141.59	141.72
6	142.34	142.39	141.86	142.27	142.93	142.41
7	142.61	142.52	142.63	142.53	142.86	142.56
8	142.31	142.14	141.57	142.23	142.11	141.93
9	142.09	141.92	141.97	141.97	142.12	142.03
10	141.85	141.82	142.41	141.47	141.56	142.04
11	143.34	143.58	143.12	142.22	142.29	141.69

EUR 23031 EN – Joint Research Centre – Institute for Reference Materials and Measurements

Title: Certification of the Deuterium-to-Hydrogen (D/H) ratio in a 1,1,3,3 – tetramethylurea master batch, IRMM-425

Author(s): R. Zeleny, H. Emteborg, F. Ulberth

Luxembourg: Office for Official Publications of the European Communities

2007 – 37 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1018-5593

ISBN 978-92-79-07550-6

Abstract

This report describes the production of a tetramethylurea reference material (IRMM-425), certified for its deuterium-to-hydrogen (D/H) ratio. The material is to be used as an internal standard in site-specific natural isotope fractionation – nuclear magnetic resonance (SNIF-NMR) spectroscopy measurements for determining the D/H ratios of ethanol distilled from wines, an important measure in wine authenticity testing (Commission Regulation 2676/90, [1]).

Commercially obtained TMU with a sufficiently high D/H ratio ($>120 \times 10^{-6}$) was purified by removing most of the residual water and filled into amber glass ampoules in 7.8 mL portions. Homogeneity was tested and no heterogeneity observed. Stability studies indicated no material degradation for 4 weeks at 60 °C and for 18 months at –20 °C. Batch characterisation was accomplished in an inter-laboratory comparison using the SNIF-NMR technique exclusively.

The certified value was obtained as the unweighted mean of the laboratory means of the accepted sets of results. The expanded uncertainty associated ($k = 2$) includes contributions from (potential) heterogeneity, potential instability, characterisation, as well as the carried-over uncertainty from BCR-656 (96% wine ethanol) that was used as the internal standard in the measurements. Due to its negligible contribution, the uncertainty associated with the purity of the material did not need to be included.

The certified value and its associated uncertainty are:

	Amount-of-substance fraction	
	Certified value ^{1,2,4)}	Uncertainty ³⁾
Deuterium-to-hydrogen (D/H) ratio	141.9×10^{-6}	0.7×10^{-6}

- 1) Traceable to V-SMOV (Vienna Standard Mean Ocean Water) by strictly adhering to the Community reference method (SNIF-NMR, site-specific natural isotope fractionation – nuclear magnetic resonance spectroscopy) as defined in Annex 8, Commission Regulation 2676/90. For V-SMOW, a value of 155.76 ppm was used (Tellus (1970) 22: 712-715).
- 2) This value was obtained by an inter-laboratory comparison (11 laboratories) employing SNIF-NMR at 46.1 (300), 61.4 (400), and 76.7 (500) MHz for ²H (¹H). The value has been corrected for the material impurities (including water).
- 3) Expanded uncertainty with a coverage factor of $k = 2$, according to the Guide to the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.
- 4) The certified value has a measurement unit of one; it is commonly expressed in parts per million (ppm).

This CRM constitutes a master batch and will be used for the certification of secondary batches; the material is not for sale.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

