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Certification of the deuterium distribution in ethanol from grape, sugar cane and sugar beet sugars' fermentation: ERM®-AE200

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Contents

Abstract 1

Acknowledgements 2

1 Introduction 3

1.1. Background 3

1.2. Choice of the material 4

1.3. Outline of the CRM project 5

2 Participants 7

2.1 Project management and data evaluation 7

2.2 Processing 7

2.3 Homogeneity measurements 7

2.4 Stability measurements 7

2.5 Characterisation measurements 7

3 Material processing and processing control 8

3.1 Origin/Purity of the starting material 8

3.2 Processing 8

3.3 Processing control 11

4 Homogeneity 13

4.1 Between-unit homogeneity 13

4.2 Within-unit homogeneity and minimum sample size 16

5 Stability 17

5.1 Transport stability 17

5.2 Storage stability 18

5.3 Estimation of uncertainties 19

6 Characterisation 21

6.1 Selection of participants 21

6.2 Study setup 21

6.3 Methods/Measurement procedures used 21

6.4 Evaluation of results 22

6.4.1 Technical evaluation 22

6.4.2 Statistical evaluation 23

7 Value Assignment 25

7.1 Certified values and their uncertainties 25

8 Metrological traceability and commutability 27

8.1 Metrological traceability 27

8.1.1 Identity 27

8.1.2 Quantity value 27

8.2 Commutability 28

9 Instructions for use 29
9.1 Safety information 29
9.2 Storage conditions 29
9.3 Use of the material 29
9.4 Minimum sample size 29
9.5 Use of the certified values 29
10 Conclusions 31
References 32
List of abbreviations 34
List of boxes 37
List of figures 38
List of tables 39
Annexes 40
Annex 1. Results of the homogeneity measurements 40
Annex 2. Data from the post-certification stability monitoring programme of BCR-123 43
Annex 3. Results of short term stability measurements 43
Annex 4: Results of the characterisation measurements 47

Abstract

ERM-AE200 is a set of three ethanol materials certified for the deuterium (D) distribution (deuterium-to-hydrogen (D/H) amount of substance ratios and the relative deuterium-to-hydrogen ratio), produced within the scope of ISO 17034 accreditation [1] and ISO Guide 35:2017 [2].

The certified reference material (CRM) comprises three NMR tubes, each containing at least 4.65 ml of a solution combining ethanol (from grape, sugar cane or sugar beet), tetramethylurea and hexafluorobenzene. The tubes are sealed under an atmosphere of argon.

Between-unit homogeneity was quantified and stability during transport and storage was assessed in accordance with ISO Guide 35:2017 [2]. The material, contained in sealed NMR tubes, is intended for direct measurement of the intact tube with SNIF-NMR®, a non-destructible analysis. Therefore, the minimum sample size for one measurement is the full content of the NMR tube and repeated use is possible.

The material was characterised by an interlaboratory comparison of laboratories of demonstrated competence and adhering to ISO/IEC 17025:2017 [3]. All results were technically valid and no outlier was eliminated unless a technical reason for the deviation was found.

Uncertainties of the certified values were calculated in accordance with ISO 17034:2016 [1] and ISO Guide 35:2017 [2] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended for the performance verification of NMR spectrometers in SNIF-NMR measurements. As with any reference material, it can be used for establishing control charts.

Before release of the ERM-A200, the certification project was subjected to peer-review involving both internal and external experts.

Acknowledgements

The authors would like to acknowledge the support received from colleagues of JRC for the processing, organising of stability studies, measuring, reviewing of the certification project and distribution of this CRM.

Azucarera del Guadalfeo, S.A, Almería, ES is acknowledged for the complimentary provision of ethanol from sugar cane used as CRM base material.

Furthermore, the authors would like to thank the experts of the Reference Material Review, Panel Katrin Vorkamp (Aarhus University, DK) and Francois Guyon (Service Commun des Laboratoires du Minefi, Marseille, FR), for their constructive comments and the external review of the certification report and certificate.

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1 Introduction

1.1. Background

The European Union is the world-leading producer of wine, and wine is the largest EU agri-food sector in terms of exports (7.6 % of agri-food value exported in 2020). Between 2016 and 2020, the average annual wine production was 165 million hectolitres. In 2020, the EU accounted for 45 % of global wine-growing areas, 64 % of production and 48 % of consumption [4].

The European Commission (EC) has adopted multiple regulations on wine and on implementing quality schemes for the wine sector [5]. The aim of the EU quality policy is to protect the names of specific agri-food products and promote their unique characteristics, linked to their geographical origin as well as traditional know-how. For wine, the application of the quality schemes is covered by EU regulation 1308/2013 [6]. The regulation states that methods of analysis applied for the control must be based on any relevant methods recommended and published by the International Organisation of Vine (OIV), unless they would be ineffective or inappropriate in view of the objective pursued by the Union[1].

The OIV is an intergovernmental organisation having as one of the main objectives to contribute to the international harmonisation of existing practices and standards for vine and wine products. In their role, the OIV published a compendium of international methods for wine analysis in 1962, which was updated at different occasions ever since, the latest edition dating from 2022 [7]. To support the implementation of the methods and aid harmonisation among laboratories working on authenticity testing for wine, the Joint Research Centre of the European Commission (EC-JRC) produced a series of dedicated certified reference materials [8, 9]. The EU also created a European wine databank of characteristic stable isotope composition in wines produced in Europe. The database is managed by the European Reference Centre for Control in the Wine Sector (ERC-CWS) and is hosted at the EC-JRC.

One of the adulteration processes that could lead to fraud in the wine sector is the so called chaptalisation. Also known as enrichment, this process involves adding sugar to unfermented grape must in order to increase the alcohol content after fermentation. The method for detecting enrichment of grape musts and wines using nuclear magnetic resonance of deuterium (D-NMR) is described in OIV-MA-AS311-05, *Determination of the deuterium distribution in ethanol derived from fermentation of grape musts, concentrated grape musts, grape sugar (rectified concentrated grape musts) and wines by application of nuclear magnetic resonance (SNIF NMR/RMN FINS)* [10]. The analysis is based on the site-specific natural isotope fractionation of the D-isotope (SNIF-NMR). The site-specific deuterium distribution in the ethanol molecule creates a botanical and geographical fingerprint that supports authentication processes.

In brief, during alcoholic fermentation, deuterium from sugars and water molecules are transferred into the methyl- and methylene position of the ethanol molecule, resulting in molecules I, II, III and IV on the ethanol skeleton [10]:

CH_2D-CH_2-OH	CH_3-CHD-OH	CH_3-CH_2-OD	HOD
I	II	III	IV

Where

$(D/H)_I$: represents the deuterium distribution (deuterium-to-hydrogen (D/H) amount of substance ratio) associated with molecule I

$(D/H)_{II}$: deuterium distribution (deuterium-to-hydrogen (D/H) amount of substance ratio) associated with molecule II

Approximately 85 % of deuterium in the sugar molecule is transferred into the methyl-group of ethanol and about 75 % of the deuterium of fermentation water into the methylene-group. Thus the resulting deuterium

[1] Article 80(5) of Regulation (EU) No 1308/2013: "The Commission shall, where necessary, adopt implementing acts laying down the methods referred to in point (d) of Article 75(5) for products listed in Part II of Annex VII. Those methods shall be based on any relevant methods recommended and published by the OIV, unless they would be ineffective or inappropriate in view of the objective pursued by the Union. Those implementing acts shall be adopted in accordance with the examination procedure referred to in Article 229(2). Pending the adoption of such implementing acts, the methods and rules to be used shall be those allowed by the Member State concerned."

isotope distribution of the methyl group which is defined as $(D/H)_I$ represents the botanical origin of the fermented sugar whereas that of the methylene group $(D/H)_{II}$ is typical for the deuterium content of the grape-water and reflects the climate conditions related to the geographical origin and year of vintage [11] The addition of exogenous sugar before fermentation of the grape must, has an effect on the deuterium distribution in the ethanol molecule.

Values for $(D/H)_I$ and $(D/H)_{II}$ are determined by SNIF-NMR in the ethanol extracted from the wine. From these values, the relative distribution of deuterium in molecules I and II is derived and expressed as *R*. This technique is often combined with stable isotope ratio analysis-mass spectrometry (SIRA-MS), which is typically used to determine stable carbon isotopes. Together, these are powerful tools for wine authentication and characterization.

In order to check the performance of the NMR spectrometer, a series of reference sample measurements with the official SNIF-NMR method is required [10]. Due to the stock exhaustion of BCR-123, one of the previously produced CRMs for this purpose, the production of a new batch became necessary. The new CRM, encoded ERM-AE200, includes three reference ethanols obtained by fermentation of sugar from different botanical species: grape, sugar cane and sugar beet. The certified characteristic site-specific deuterium to hydrogen (D/H) amount of substance ratios in these ethanols allow for the necessary check of the spectrometer standardisation (performance check on the calibration of SNIF-NMR systems) to detect potential enrichment of wine and grape musts.

1.2. Choice of the material

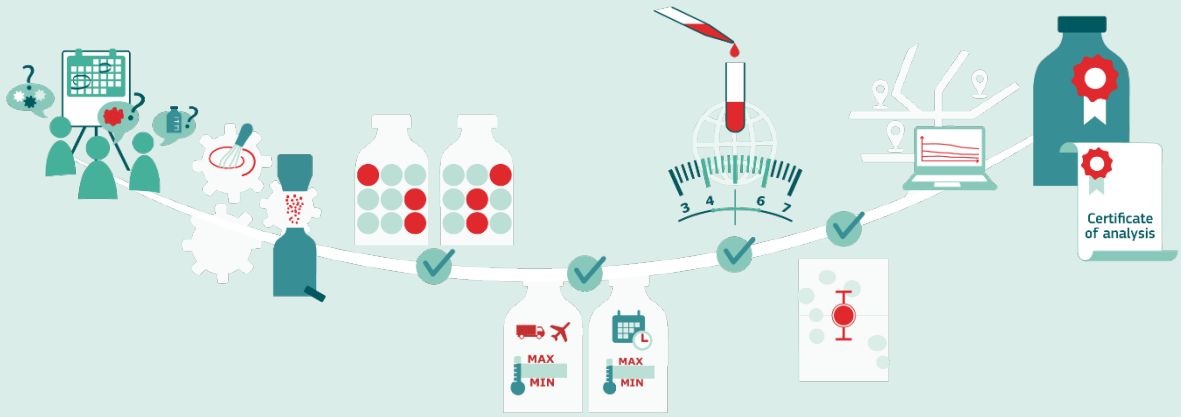
Ethanols obtained during fermentation of sugar cane, sugar beet and grape sugars were selected as base materials for ERM-AE200 in order to resemble the composition of the BCR-123 set of reference ethanols [8]. The different botanical origin provides characteristic natural deuterium distribution associated to ethanol molecule I covering a large range, i.e.

- Ethanol from sugar cane has a high (H) deuterium-to-hydrogen (D/H) amount-of-substance ratio
- Ethanol from grape has a medium (M) deuterium-to-hydrogen (D/H) amount-of-substance ratio
- Ethanol from sugar beet has a low (L) deuterium-to-hydrogen (D/H) amount-of-substance ratio

After further purification, each ethanol was separately mixed with pure tetramethylurea (TMU) (ERM-AE003) [12], which acts as an internal standard. Each mixture was completed by adding hexafluorobenzene (HFB), used as a field frequency stabilisation substance (lock) in ratios matching the protocol for sample preparation of alcohol samples for NMR measurements indicated in the standard method [10].

1.3. Outline of the CRM project

Box 1. Reference material production



Reference material (RM) production is defined in ISO 17034 [1] as a project comprising planning and processing of the material, followed by homogeneity and stability testing, characterisation and assigning of one or more property values. Depending on the intended use of the RM a commutability study is carried out.

For certified reference materials (CRMs) a certificate is issued while for RMs a product information sheet is issued by the reference material producer (RMP).

CRMs and RMs are distributed globally and the stability of their assigned values is monitored throughout the life-time of the material.

ERM-AE200 was produced to support the application of the OIV-MA-AS311-05 method and to replace the exhausted stock of material BCR-123.

The production of ERM-AE200 reproduces the main lines formerly followed for BCR-123 [8] with two particularities. First, the composition of the new certified solutions includes the recently produced TMU material (ERM-AE003) [12]. The TMU material has a certified (D/H) value and is used as an internal standard during the measurement procedure, providing the necessary traceability links for the new CRM. The second particular aspect involves the use of the BCR-123 stability data acquired over years of systematic monitoring. Besides confirming the long-lasting stability of the reference ethanols, these results served to predict the stability of ERM-AE200 over a period of one year and to estimate the uncertainty associated to it.

A set of three sealed NMR tubes composes one unit of ERM-AE200. The tubes containing ethanol from sugar cane (H), grape (M) or sugar beet (L) were processed at the EC-JRC Geel. Whenever required, base ethanols were further purified in-house before mixing with tetramethylurea and hexafluorobenzene. Analytical measurements necessary for quality control during processing, homogeneity and stability studies were performed at the laboratories of the EC-JRC Geel, holding the appropriate accreditation to ISO/IEC 17025.

ERM-AE200 was characterised in an inter-laboratory comparison. Laboratories with expertise in the field of wine analysis strictly applied the OIV method of analysis [10] for the determination of the site-specific deuterium to hydrogen (D/H) amount of substance ratios, $(D/H)_I$ and $(D/H)_{II}$, as well as the relative deuterium isotope ratio (R) for the three ethanols. The deuterium isotope abundance is expressed as the difference of the isotope ratios for ethanol and the isotope ratio of TMU and expressed as amount-of-substance ratio in mol/mol. R is dimensionless.

Uncertainties of certified and indicative values were estimated in compliance with ISO 17034 [1], which implements the basic principles of ISO/IEC Guide 98 (GUM) [13].

The CRM project, including the certification approach and the evaluation of the obtained measurement data, was subjected to peer-review involving both internal and external experts.

Certain commercial equipment, instruments, and materials are identified in this report to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the

European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

2 Participants

2.1 Project management and data evaluation

European Commission, Joint Research Centre, Directorate F – Health and Food, Geel, BE
(accredited to ISO 17034:2016 for production of certified reference materials, BELAC No. 268-RM).

2.2 Processing

European Commission, Joint Research Centre, Directorate F – Health and Food, Geel, BE.

2.3 Homogeneity measurements

European Commission, Joint Research Centre, Directorate F – Health and Food, Geel, BE
(measurements under the scope of ISO/IEC 17025:2017 accreditation BELAC nr 268-TEST).

2.4 Stability measurements

European Commission, Joint Research Centre, Directorate F – Health and Food, Geel, BE
(measurements under the scope of ISO/IEC 17025:2017 accreditation BELAC nr 268-TEST).

2.5 Characterisation measurements

European Commission, Joint Research Centre, Directorate F – Health and Food, Geel, BE
(measurements under the scope of ISO/IEC 17025:2017 accreditation BELAC nr 268-TEST).

Fondazione Edmund Mach, San Michele all'Adige, IT
(measurements under the scope of ISO/IEC 17025:2017 accreditation ACCREDIA nr 0193L)

General Directorate of Customs - Customs Technical Laboratory, Prague, CZ
(measurements under the scope of ISO/IEC 17025:2017 accreditation Český institute pro akreditaci nr 580/2019)

National Food Chain Safety Office Directorate of Oenology and Alcoholic Beverages, Budapest, HU
(measurements under the scope of ISO/IEC 17025:2017 accreditation NAH nr 1-1673/2019)

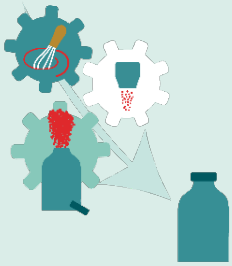
Service Commun des Laboratoires du Minefi, Montpellier, FR
(measurements under the scope of ISO/IEC 17025:2017 accreditation COFRAC nr 1-0154)

Service Commun des Laboratoires du Minefi, Pessac, FR
(measurements under the scope of ISO/IEC 17025:2017 accreditation COFRAC nr 1-0152)

All datasets are identified by a code (e.g. L01). The numbering is not related to the order of the laboratories presented above.

3 Material processing and processing control

Box 2. Reference material processing



RM processing covers the conversion of the raw material into a homogenous and stable material. It typically includes processing steps such as grinding or sieving and drying steps to enhance stability. When the processed material fulfils the specifications, it is filled into individual containers, such as bottles or ampoules, and labelled. These containers are referred to as RM units.

3.1 Origin/Purity of the starting material

Starting materials were ethanols distilled from three different botanical sources: grape, sugar cane and sugar beet. Ethanols from grape and sugar cane had alcoholic grades > 96 % vol., whereas ethanol from sugar beet had an alcoholic grade > 99,5 % vol. Grape and sugar beet ethanols were provided by Iberalcohol, Valdemoro, ES. The ethanol obtained from sugar cane was provided by Azucarera del Guadalfeo, S.A., Almería, ES.

On arrival, the three ethanols were tested for their identity by SNIF-NMR, and for their alcoholic strength by water content determination using Coulometric Karl Fischer Titration (C-KFT). The analyses were performed in house at JRC-Geel laboratories using accredited methods. The results (Table 1) confirmed site-specific deuterium distribution in ethanol characteristic of the three botanical species.

Table 1. SNIF-NMR and C-KFT results obtained in-house for ethanol starting materials on arrival to the EC-JRC

	Ethanol (sugar beet)	Ethanol (grape)	Ethanol (sugar cane)
$(D/H)_I$ [$x10^{-6}$ mol/mol]	93.17 ± 0.35	103.71 ± 0.39	110.69 ± 0.42
$(D/H)_{II}$ [$x10^{-6}$ mol/mol]	126.36 ± 0.53	131.07 ± 0.55	130.18 ± 0.55
R	2.713 ± 0.011	2.528 ± 0.011	2.352 ± 0.009
Water content [g/100g]	0.180 ± 0.004	5.83 ± 0.12	6.53 ± 0.13

Tetramethylurea (TMU) CRM ERM-AE003 was provided by the European Commission, Joint Research Centre, Geel, Belgium. Pure hexafluorobenzene (HFB) > 99.5 % NMR grade, was purchased from Aldrich (St. Louis, Missouri, USA) and Acros Organics™.

3.2 Processing

For the purpose of this report, the term 'unit of ERM-AE200´ refers to one set of three NMR tubes. One unit of ERM-AE200 is shown in Figure 1.

The CRM is prepared by mixing ethanol (from grape, sugar cane or sugar beet) with TMU and HFB. Prior to mixing, a preliminary step involved the dehydration of the ethanol base materials. Molecular sieves were employed to separate the remaining water from the ethanol and obtain a grade > 99 % vol. The solution containing the mixture of the three compounds was filled in NMR tubes and immediately flame-sealed (argon was purged into the tubes before and after filling of the solution).

Figure 1. ERM-AE200.

Purification (drying) of ethanol

The ethanol base materials obtained from external providers were further purified (dehydrated) to obtain alcoholic grades higher than 99 % vol.

The dehydration process which involved the use of molecular sieves type 3A, is briefly described as follows. The selected molecular sieves are zeolites with a pore size of 0.3 nm diameter, activated at 300 ºC overnight before use. Once cooled down to room temperature and put in contact with the ethanol, the dry molecular sieve adsorbed the water present in the media until saturation of the cavities. Sufficient contact time is necessary to complete the process, which takes typically 24 to 48 h. The next step was filtration through a 5 µm filter (Ahlstrom-Munksjö cellulose nitrate filter) followed by a second filtration step through a finer particle size filter (a 0.45 µm Ahlstrom-Munksjö cellulose acetate filter) separating all inert zeolite particles from the ethanol solution. The filtration was performed under-pressure with a VP100 Vaccubrand vacuum pump from VWR. After a first drying cycle, the molecular sieves could be reactivated in the oven at 300 ºC and the full process repeated until the target alcoholic grade values were reached.

Two cycles were necessary to obtain a water content of 0.05 % m/m in ethanol from grape and sugar beet. Ethanol from sugar cane, with a higher starting water content, required three full cycles to achieve a final value of 0.063 % m/m. The water content was obtained by C-KFT analysis of the ethanols.

Mixing of ethanol solution with TMU and HFB

For the preparation of the bulk mixtures, tetramethylurea (TMU) was added to each one of the three ethanols (H, M or L) followed by incorporation of hexafluorobenzene (HFB). Table 2 includes the composition for the three bulk solutions.

Table 2. Composition of bulk materials for ERM-AE200

Composition of bulk solutions	Ethanol[1)] [g]	TMU[2)] [g]	HFB[3)] [g]
ERM-AE200a (H; sugar cane)	522.36	260.30	50.07
ERM-AE200b (M; grape)	851.53	424.19	81.74
ERM-AE200c (L; sugar beet)	605.00	301.90	58.03

[1)] Ethanol density of 0.807 g/ml at 20 °C
[2)] TMU density 0.968 g/ml at 20 °C
[3)] HFB density 1.612 g/ml at 25 °C

The containers used for the mixtures were borosilicate glass NMR precision quartz tubes (Wilmad-LabGlass, Vineland, New Jersey, USA) of 10 mm outer diameter, thin wall, 8" length and 500 MHz. They were pre-cleaned according to the following procedure:

- Rinsing inside and outside with sulfuric acid 20 %
- Rinsing with Milli-Q water (two times) and drying in fume hood
- Rinsing inside and outside with acetone 99.9 % and drying at 50 °C for 3 hours (two times)

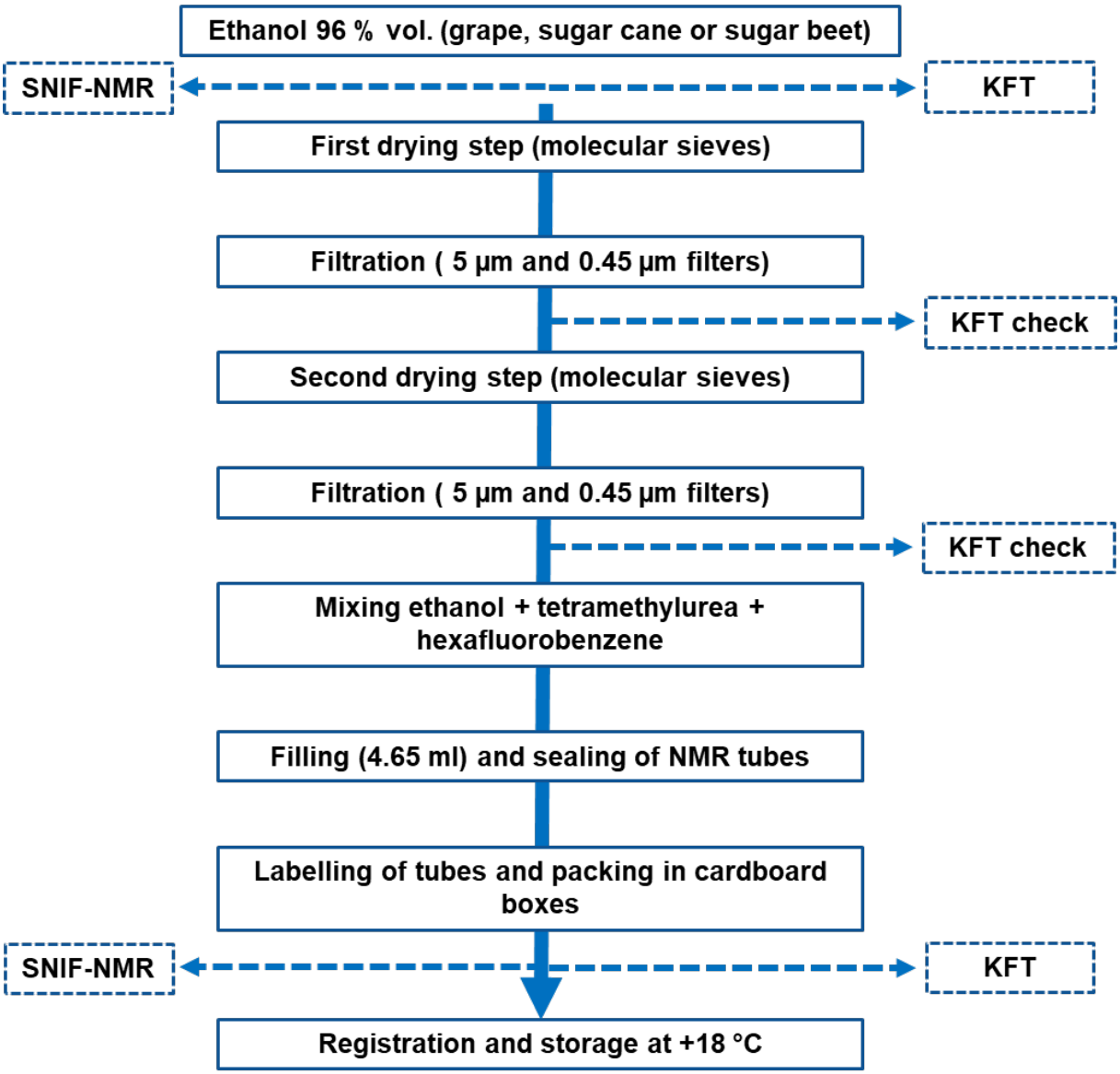
After cleaning, the tubes were recapped and kept in a fumehood until use a few days later.

The NMR tubes were pre-filled with argon (Ar) before filling an amount of 4.65 ml of the bulk solution at the Rota R910 ampouling machine (Wehr, Germany). Subsequently, the quartz tubes were flushed again with a gentle flow of argon, and flame sealed.

Labelling of the tubes was done manually. The three tubes with the same sample number (ERM-AE200a, b and c, corresponding to ethanols H, M and L respectively), were packed as a set in a cardboard box, and the box was labelled with the same set number as the tubes. All samples were stored at 18 °C except the reference samples that were placed at 4 °C.

Figure 2 illustrates the processing of the CRM as a flow chart.

Figure 2. Flow diagram, processing of ERM-AE200



Note that for ethanol from sugar cane, ERM-AE200a (H), a third drying cycle not shown above was applied.

3.3 Processing control

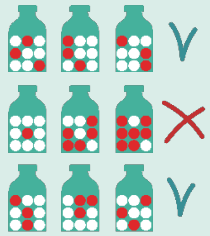
Water content determined by C-KFT and deuterium distribution determined by SNIF-NMR were measured at different stages of the CRM processing. The incoming ethanols were checked prior to further purification and C-KFT was applied during the intermediate ethanol drying cycles to test the efficiency of the different drying steps. Results obtained for the final check are shown in Table 3.

Table 3. Deuterium distribution in the ERM-AE200 bulk solutions containing TMU, HFB and one of the ethanols (H; sugar cane, M; grape or L; sugar beet). Water content values correspond to the C-KFT values obtained for the purified ethanols only.

	Ethanol (H), TMU and HFB	Ethanol (M), TMU and HFB	Ethanol (L), TMU and HFB
$(D/H)_I$ [x10^{-6} mol/mol]	110.42 ± 0.32	103.26 ± 0.56	92.54 ± 0.23
$(D/H)_{II}$ [x10^{-6} mol/mol]	129.65 ± 0.44	130.55 ± 1.10	125.49 ± 0.36
Water content ethanol only [g/100g]	0.063 ± 0.001	0.050 ± 0.007	0.040 ± 0.001

4 Homogeneity

Box 3. Homogeneity assessment



A key requirement for any RM produced as a batch of units is equivalence between those units. It is important to know how much the variation between units contributes to the uncertainty of the certified value. Consequently, ISO 17034 [1] requires RMPs to quantify the between-unit variation in homogeneity studies.

The within-unit homogeneity is correlated to the minimum sample size, which is the minimum amount of sample that is, for a given measurand, representative of the whole unit and that should be used in an analysis. Using sample intakes equal to or above the minimum sample size guarantees the assigned value within its stated uncertainty.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample size is respected, but determines the minimum size of sample that is representative for the whole unit. ERM-AE200 is a solution provided in sealed tubes and intended for measurement with a non-destructive technique directly on the sealed tubes. Therefore the minimum sample size is one tube.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all units of the material, within the stated uncertainties.

The number of units selected corresponds to approximately the cube root of the total number of units produced. Ten units were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. Random stratified sampling involves dividing the batch into ten groups (with a similar number of units in each group) and selecting one unit randomly from each group. Two independent analyses were performed from each selected unit by SNIF-NMR [10]. The measurements were performed under repeatability conditions, and in a randomised manner to separate a potential drift in the measurement results from a potential trend in the filling sequence. The results are shown as graphs in Annex 1.

Regression analyses were performed to evaluate potential trends in the measurement sequence as well as trends in the filling sequence. No trends in the filling sequence or the measurement sequence were observed at a 95 % confidence level.

The dataset was assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the unit means. One outlying individual result and outlying unit mean were detected for measurands $(D/H)_I$ and $(D/H)_{II}$ in ERM-AE200c (L). They all correspond to the same NMR tube (no. 116). No technical reason for the outliers could be found. However, the particularity of this material that allows repeated use by the non-destructive technique SNIF-NMR, made it possible to screen the complete set of ERM-AE200 units processed (data not shown). For the screening measurements, the NMR method was slightly adapted to shorten the measurement time per sample while providing sufficient repeatability of the results. The purpose was to identify any other potential outlying CRM units. The additional quality control test confirmed the values for unit no. 116 and did not flag any other outlier cases within the full batch processed. Unit no. 116 was then withdrawn from the material batch. Moreover, the analytical data obtained for unit no. 116 were excluded from the statistical evaluation, and consequently, from the estimation of the uncertainty contribution of inhomogeneity to the total uncertainty budget. Once this unit was removed, the homogeneity assessment was based on results from the remaining nine ERM-AE200 units originally analysed strictly following the SNIF NMR standard method [10].

Quantification of between-unit inhomogeneity was undertaken by analysis of variance (ANOVA), which separates the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples were representative for the whole unit.

Evaluation by ANOVA requires mean values per unit, which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per unit was visually tested using histograms and normal probability plots. Too few data are available for the unit means to make a clear statement of the distribution. Therefore, it was checked visually whether all individual data follow a unimodal distribution using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are given in Table 4.

Table 4. Results of the statistical evaluation of the homogeneity studies

Measurand	Trends (before correction) [1)]		Outliers [2)]		Distribution	
	Measurement sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
ERM-AE200a Ethanol (H; sugar cane)						
$(D/H)_I$	no	no	none	none	normal	normal
$(D/H)_{II}$	no	no	none	none	unimodal	normal
R	no	no	none	none	normal	normal
ERM-AE200b Ethanol (M; grape)						
$(D/H)_I$	no	no	none	none	normal	normal
$(D/H)_{II}$	no	no	none	none	unimodal	normal
R	no	no	none	none	unimodal	unimodal
ERM-AE200c Ethanol (L; sugar beet)						
$(D/H)_I$	no	no	1 (removed)[3)]	1(removed)[4)]	unimodal	normal
$(D/H)_{II}$	no	no	1 (removed)[3)]	1(removed)[4)]	normal	normal
R	no	no	none	none	normal	normal

[1)] 95 % confidence level
[2)] 99 % confidence level
[3)] Corresponds to one replicate result for ERM-AE200 unit no. 116. When removed, a new outlier is statistically detected, corresponding to the second replicate result obtained for unit no. 116
[4)] Corresponds to ERM-AE200 unit no. 116, which was physically removed before the estimation of u_{bb}

It should be noted that $s_{bb,\,rel}$ (Equation 2) and $s_{wb,\,rel}$ (Equation 1) are estimates of the standard deviations and are therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in a negative number under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be less than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [14]. u^*_{bb} is comparable to the limit of detection (LOD) of a measurement method yielding the maximum degree of inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,\,rel}$) (equivalent to the within-unit standard deviation), between–unit standard deviation ($s_{bb,\,rel}$) and $u^*_{bb,\,rel}$ were calculated as:

$$s_{wb,\,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}}$$ **Equation 1**

$$s_{bb,\,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}}$$ **Equation 2**

$$u^{*}_{bb,\,rel} = \frac{\sqrt{\frac{MS_{within}}{n}} \cdot 4\sqrt{\frac{2}{\nu_{MS_{within}}}}}{\bar{y}}$$ **Equation 3**

MS_{within} mean of squares within-unit from ANOVA
$MS_{between}$ mean of squares between-unit from ANOVA
$\bar{y}$ mean of all results of the homogeneity study
n mean number of replicate analysis per unit
$\nu_{MSwithin}$ degrees of freedom of MS_{within}

Table 5. Results of the homogeneity study

Measurand	$s_{wb,\,rel}$ [%]	$s_{bb,\,rel}$ [%]	$u^{*}_{bb,\,rel}$ [%]	$u_{bb,\,rel}$ [%]
ERM-AE200a Ethanol (H; sugar cane)				
$(D/H)_I$	0.154	n.c.	0.073	0.073
$(D/H)_{II}$	0.196	n.c.	0.093	0.093
R	0.161	n.c.	0.076	0.076
ERM-AE200b Ethanol (M; grape)				
$(D/H)_I$	0.155	n.c.	0.073	0.073
$(D/H)_{II}$	0.148	n.c	0.070	0.070
R	0.161	n.c.	0.076	0.076
ERM-AE200c Ethanol (L; sugar beet)				
$(D/H)_I$	0.151	0.052	0.073	0.073
$(D/H)_{II}$	0.167	n.c.	0.081	0.081
R	0.069	0.075	0.033	0.075

[1)] n.c.: cannot be calculated as $MS_{between} < MS_{within}$.

Except for the one case explained above, the homogeneity studies showed no outlying unit means or trends in the filling sequence. Therefore, the between-unit standard deviation can be used as an estimate of u_{bb}. As u^{*}_{bb}

sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

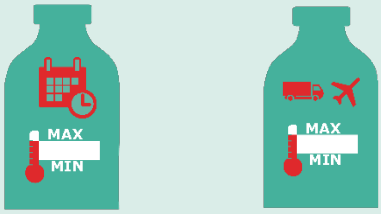
4.2 Within-unit homogeneity and minimum sample size

The within-unit homogeneity is correlated to the minimum sample size. If the sample size is too small, individual samples of a unit might not contain the same amount of analyte. The minimum sample size is the minimum amount of sample that is, for a given measurand, representative of the whole unit and thus should be used in an analysis. Using sample intakes equal to or above the minimum sample size guarantees the certified value within its stated uncertainty.

The certified material is a solution and is not expected to have any relevant within-unit inhomogeneity. When analysed by SNIF-NMR, an entire sealed tube is used.

5 Stability

Box 4. Stability assessment



Stability testing is necessary to establish the conditions for storage as well as the transport conditions of the RMs to the customers. During transport, especially in summer, temperatures up to 60 °C can be reached, and stability under these conditions must be demonstrated if the RMs are to be transported without any additional cooling.

Time, temperature and light (including ultraviolet radiation) were regarded as the most relevant influences on the stability of the materials. Materials are stored in the dark and transported in boxes, thus removing any possibility of degradation by light. Therefore, only the influences of time and temperature needed to be investigated.

To set the transport conditions, stability studies were carried out using an isochronous design [15]. In this approach, units were stored for a defined duration under different temperature conditions. Afterwards, the units were moved to conditions where further degradation can be assumed negligible (reference conditions). At the end of the isochronous storage, the samples were analysed simultaneously under repeatability/intermediate precision conditions. Analysis of the material (after various exposure times and temperatures) under repeatability precision conditions greatly improves the sensitivity of the stability tests.

ERM-AE200 consists of sealed NMR tubes containing solutions with a composition equivalent to that of BCR-123 material. The latter was produced in 2002 and monitored for stability for 18 years. Results from the regular BCR-123 stability monitoring confirm the validity for the certified parameters within the established uncertainties when stored at room temperature conditions (results shown in Annex 2). Those studies served as solid basis to expect an equivalent degree of stability for ERM-AE200 when stored under the same conditions. Consequently, a dedicated storage stability study was not required as part of the production for this material.

5.1 Transport stability

The conditions for the transport of the material to the customers were established in a short-term stability study. To this end, units were stored at 60 °C for 0, 1, 2 and 3 weeks. The reference temperature was set to 4 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, samples were measured twice by the SNIF-NMR standard method [10]. The measurements were performed under repeatability conditions, and a randomised sequence was used to differentiate any potential drift in the measurement results from a potential trend over storage time. The data were evaluated individually for each temperature.

The results were screened for outliers using the single and double Grubbs test with a confidence level of 99 %.

In addition, the data were evaluated against storage time, and regression lines of the target parameters *versus* time were calculated, to test for potential increases or decreases of the measurands due to shipping conditions. The slopes of the regression lines were tested for statistical significance.

The results of the measurements are shown in Annex 3. The results of the statistical evaluation of the short-term stability are summarised in Table 6.

Table 6. Results of the short-term stability tests.

Measurand	Number of individual outlying results [1)]	Significance of the trend [2)]
	60 °C	60 °C
ERM-AE200a Ethanol (H; sugar cane)		
$(D/H)_I$	none	no
$(D/H)_{II}$	none	no
R	none	no
ERM-AE200b Ethanol (M; grape)		
$(D/H)_I$	none	no
$(D/H)_{II}$	none	no
R	none	no
ERM-AE200c Ethanol (L; sugar beet)		
$(D/H)_I$	none	no
$(D/H)_{II}$	none	no
R	none	no

[1)] 99 % confidence level.
[2)] 95 % confidence level.

No statistical outliers were detected for the measurands. None of the trends was statistically significant at a 95 % confidence level for the temperature tested.

The material can be dispatched without further temperature precautions under ambient conditions.

5.2 Storage stability

Long-term storage conditions and shelf life guaranteeing the stability of the material and the certified values were established.

Data from the post-certification stability monitoring programme for the analogous CRM BCR-123 are available. The previously released CRM was analysed for the site-specific deuterium distributions on two occasions over a period of nearly 18 years. At each time point, measurements were performed on units stored at normal storage temperature (18 °C) and at a reference temperature (4 °C). Each of these studies can be viewed as a two-point stability study. The evaluation was based on the ratio of samples stored at 18 °C and 4 °C after 18 years of storage and the results, which confirm the stability of the certified parameters at 18 °C, are presented in Annex 2.

To additionally verify that the data obtained from stability monitoring of a similar CRM produced and stored in the same way could be used to estimate the stability uncertainty contribution for ERM-AE200, the data of the short-term stability study were compared to the stability monitoring data. The data of the transport stability study at 60 °C for ERM-AE200 supports the assumption that EMR-AE200 is as stable as BCR-123. Therefore uncertainty estimations based on stability monitoring data can be used to estimate the uncertainty contribution relating to the storage of the CRM. The estimation was based on the analysis of the slopes produced by stability monitoring data obtained at three time points spread over the 18 years period. No significant slopes at 95 % confidence level were detected for any of the measurands apart from $(D/H)_{II}$ in

ethanol (L). Despite being small, the slope was considered as a potential instability and therefore the degradation is accounted as a contribution to the uncertainty of the stability during storage.

The storage temperature set for ERM-AE200 is 18 °C.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can entirely rule out degradation of materials, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be that there is no detectable degradation within an uncertainty to be estimated.

The uncertainties of stability during transport and storage were estimated, as described in [16] for each measurand. In this approach, the uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,\,rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt} \qquad \textbf{Equation 4}$$

$$u_{lts,\,rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl} \qquad \textbf{Equation 5}$$

s_{rel} relative standard deviation of all results of the stability study
t_i time elapsed at time point *i*
$\bar{t}$ mean of all t_i
t_{tt} chosen transport time (1 week at 60 °C)
t_{sl} chosen shelf life (3 years at 18 °C)

For measurand $(D/H)_{II}$ in ethanol (L) the u_{lts} is increased by an additional uncertainty contribution (u_{deg}) to cover for the small potential degradation associated to a significant trend detected for this measurand:

$$u_{lts,\ rel} = \left(\sqrt{u_{deg}{}^2 + u_b{}^2}\right) \cdot t_{st} \qquad \textbf{Equation 6}$$

u_{deg} uncertainty contribution associated to the significant slope, estimated as $b/\sqrt{3}$

b slope of the regression line

u_b standard error of the slope of the regression line

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of stability during transport. This was estimated from the 60 °C study. The uncertainty describes the possible change during a transport at 60 °C lasting for one week.
- $u_{lts,rel}$, the uncertainty of stability during storage. This uncertainty contribution was estimated from the application of Equation 4 to data from the stability monitoring programme for CRM BCR-123. Exceptionally for measurand $(D/H)_{II}$ in ethanol (L), a degradation contribution (u_{deg}) is

additionally included in the uncertainty estimation according to Equation 6. The uncertainty contribution describes the possible degradation during storage for 3 years at 18 °C.

The results of these evaluations are summarised in Table 7.

Table 7. Uncertainties of stability during transport and storage. $u_{sts,rel}$ was calculated for a temperature of 60 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of BCR-123 at 18 °C for 3 years.

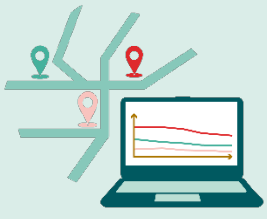
Measurand	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
ERM-AE200a Ethanol (H; sugar cane)		
$(D/H)_I$	0.030	0.010
$(D/H)_{II}$	0.032	0.018
R	0.023	0.024
ERM-AE200b Ethanol (M; grape)		
$(D/H)_I$	0.031	0.013
$(D/H)_{II}$	0.030	0.019
R	0.027	0.021
ERM-AE200c Ethanol (L; sugar beet)		
$(D/H)_I$	0.023	0.023
$(D/H)_{II}$	0.036	0.225*
R	0.032	0.029

*Based on the combination of results of studies performed at different time points. A small positive significant trend is detected, and, as a conservative approach, this degradation (u_{deg}) is included in the estimation of u_{lts}.

The outcome of the different stability studies show that no significant degradation is observed at the conditions tested, even at high temperatures (60 °C). For this reason the material can be shipped at ambient temperature conditions.

The material is included in the JRC's regular stability monitoring programme, to control its further stability.

Box 5. Stability monitoring



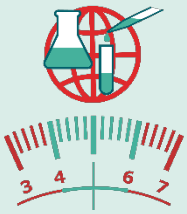
RMs are produced as batches that should last for ten years or longer. This long lifetime means that a storage stability study of limited duration cannot provide a definite “use by” date for the material. It therefore needs to be complemented by stability monitoring throughout the lifetime of the RM.

Therefore, the stability of RMs whose assigned values might change is regularly monitored. The monitoring frequency depends on the outcome of the storage stability assessment.

If the tests confirm the stability of the assigned values, the material remains on sale. If not, possible actions include the retraction of the value in question, retraction of the complete material or a change of the certified value. Customers are notified if the change is larger than the uncertainty of the assigned value.

6 Characterisation

Box 6. Reference material characterisation



Material characterisation is the process of determining the property value(s) of a RM. While ISO 17034 [1] allows to characterise a RM in various ways, quality management procedures of the JRC are more stringent and allow characterisation only by either interlaboratory comparison or the use of a primary method confirmed by independent analysis.

The material characterisation was based on an interlaboratory comparison, i.e. the properties of the material were determined in different laboratories with documented expertise. Due to the nature of the measurands and the requirements from the certification campaign, all participants used the same method for the measurements. This approach converts the systematic bias of each laboratory into a random variable, the combined effect of which is reduced by averaging over several laboratories.

6.1 Selection of participants

Six laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participating laboratory was required to operate a quality system and to deliver documented evidence of its proficiency in the field of site-specific deuterium distribution measurements of ethanol, by submitting results for interlaboratory comparison exercises or method validation reports. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 [3] was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.2 Study setup

Laboratories received two units of ERM-AE200 and were requested to provide six independent results for each dataset, three per unit. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample measurements had to be done on at least two days (or independent analytical sequences) to ensure intermediate precision conditions, i.e. each material unit analysed in triplicate in one analytical sequence.

Each participant additionally measured a sample of BCR-123 as a quality control (QC) sample. The results for this sample were used to support the evaluation of the characterisation results.

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the six results. No approach for the estimation was prescribed, i.e. top-down and bottom-up [13] were regarded as equally valid procedures.

6.3 Methods/Measurement procedures used

All laboratories used the same measurement procedure for the determination of site-specific deuterium distribution of ethanol.

Laboratories did not deviate from the measurement procedure described in OIV-MA-AS311-05 [10].

The ERM-AE200 sealed tubes containing a mixture of ethanol, TMU and HFB, were measured directly by SNIF-NMR. The site-specific deuterium isotope ratios were calculated according to the expressions below.

$$(D/H)_I = 1.5866 \cdot \frac{n_{TMU}}{n_A} \cdot \frac{(D/H)_{TMU}}{\quad} \qquad \textbf{Equation 7}$$

$$(D/H)_{II} = 2.3799 \cdot T_{II} \frac{m_{TMU}}{m_A} \cdot \frac{(D/H)_{TMU}}{t_m^D}$$ **Equation 8**

Where

$(D/H)_I$ site specific deuterium-to-hydrogen (D/H) amount of substance ratio of ethanol (CH_2DCH_2OH, molecule I)

$(D/H)_{II}$ site specific deuterium-to-hydrogen (D/H) amount of substance ratio of ethanol (CH_3CHDOH, molecule II)

T_I height of signal I (CH_2DCH_2OH) divided by height of TMU signal, used as internal standard ($T_I = h_I/h_{TMU}$)

T_{II} height of signal II (CH_3CHDOH) divided by height of TMU signal, used as internal standard ($T_{II} = h_{II}/h_{TMU}$)

m_{TMU} mass of TMU to nearest 0.1 mg

m_A mass of ethanol to nearest 0.1 mg

$(D/H)_{TMU}$ certified value of TMU [12]

t_m^D alcoholic strength mass fraction in % (m/m), obtained by

$$t_m^D = \frac{m-m'}{m} \cdot 100$$ **Equation 9**

Where

m mass of ethanol solution

m' mass of water in the ethanol solution as determined by KFT

$$S = \frac{3I_{II}}{I_I}$$ **Equation 10**

Where

h_I height of signal I (CH_2DCH_2OH)

h_{II} height of signal II (CH_3CHDOH)

Details on the preparation of the solutions, such as masses of ethanol, mass of water in ethanol determined by C-KFT and mass of TMU, were provided to the laboratories.

6.4 Evaluation of results

The characterisation study resulted in seven datasets per measurand. One laboratory reported two sets of results obtained with different NMR instruments. These are considered as independent datasets for further evaluation. All individual datasets of the participating laboratories, grouped per measurand, are displayed in tabular and graphical form in Annex 4.

6.4.1 Technical evaluation

The obtained data were first checked for compliance with the requested instructions and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- compliance with the instructions given: sample measurements performed on two analytical runs.
- method performance, i.e. agreement of the measurement results with the assigned value of the QC sample

Based on the above criteria, all datasets were accepted on technical grounds for further statistical evaluation.

6.4.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 8.

Table 8. Statistical evaluation of the technically accepted datasets for ERM-AE200. *p*: number of technically valid datasets.

Measurand	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean	*s*	$s_{between}$	s_{within}
ERM-AE200a Ethanol (H; sugar cane)								
$(D/H)_I$ [x10^{-6} mol/mol]	7	0	0	yes	110.468	0.182	0.172	0.148
$(D/H)_{II}$ [x10^{-6} mol/mol]	7	0	0	yes	129.900	0.201	0.185	0.193
R	7	0	0	yes	2.352	0.002	0.002	0.004
ERM-AE200b Ethanol (M; grape)								
$(D/H)_I$ [x10^{-6} mol/mol]	7	0	1	yes	103.326	0.168	0.162	0.108
$(D/H)_{II}$ [x10^{-6} mol/mol]	7	0	0	yes	130.520	0.177	0.164	0.163
R	7	0	0	yes	2.526	0.003	0.003	0.003
ERM-AE200c Ethanol (L; sugar beet)								
$(D/H)_I$ [x10^{-6} mol/mol]	7	0	0	yes	92.597	0.156	0.148	0.123
$(D/H)_{II}$ [x10^{-6} mol/mol]	7	0	0	yes	125.624	0.178	0.166	0.156
R	7	0	5	yes	2.714	0.003	0.002	0.005

The laboratory means follow normal distributions. None of the data contains outlying means. The datasets are therefore consistent and the mean of laboratory means is a good estimate of the true value. Standard deviations between laboratories are comparable to the standard deviation within laboratories, showing that confidence intervals of replicate measurement results can be suitable as estimates of measurement uncertainty.

The statistical evaluation flags laboratory L01 as outlying variance for measurand $(D/H)_I$ in ethanol (M) and five outliers for *R* in ethanol (L). As all laboratories used the SNIF-NMR method from [10], this indicates that laboratory L01's proficiency in applying the measurement procedure is significantly different from that of the other laboratories, and still technically acceptable, when considering the magnitude of variability of the overall values. The dataset of laboratory L01 was therefore retained.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means ($s/\sqrt{p}$) (Table 9).

Table 9. Uncertainty of characterisation for ERM-AE200.

Measurand	*p*	Mean	*s*	u_{char}
ERM-AE200a Ethanol (H; sugar cane)				
$(D/H)_I$ [x10^{-6} mol/mol]	7	110.468	0.182	0.069
$(D/H)_{II}$ [x10^{-6} mol/mol]	7	129.900	0.201	0.076
R	7	2.352	0.002	0.0009
ERM-AE200b Ethanol (M; grape)				
$(D/H)_I$ [x10^{-6} mol/mol]	7	103.326	0.168	0.063
$(D/H)_{II}$ [x10^{-6} mol/mol]	7	130.520	0.177	0.067
R	7	2.526	0.003	0.0011
ERM-AE200c Ethanol (L; sugar beet)				
$(D/H)_I$ [x10^{-6} mol/mol]	7	92.597	0.156	0.059
$(D/H)_{II}$ [x10^{-6} mol/mol]	7	125.624	0.178	0.067
R	7	2.714	0.003	0.0010

7 Value Assignment

Box 7. Assignment of values to a reference material

Based on the outcome of characterisation measurements three types of values can be assigned, namely certified, indicative or additional material information values.

Certified values are values that fulfil the highest standards of accuracy. Procedures at JRC Directorate F require a sufficient number of datasets to assign certified values. Full uncertainty budgets in accordance with ISO 17034 [1] and ISO Guide 35 [2] are required. Certified values of a CRM can be used for calibration and trueness controls.

Indicative values are values where either the uncertainty is deemed too large or too few independent datasets are available to allow certification. Indicative values of an RM can be used for statistical quality control (homogeneity and stability has been assessed) but not for calibration, demonstration of method or laboratory proficiency or method trueness.

Additional material information values are values for which homogeneity and stability have usually not been assessed and insufficient data for characterisation are available. Consequently, an estimate of the reliability of the values is not possible and no uncertainty is given. Additional material information values cannot be used for calibration, demonstration of method or laboratory proficiency or method trueness. They can be used to e.g. anticipate possible interferences in measurement processes.

Only certified values were assigned for this material.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 10 was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties relating to characterisation (u_{char}), potential between-unit inhomogeneity (u_{bb}), and potential degradation during transport (u_{sts}) and long-term storage (u_{lts}). The uncertainty related to degradation during long-term storage was found negligible, nonetheless it is included in the calculations. The uncertainty contribution for weighing related to sample preparation is negligible. These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM,\,rel}$) with a coverage factor k given as:

$$U_{CRM,\,rel} = k \cdot \sqrt{u^2_{bb,\,rel} + u^2_{sts,\,rel} + u^2_{lts,\,rel} + u^2_{char,\,rel}} \qquad \textbf{Equation 11}$$

- u_{char} was estimated as described in Section 6.4.2
- u_{bb} was estimated as described in Section 4.1
- u_{sts} and u_{lts} were estimated as described in Section 5.3

Following JRC's procedures for assigning uncertainties to certified values, a coverage (k) factor of 2 can be chosen if the main uncertainty component has at least five degrees of freedom. As can be seen in Table 10, the $u_{bb,rel}$ is the dominant contribution to the combined uncertainty and it has eight degrees of freedom. Therefore, a k-factor of 2 was applied to obtain the expanded uncertainties.

The certified values and their uncertainties are summarised in Table 10.

Table 10. Certified values and their uncertainties for ERM-AE200

Certified property	Certified value	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [1)]
ERM-AE200a Ethanol (H; sugar cane)							
$(D/H)_I$ [x10^{-6} mol/mol]	110.47	0.062	0.073	0.030	0.010	0.202	0.23
$(D/H)_{II}$ [x10^{-6} mol/mol]	129.9	0.059	0.093	0.032	0.018	0.232	0.3
R	2.352	0.037	0.076	0.023	0.024	0.182	0.005
ERM-AE200b Ethanol (M; grape)							
$(D/H)_I$ [x10^{-6} mol/mol]	103.33	0.061	0.073	0.031	0.013	0.202	0.21
$(D/H)_{II}$ [x10^{-6} mol/mol]	130.52	0.051	0.07	0.030	0.019	0.187	0.25
R	2.526	0.044	0.076	0.027	0.021	0.189	0.005
ERM-AE200c Ethanol (L; sugar beet)							
$(D/H)_I$ [x10^{-6} mol/mol]	92.60	0.064	0.073	0.023	0.023	0.204	0.19
$(D/H)_{II}$ [x10^{-6} mol/mol]	125.6	0.053	0.081	0.036	0.225	0.495	0.7
R	2.714	0.036	0.075	0.032	0.029	0.188	0.006

[1)] Expanded (k = 2) and rounded uncertainty; uncertainties are always rounded up [17] and in a way that the rounding error corresponds to 3 % to 30 % of the uncertainty.

8 Metrological traceability and commutability

8.1 Metrological traceability

Box 8. Metrological traceability

Metrological traceability of measurement results is a key requirement for ensuring the comparability of data. As CRMs are used to make measurement results traceable, metrological traceability of their certified values to a stated reference is essential.

The certified value of a CRM is metrologically traceable if the measurements used for establishing it can be related to a reference through an unbroken chain of calibrations.

This requires that these measurements

- refer to the same property (e.g. Pb) and the same (kind of) quantity (e.g. Pb content),
- result in a number and its uncertainty (e.g. 6 ± 2) expressed in the same measurement unit (e.g. µg/kg).

The concept of traceability rests on several anchor points, namely identity, quantity value and measurement unit. The identity of a measurand can be defined by its structure alone or can be operationally defined, the quantity value of the measurand can refer to the SI, or to other appropriate references.

8.1.1 Identity

Site-specific deuterium isotope ratios $(D/H)_I$ and $(D/H)_{II}$, as well as the relative deuterium isotope ratio R, are operationally defined measurands and can only be obtained by following the measurement procedure specified in the standard method described in OIV-MA-AS311-05, *Determination of the deuterium distribution in ethanol derived from fermentation of grape musts, concentrated grape musts, grape sugar (rectified concentrated grape musts) and wines by application of nuclear magnetic resonance (SNIF-NMR/RMN-FINS)* [10]. Adherence to this measurement procedure was confirmed by agreement of the laboratories' results with the assigned value for the CRM that was used as a quality control sample. The measurands are therefore operationally defined by the SNIF-NMR method.

8.1.2 Quantity value

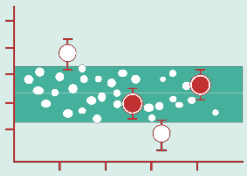
Traceability of the obtained results is based on the traceability of all relevant input factors. Investigation of the method and measurement details of the individual results show that all relevant input parameters of each technically accepted dataset have been properly calibrated. All laboratories used TMU (ERM-AE003), included in the mixture, as internal working standard. The TMU is a CRM whose value for the site-specific deuterium-to-hydrogen (D/H) amount of substance ratio is traceable to the value of VSMOW (Vienna Standard Mean Ocean Water). That makes the individual results traceable to the internationally accepted scale of VSMOW through an unbroken chain of reference values obtained by SNIF-NMR. All technically accepted datasets are therefore traceable to the same reference, namely the value of VSMOW, 155.76×10^{-6} for the (D/H) amount-of-substance ratio [18]. This traceability to the same reference is also confirmed by the agreement of results within their respective uncertainties. As the assigned values are combinations of agreeing results individually traceable to VSMOV, the assigned quantity values themselves are also traceable to the value of VSMOW.

8.2 Commutability

Box 9. Commutability

Commutability is a prerequisite for RMs intended to be used for calibration or quality control of different measurement procedures targeting the same measurand. The concept of commutability of a RM is defined by the VIM [12] as:

"*property of a reference material, demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specified materials*"



Commutability is a property of an RM indicating how well an RM mimics the characteristics of a typical routine sample in various measurement procedures for a stated measurand.

The same RM may be commutable for some measurement procedures but non-commutable for others. A commutability statement is therefore only valid for the mentioned measurement procedure(s).

The certified value of this CRM is operationally defined and can only be obtained when tested in accordance with the method specified. Therefore, commutability of this reference material has not been assessed.

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

Hazard statements

H225 Highly flammable liquid or vapour.

H302 Harmful if swallowed.

H361 Suspected of damaging fertility or the unborn child.

Precautionary statement

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P233 Keep container tightly closed.

P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor if you feel unwell.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.

P403 + P235 Store in a well-ventilated place. Keep cool.

P405 Store locked up.

9.2 Storage conditions

The material should be stored at (18 ± 5) °C in the dark.

For more information regarding the shelf life of reference materials please consult ERM Application Note 7 [19].

Note that the European Commission cannot be held responsible for changes that may happen to samples after opening or when the material is stored differently from the stated storage conditions at the customer's premises.

9.3 Use of the material

The material is ready for use. No sample preparation step is required; the sealed tubes can be inserted in the NMR instrument and can be measured directly. The results are used for performance verification of the NMR spectrometers. It is recommended to routinely run the spectra of two of the reference ethanols which have $(D/H)_I$ values at the two ends of the range of isotopic ratios usually determined in the laboratory. Weekly calibrations are appropriate to detect any potential drift in the overall stability of the spectrometer.

For general information on handling of reference materials, please consult ERM Application Note 6 [20].

9.4 Minimum sample size

The minimum sample size is the full sealed NMR tube.

9.5 Use of the certified values

The intended use of this material is the standardisation (performance verification) of NMR spectrometers in SNIF-NMR measurements. As with any reference material, it can be used for establishing control charts.

Comparing a measurement result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1 [21]).

When assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is summarised here:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine the measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty (u_{Δ}) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %.
- If $\Delta_{meas} \leq U_{\Delta}$ then no significant difference exists between the measurement result and the certified value, at a confidence level of approximately 95 %.

Use in quality control charts

The material(s) can be used for quality control charts. Using CRMs for quality control charts has the added value that a trueness assessment is built into the chart.

10 Conclusions

ERM-AE200 is a set of three ethanol materials certified for the deuterium distribution content. This material was produced and certified in accordance with ISO 17034:2016 [1] and ISO Guide 35:2017 [2]. ERM-AE200 was produced within the scope of ISO 17034 accreditation.

The CRM supports the application of the method for detecting enrichment of grape musts and wines by application of nuclear magnetic resonance of deuterium (D-NMR or SNIF-NMR), described in [10].

The following values were assigned:

Table 11 Values assigned to ERM-AE200.

Deuterium distribution		
	Certified value [2)]	Uncertainty [3)]
ERM-AE200a Ethanol (H; sugar cane)		
$(D/H)_I$ [$x10^{-6}$ mol/mol][1)]	110.47	0.23
$(D/H)_{II}$ [$x10^{-6}$ mol/mol][1)]	129.9	0.3
R[1)]	2.352	0.005
ERM-AE200b Ethanol (M; grape)		
$(D/H)_I$ [$x10^{-6}$ mol/mol][1)]	103.33	0.21
$(D/H)_{II}$ [$x10^{-6}$ mol/mol][1)]	130.52	0.25
R[1)]	2.526	0.005
ERM-AE200c Ethanol (L; sugar beet)		
$(D/H)_I$ [$x10^{-6}$ mol/mol][1)]	92.60	0.19
$(D/H)_{II}$ [$x10^{-6}$ mol/mol][1)]	125.62	0. 7
R[1)]	2.714	0.006

[1)] As defined by the procedure according to the OIV-MA-AS311-05 method [10], by application of site-specific natural isotope fractionation nuclear magnetic resonance of deuterium (SNIF-NMR). H, M and L refer to high, medium and low deuterium-to-hydrogen (D/H) amount-of-substance ratio $(D/H)_I$ that correspond to sugar cane ethanol, ethanol from grapes, and ethanol from sugar beet, respectively.

[2)] Certified values are values that fulfil the highest standards of accuracy. The given values represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and with the SNIF-NMR method defined in OIV-MA-AS311-05. The certified values and their uncertainties are traceable to the value of VSMOV (Vienna Standard Mean Ocean Water) via the use of TMU CRM ERM-AE003 where an absolute (D/H) amount-of-substance ratio value of $(D/H)_{VSMOW}$ =155.76 $x10^{-6}$ was used [18]. $(D/H)_I$ and $(D/H)_{II}$ are expressed as amount-of-substance ratio in [mol/mol]. R is dimensionless.

[3)] The uncertainty of the certified value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of 95 %, estimated in accordance with ISO 17034:2016 and ISO Guide 35:2017. $(D/H)_I$ and $(D/H)_{II}$ are expressed as amount-of-substance in [mol/mol]. R is dimensionless.

The material is intended for the performance verification of NMR spectrometers in SNIF-NMR measurements. As with any reference material, it can be used for establishing control charts.

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List of abbreviations

ANOVA	Analysis of variance
b	Slope in the equation of linear regression $y = a + bx$
BCR®	Trademark owned by the European Commission; used by the JRC for reference materials
CI	Confidence interval
C-KFT	Coulometric Karl Fischer titration
CRM	Certified reference material
D	Deuterium (^{2}H)
EC	European Commission
EN	European norm (standard)
ERC-CWS	European Reference Centre for Control in the Wine Sector
ERM®	Trademark owned by the European Commission; used by the JRC for reference materials
EU	European Union
GUM	Guide to the Expression of Uncertainty in Measurement
H	Ethanol with high deuterium-to-hydrogen (D/H) amount-of-substance ratio (CH_2DCH_2OH, molecule I)
h_I	Height of NMR signal I (CH_2DCH_2OH)
h_{II}	Height of NMR signal II (CH_3CHDOH)
HFB	Hexafluorobenzene (C_6F_6)
IEC	International Electrotechnical Commission
IRMS	Isotope Ratio Mass Spectrometry
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
KFT	Karl Fischer titration
L	Ethanol with low deuterium-to-hydrogen (D/H) amount-of-substance ratio (CH_2DCH_2OH, molecule I)
$\bar{m}$	Mean sample mass
m	Mass of ethanol solution
m'	Mass of water in the ethanol solution as determined by KFT
m_{TMU}	Mass of TMU to nearest 0.1 mg
m_A	Mass of ethanol to nearest 0.1 mg
M	Ethanol with intermediate deuterium-to-hydrogen (D/H) amount-of-substance ratio (CH_2DCH_2OH, molecule I)
MS	Mass spectrometry

$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicate analysis per unit
N	Number of units analysed
NMR	Nuclear Magnetic Resonance
n.a.	Not applicable
n.c.	Not calculated
OIV	International Organisation of Vine and Wine
p	Number of technically valid datasets
QC	Quality control
rel	Index denoting relative figures (uncertainties etc.)
R	Relative deuterium-to-hydrogen ratio
RM	Reference material
RMP	Reference material producer
RSD	Relative standard deviation
RSE	Relative standard error (=$RSD/\sqrt{n}$)
s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate; this parameter is linked to the homogeneity of the material
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
se	Standard error of the mean
SI	International System of Units
SIRA	Stable Isotope Ratio Analysis
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
SNIF-NMR®	Site-specific Natural Isotope Fractionation-Nuclear Magnetic Resonance Spectroscopy (^{2}H-NMR)
s_{wb}	Within-unit standard deviation; this parameter is linked to the homogeneity of the material
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
T	Temperature
t	Time
t_i	Time point for each replicate
T_I	Height of NRM signal I (CH2DCH2OH) divided by height of TMU NRM signal
T_{II}	Height of NRM signal II (CH3CHDOH) divided by height of TMU NRM signal
TMU	Tetramethylurea
t_{sl}	Proposed shelf life
t_{tt}	Proposed transport time
u	Standard uncertainty

U	Expanded uncertainty
u_b	Standard error of the slope of the regression line
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	Combined standard uncertainty; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{deg}	Standard uncertainty related to degradation from a significant slope of the regression line
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_{deg}	Standard uncertainty of degradation
u_Δ	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
V	Volume
VSMOW	Vienna Standard Mean Ocean Water
$\bar{x}$	Arithmetic mean
$\bar{x}_{max}$	Highest unit mean of the homogeneity study
$\bar{x}_{min}$	Lowest unit mean of the homogeneity study
$\bar{x}_{ns}$	Arithmetic mean of all results of normal stock samples
$\bar{x}_{ref}$	Arithmetic mean of results of reference samples
α	Significance level
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{s,meas}$	Degrees of freedom for the determination of the standard deviation s_{meas}
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}
$\bar{y}$	Mean of all results of the homogeneity study

List of boxes

Box 1. Reference material production 5
Box 2. Reference material processing 8
Box 3. Homogeneity assessment 13
Box 4. Stability assessment 17
Box 5. Stability monitoring 20
Box 6. Reference material characterisation 21
Box 7. Assignment of values to a reference material 25
Box 8. Metrological traceability 27
Box 9. Commutability 28

List of figures

Figure 1. ERM-AE200 9
Figure 2. Flow diagram, processing of ERM-AE200 11
Figure 3. Homogeneity results for ERM-AE200a ethanol (H; sugar cane) 40
Figure 4. Homogeneity results for ERM-AE200b ethanol (M; grape) 41
Figure 5. Homogeneity results for ERM-AE200c ethanol (L; sugar beet) 42
Figure 6. Short-term stability results for ERM-AE200a ethanol (L; sugar cane) 44
Figure 7. Short-term stability results for ERM-AE200b ethanol (L; grape) 45
Figure 8. Short-term stability results for ERM-AE200c ethanol (L; sugar beet) 46
Figure 9. Results of characterisation study for $(D/H)_I$ in ERM-AE200a [H; sugar cane] 47
Figure 10. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200a [H; sugar cane] 48
Figure 11. Results of characterisation study for R in ERM-AE200a [H; sugar cane] 49
Figure 12. Results of characterisation study for R in ERM-AE200a [H; sugar cane] 49
Figure 13. Results of characterisation study for $(D/H)_I$ in ERM-AE200b [M; grape] 50
Figure 14. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200b [M; grape] 51
Figure 15. Results of characterisation study for R in ERM-AE200b [M; grape] 52
Figure 16. Results of characterisation study for R in ERM-AE200b [M; grape] 52
Figure 17. Results of characterisation study for $(D/H)_I$ in ERM-AE200c [L; sugar beet] 53
Figure 18. Results of characterisation study for $(D/H)_{II}$ in ERM- AE200c [L; sugar beet] 54
Figure 19. Results of characterisation study for R in ERM- AE200c [L; sugar beet] 55
Figure 20. Results of characterisation study for R in ERM- AE200c [L; sugar beet]. 55

List of tables

Table 1. SNIF-NMR and C-KFT results obtained in-house for ethanol starting materials on arrival to the EC-JRC 8
Table 2. Composition of bulk materials for ERM-AE200 10
Table 3. Deuterium distribution in the ERM-AE200 bulk solutions. 12
Table 4. Results of the statistical evaluation of the homogeneity studies 14
Table 5. Results of the homogeneity study 15
Table 6. Results of the short-term stability tests 18
Table 7. Uncertainties of stability during transport and storage. 20
Table 8. Statistical evaluation of the technically accepted datasets for ERM-AE200. 23
Table 9. Uncertainty of characterisation for ERM-AE200. 24
Table 10. Certified values and their uncertainties for ERM-AE200 26
Table 11 Values assigned to ERM-AE200 31
Table 12 Results from measurements performed on units stored at normal storage temperature (18 °C) and at a reference temperature (4 °C) after 18 years of storage 43
Table 13. Results of characterisation study for $(D/H)_I$ in ERM-AE200a [H; sugar cane] 47
Table 14. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200a [H; sugar cane] 48
Table 15. Results of characterisation study for R in ERM-AE200a [H; sugar cane] 49
Table 16. Results of characterisation study for $(D/H)_I$ in ERM-AE200b [M; grape] 50
Table 17. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200b [M; grape] 51
Table 18. Results of characterisation study for R in ERM-AE200b [M; grape] 52
Table 19. Results of characterisation study for $(D/H)_I$ in ERM-AE200c [L; sugar beet] 53
Table 20. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200c [L; sugar beet] 54
Table 21. Results of characterisation study for R in ERM-AE200c [L; sugar beet] 55

Annexes

Annex 1. Results of the homogeneity measurements

Results of the homogeneity measurements for ERM-AE200. Graphs represent unit means ± confidence interval of the means (CI calculated from s_{wb} from ANOVA for all units).

Figure 3. Homogeneity results for ERM-AE200a ethanol (H; sugar cane)

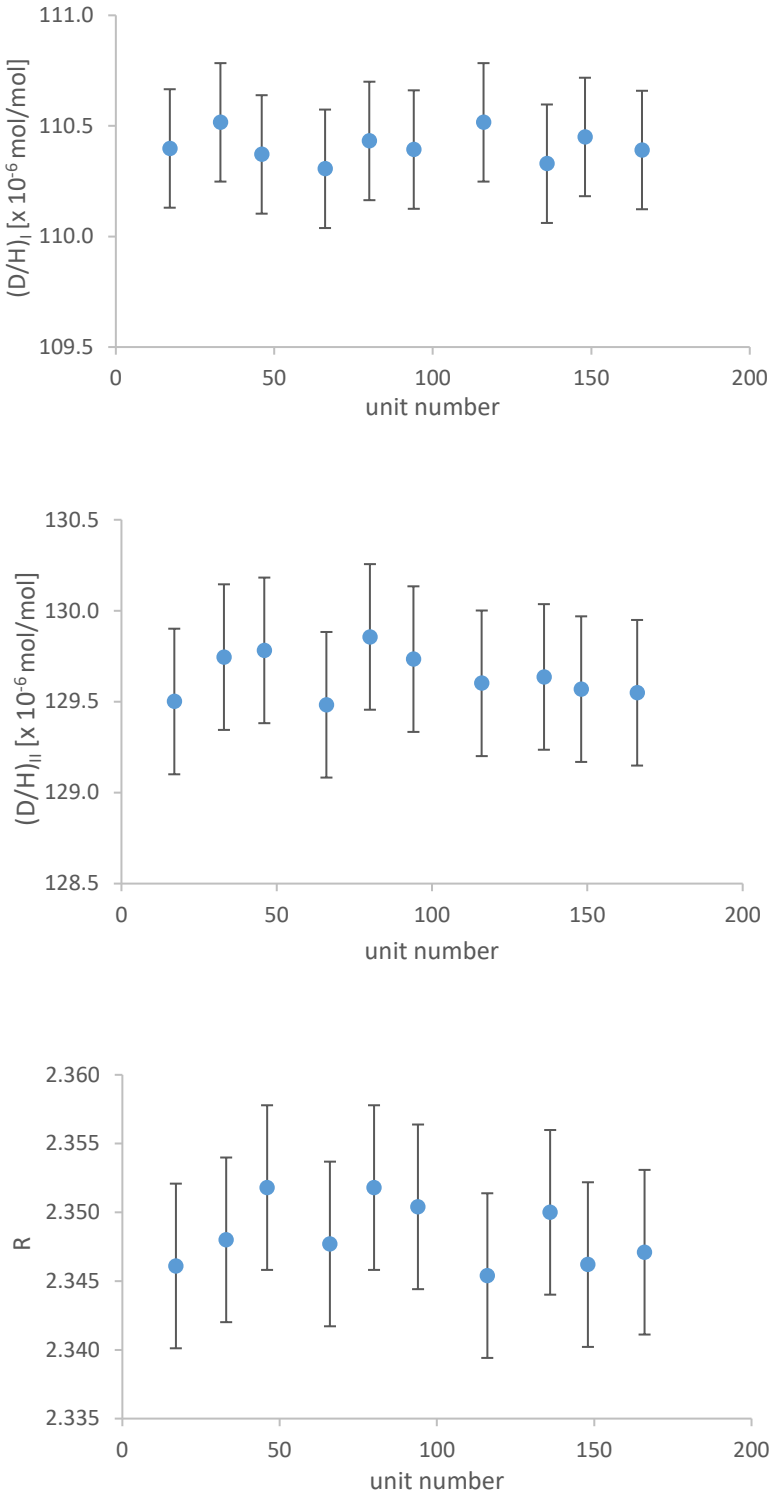


Figure 4. Homogeneity results for ERM-AE200b ethanol (M; grape)

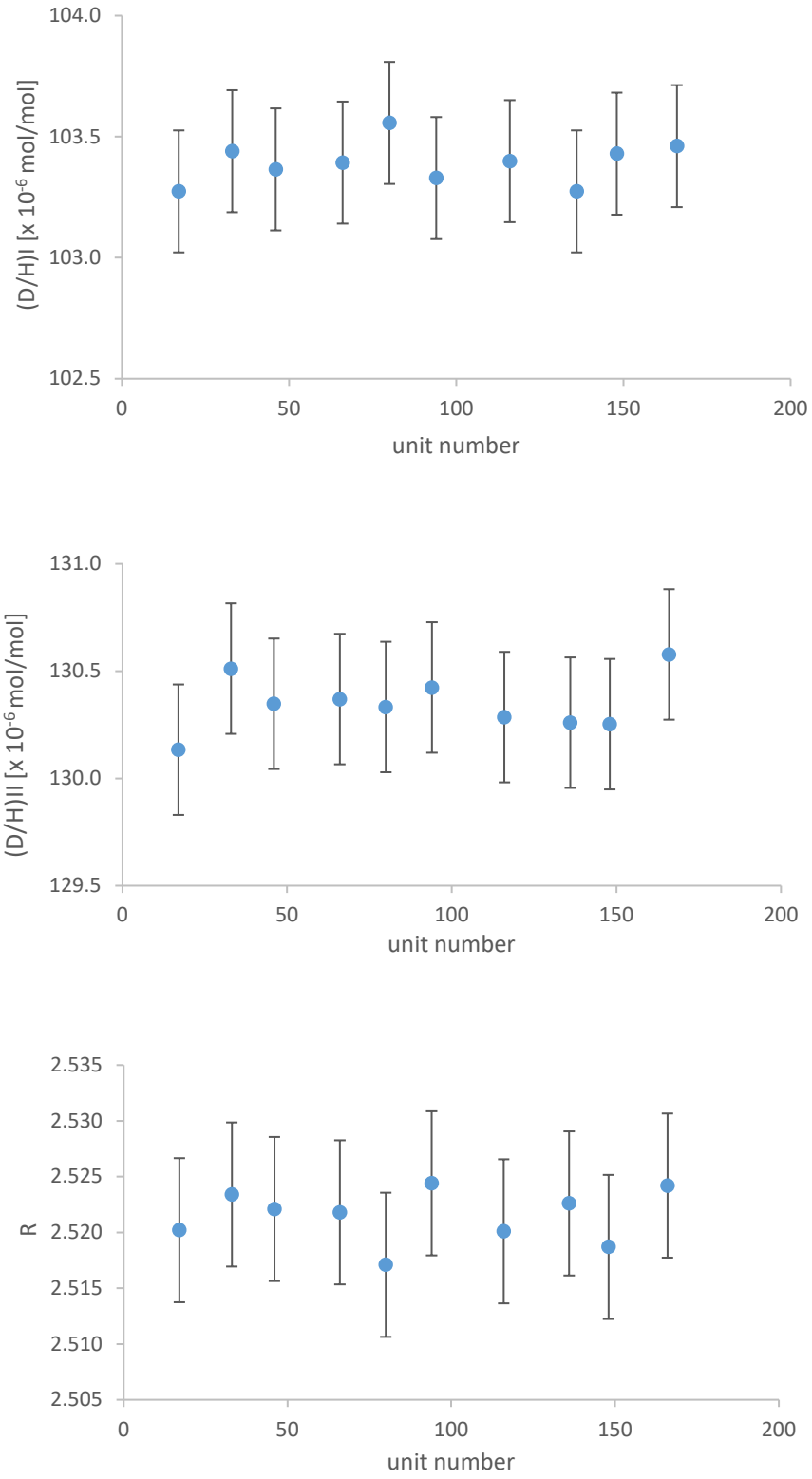
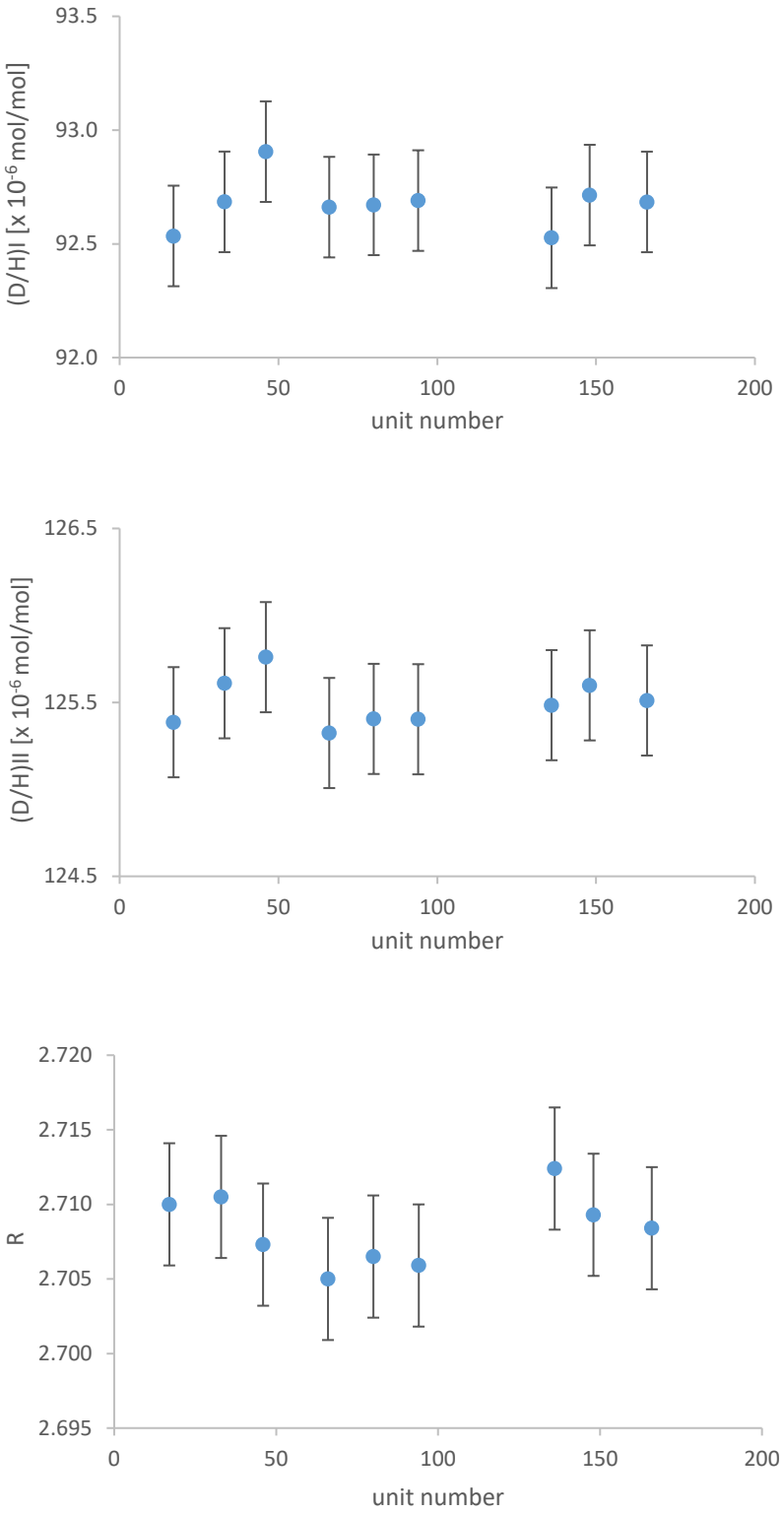


Figure 5. Homogeneity results for ERM-AE200c ethanol (L; sugar beet)



Annex 2. Data from the post-certification stability monitoring programme of BCR-123.

Table 12 Results from measurements performed on units stored at normal storage temperature (18 °C) and at a reference temperature (4 °C) after 18 years of storage

		Certified value ± U	Mean samples at 18 °C ± U	Mean samples at 4 °C ± U	Ratio ± U (k = 2)
BCR-123 (H; sugar cane)	$(D/H)_I$ [x10^{-6} mol/mol]	109.65 ± 0.20	109.65 ± 1.50	109.85 ± 1.50	0.998 ± 0.008
	$(D/H)_{II}$ [x10^{-6} mol/mol]	119.76 ± 0.25	119.95 ± 1.70	120.15 ± 1.70	0.998 ± 0.006
	R	2.184 ± 0.005	2.188 ± 0.020	2.188 ± 0.020	1.000 ± 0.007
BCR-123 (M; grape)	$(D/H)_I$ [x10^{-6} mol/mol]	101.69 ± 0.17	101.75 ± 1.50	101.80 ± 1.50	1.000 ± 0.006
	$(D/H)_{II}$ [x10^{-6} mol/mol	130.94 ± 0.21	130.85 ± 1.70	131.10 ± 1.70	0.998 ± 0.006
	R	2.575 ± 0.006	2.571 ± 0.020	2.576 ± 0.020	0.998 ± 0.007
BCR-123 (L; sugar beet)	$(D/H)_I$ [x10^{-6} mol/mol]	90.30 ± 0.18	90.40 ± 1.50	90.30 ± 1.50	1.001 ± 0.010
	$(D/H)_{II}$ [x10^{-6} mol/mol]	122.20 ± 0.40	122.80 ± 1.70	122.80 ± 1.70	1.000 ± 0.007
	R	2.708 ± 0.009	2.717 ± 0.022	2.719 ± 0.022	0.999 ± 0.008

Annex 3. Results of the short-term stability measurements for ERM-AE200

Short-term stability graphs represent means per time [week] ± confidence interval (CI) of the means

Figure 6. Short-term stability results for ERM-AE200a ethanol (L; sugar cane)

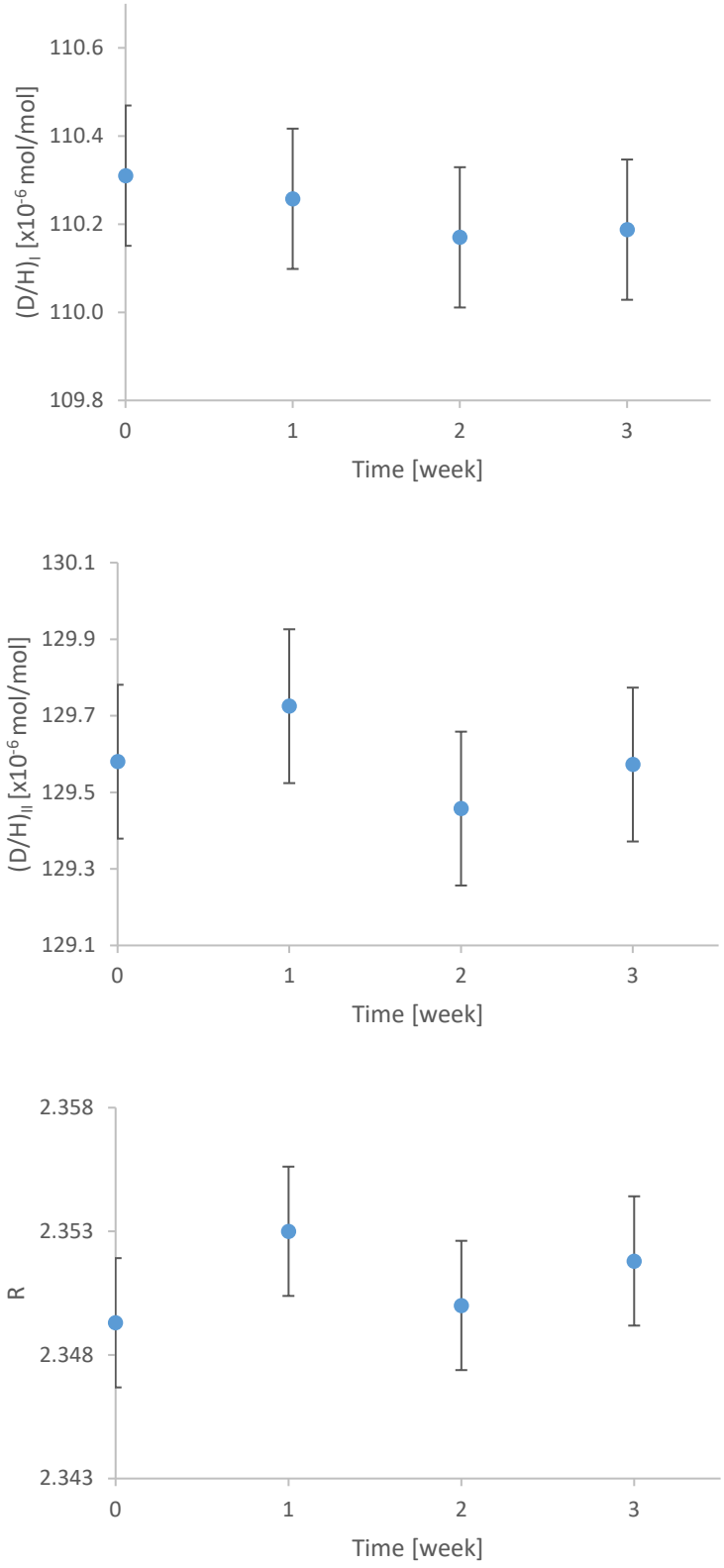


Figure 7. Short-term stability results for ERM-AE200b ethanol (L; grape)

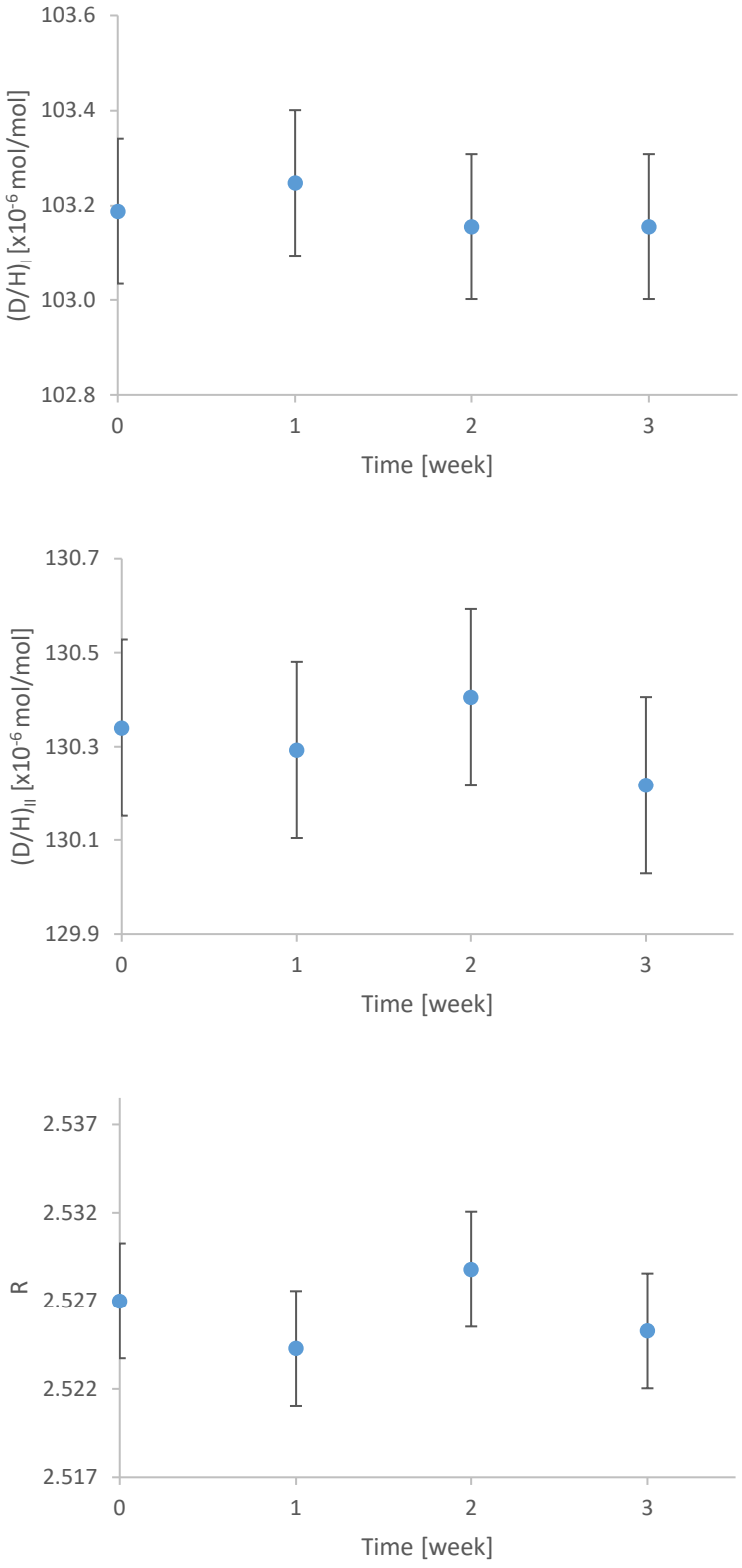
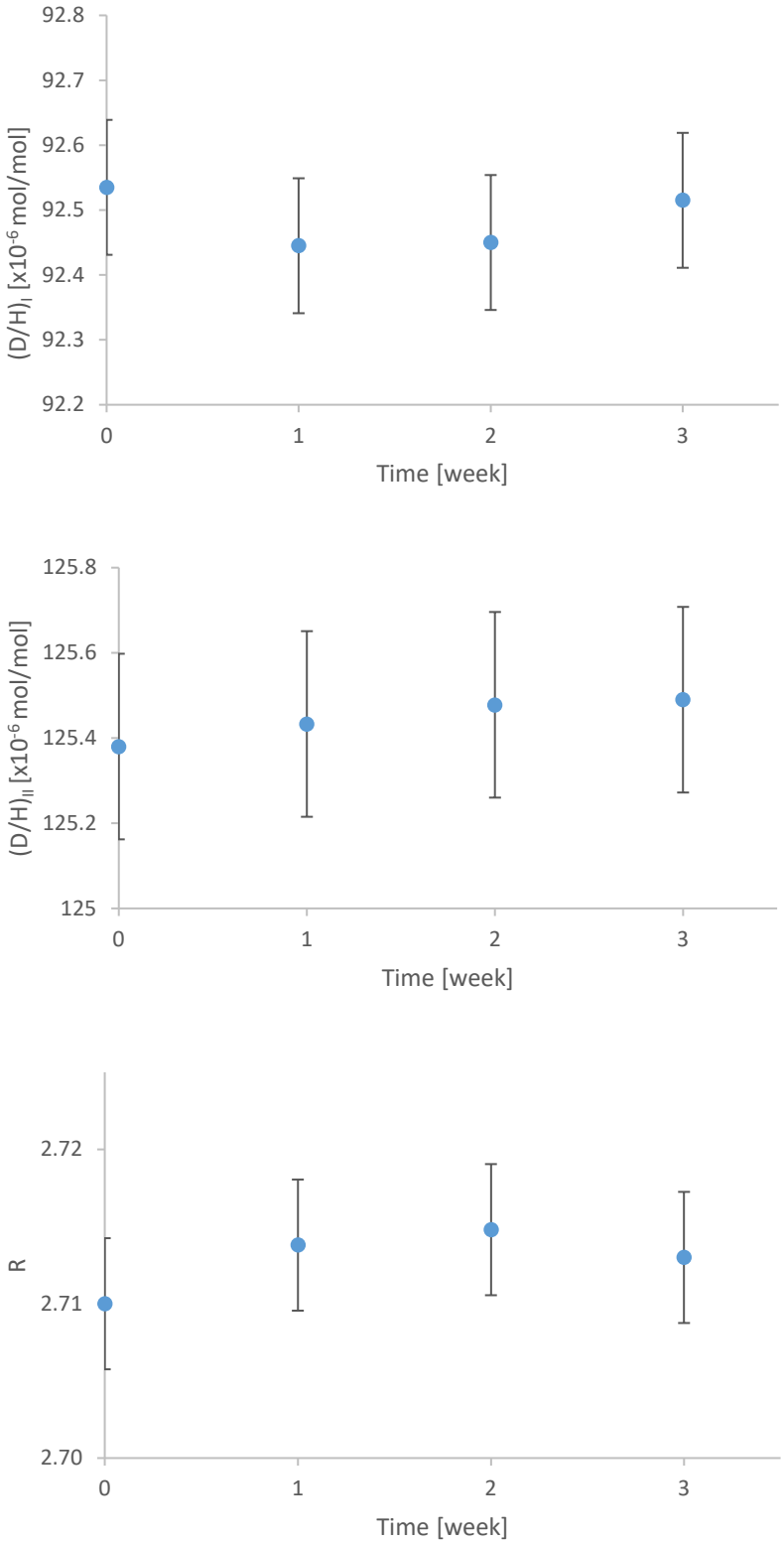


Figure 8. Short-term stability results for ERM-AE200c ethanol (L; sugar beet)



Annex 4: Results of the characterisation measurements.

Graphs represent mean values with associated uncertainties as reported by the participant laboratories. Red lines illustrate the certified value range for the material.

Table 13 Results of characterisation study for $(D/H)_I$ in ERM-AE200a [H; sugar cane]

Laboratory code	replicate 1 [x10^{-6}]	replicate 2 [x10^{-6}]	replicate 3 [x10^{-6}]	replicate 4 [x10^{-6}]	replicate 5 [x10^{-6}]	replicate 6 [x10^{-6}]	Mean [x10^{-6} mol/mol]	Expanded uncertainty [x10^{-6} mol/mol]
L1	109.95	110.27	110.21	110.37	110.21	110.39	110.233	0.27
L2	110.12	110.22	110.5	110.47	110.42	110.18	110.318	0.7
L3	110.7	110.6	110.8	110.7	110.5	110.7	110.667	0.8
L4	110.4	110.4	110.5	110.4	110.1	110	110.300	0.6
L5	110.47	110.69	110.77	110.84	110.6	110.44	110.635	0.86
L6	110.72	110.85	110.45	110.6	110.59	110.54	110.625	0.7
L7	110.43	110.54	110.57	110.41	110.48	110.56	110.498	0.7

Figure 9. Results of characterisation study for $(D/H)_I$ in ERM-AE200a [H; sugar cane] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material

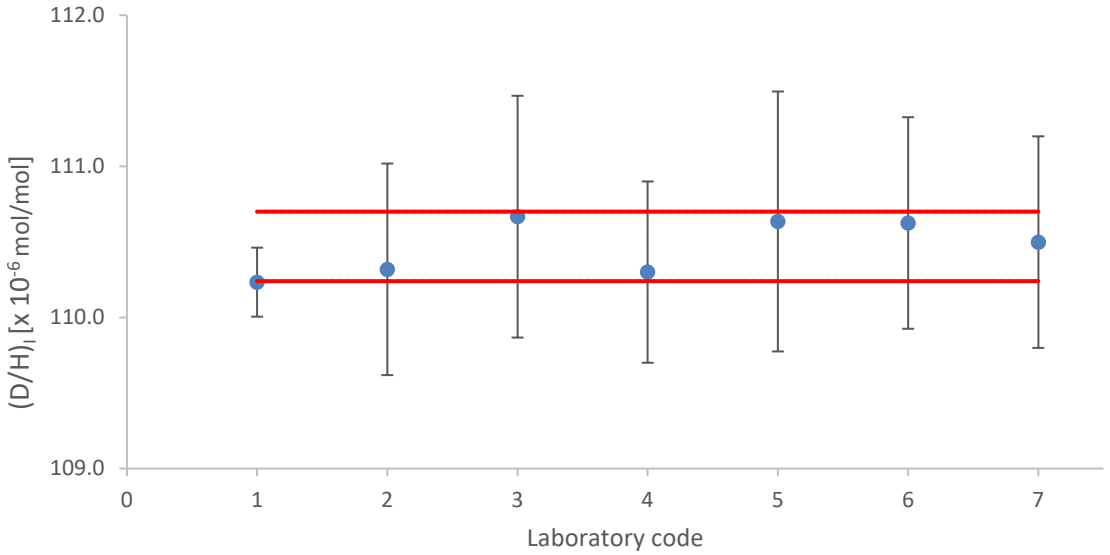


Table 14. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200a [H; sugar cane]

Laboratory code	replicate 1 [x10^{-6}]	replicate 2 [x10^{-6}]	replicate 3 [x10^{-6}]	replicate 4 [x10^{-6}]	replicate 5 [x10^{-6}]	replicate 6 [x10^{-6}]	Mean [x10^{-6} mol/mol]	Expanded uncertainty [x10^{-6} mol/mol]
L1	129.43	129.64	129.35	129.98	130.1	129.71	129.702	0.32667
L2	129.49	129.55	129.42	129.76	129.72	129.72	129.610	1.3
L3	130.2	130.1	130.3	129.9	129.9	129.9	130.050	1.2
L4	129.9	129.9	130.1	129.9	129.7	129.6	129.850	1.2
L5	130.08	129.8	130.27	130.42	130.01	130.06	130.107	1.36
L6	130.24	130.2	130.1	130.07	129.95	130.18	130.123	1.9
L7	130.02	129.85	130.1	129.73	129.87	129.59	129.860	1.9

Figure 10. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200a [H; sugar cane] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material

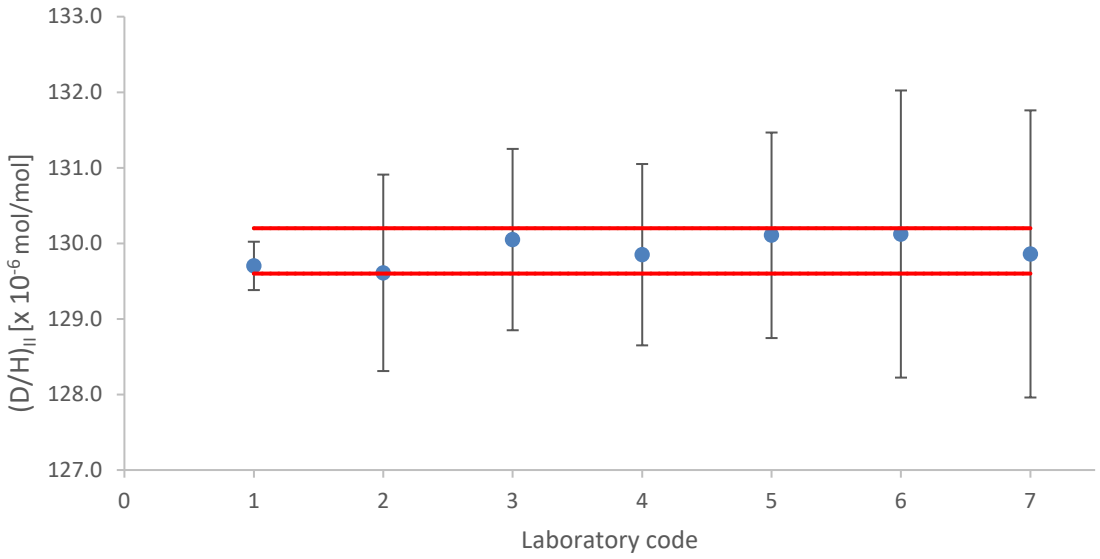


Table 15. Results of characterisation study for *R* in ERM-AE200a [H; sugar cane]

Laboratory code	replicate 1	replicate 2	replicate 3	replicate 4	replicate 5	replicate 6	Mean	Expanded uncertainty
L1	2.354	2.351	2.347	2.355	2.361	2.35	2.353	0.0075
L2	2.3518	2.3508	2.3426	2.3493	2.3495	2.3548	2.3498	n.p.
L3	2.353	2.354	2.352	2.347	2.351	2.348	2.3508	0.012
L4	2.35	2.35	2.36	2.36	2.36	2.36	2.3567	0.02
L5	2.355	2.345	2.352	2.353	2.351	2.355	2.3518	n.p.
L6	2.353	2.349	2.356	2.352	2.35	2.355	2.3525	n.p.
L7	2.355	2.349	2.353	2.35	2.351	2.344	2.3503	n.p.

n.p. not provided

Figure 11. Results of characterisation study for *R* in ERM-AE200a [H; sugar cane] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material

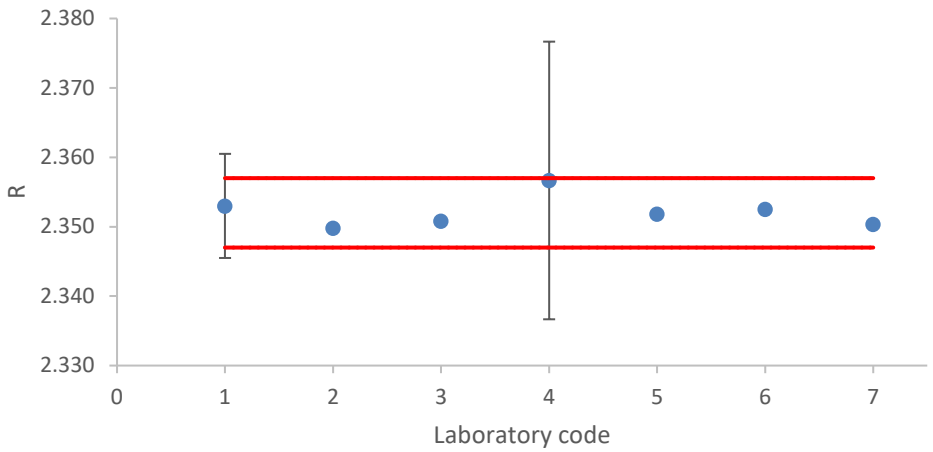


Figure 12. Results of characterisation study for *R* in ERM-AE200a [H; sugar cane]. Error bars represent standard deviations of data sets. Red lines illustrate the certified value range for the material.

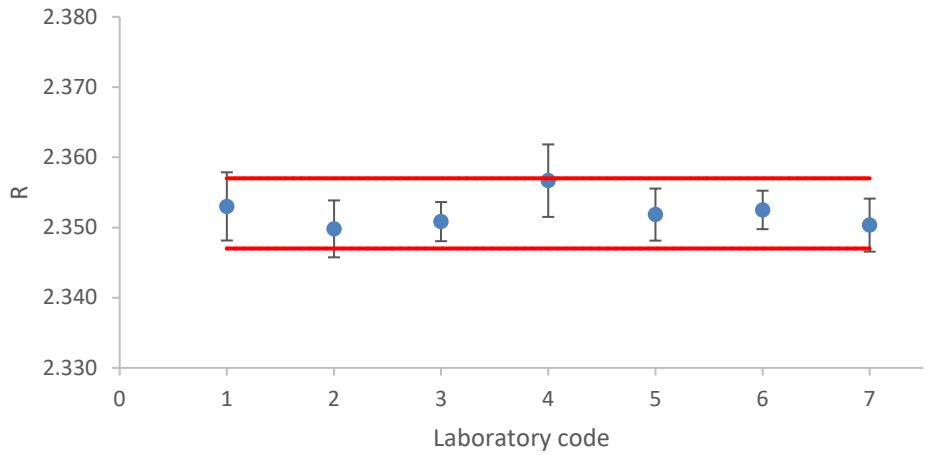


Table 16. Results of characterisation study for $(D/H)_I$ in ERM-AE200b [M; grape]

Laboratory code	replicate 1 [x10^{-6}]	replicate 2 [x10^{-6}]	replicate 3 [x10^{-6}]	replicate 4 [x10^{-6}]	replicate 5 [x10^{-6}]	replicate 6 [x10^{-6}]	Mean [x10^{-6} mol/mol]	Expanded uncertainty [x10^{-6} mol/mol]
L1	103.06	103.16	103.17	103.26	103.04	103.06	103.125	0.27
L2	103.23	103.04	103.08	103.25	103.10	103.26	103.16	0.7
L3	103.6	103.4	103.5	103.7	103.5	103.5	103.533	0.8
L4	103.2	103.2	103.2	103.3	103.2	103.2	103.217	0.6
L5	103.71	103.6	103.4	103.38	103.26	103.43	103.463	0.86
L6	103.5	103.56	103.49	103.34	103.51	103.53	103.488	0.7
L7	103.27	103.39	103.42	103.07	103.42	103.22	103.298	0.7

Figure 13. Results of characterisation study for $(D/H)_I$ in ERM-AE200b [M; grape] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material.

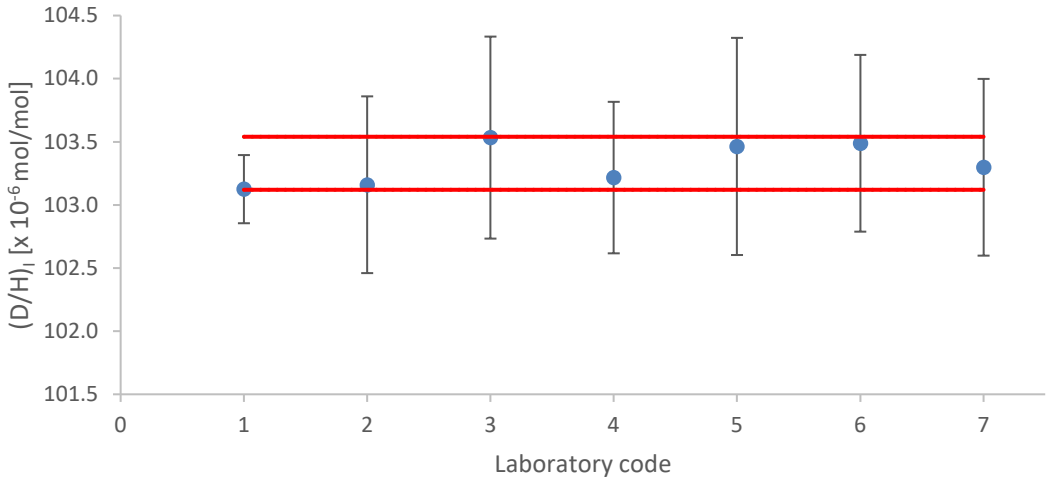


Table 17. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200b [M; grape]

Laboratory code	replicate 1 [x10^{-6}]	replicate 2 [x10^{-6}]	replicate 3 [x10^{-6}]	replicate 4 [x10^{-6}]	replicate 5 [x10^{-6}]	replicate 6 [x10^{-6}]	Mean [x10^{-6} mol/mol]	Expanded uncertainty [x10^{-6} mol/mol]
L1	129.92	130.34	130.31	130.15	130.19	130.34	130.208	0.32667
L2	130.5	130.18	130.57	130.32	130.46	130.70	130.455	1.3
L3	130.8	130.5	130.7	130.7	130.5	130.5	130.617	1.2
L4	130.8	130.7	130.7	130.8	131	130.3	130.717	1.2
L5	130.68	130.74	130.48	130.65	130.51	130.68	130.623	1.36
L6	130.49	130.81	130.52	130.49	130.78	130.66	130.625	1.9
L7	130.39	130.55	130.3	130.19	130.59	130.34	130.393	1.9

Figure 14. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200b [M; grape] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material.

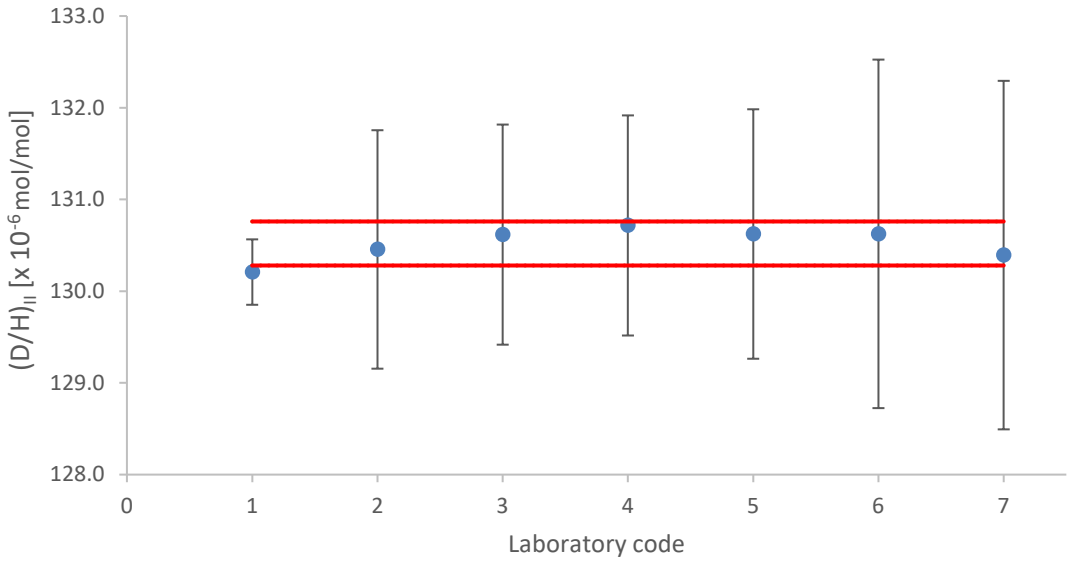


Table 18. Results of characterisation study for *R* in ERM-AE200b [M; grape]

Laboratory code	replicate 1	replicate 2	replicate 3	replicate 4	replicate 5	replicate 6	Mean [x10^{-6} mol/mol]	Expanded uncertainty [x10^{-6} mol/mol]
L1	2.521	2.527	2.526	2.521	2.527	2.529	2.52517	0.023
L2	2.5283	2.5269	2.5336	2.5245	2.5308	2.5316	2.52928	n.p.
L3	2.525	2.523	2.528	2.523	2.521	2.523	2.52383	0.012
L4	2.53	2.53	2.53	2.53	2.54	2.53	2.53167	0.02
L5	2.52	2.524	2.524	2.528	2.528	2.527	2.52517	n.p.
L6	2.522	2.526	2.522	2.526	2.527	2.524	2.5245	n.p.
L7	2.525	2.526	2.52	2.526	2.525	2.526	2.52467	n.p.

n.p. not provided

Figure 15. Results of characterisation study for *R* in ERM-AE200b [M; grape] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material.

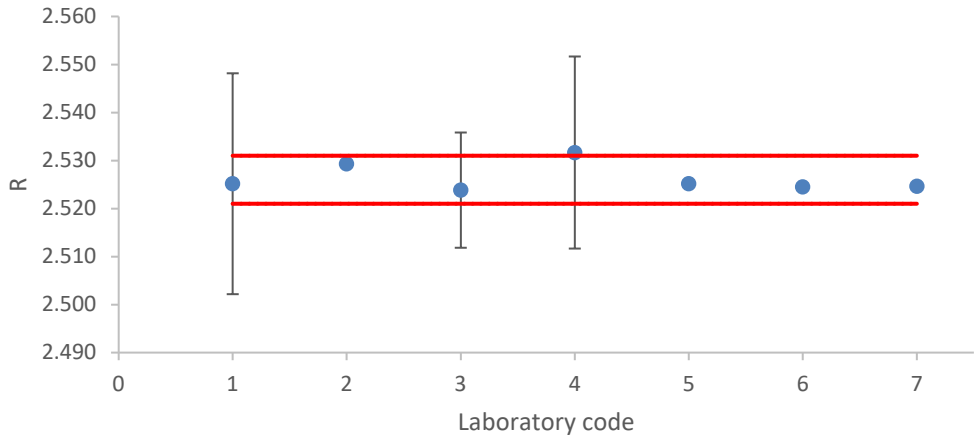


Figure 16. Results of characterisation study for *R* in ERM-AE200b [M; grape]. Error bars represent standard deviations of data sets. Red lines illustrate the certified value range for the material.

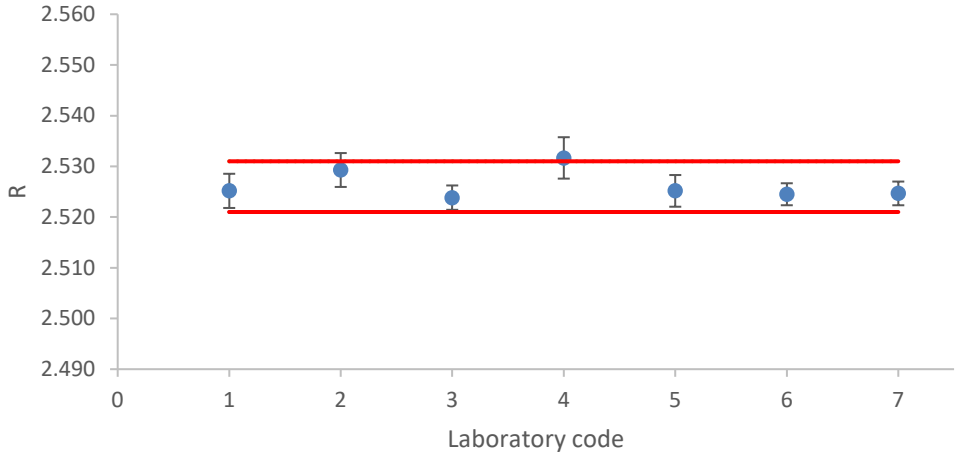


Table 19. Results of characterisation study for $(D/H)_I$ in ERM-AE200c [L; sugar beet]

Laboratory code	replicate 1 [x10^{-6}]	replicate 2 [x10^{-6}]	replicate 3 [x10^{-6}]	replicate 4 [x10^{-6}]	replicate 5 [x10^{-6}]	replicate 6 [x10^{-6}]	Mean [x10^{-6} mol/mol]	Expanded uncertainty [x10^{-6} mol/mol]
L1	92.56	92.78	92.71	92.37	92.48	92.28	92.53	0.245
L2	92.39	92.32	92.59	92.22	92.44	92.34	92.3833	0.7
L3	92.9	92.8	92.8	92.8	92.6	92.7	92.7667	0.8
L4	92.6	92.3	92.4	92.5	92.3	92.4	92.4167	0.6
L5	92.78	92.74	92.68	92.58	92.55	92.52	92.6417	0.86
L6	92.75	92.82	92.71	92.7	92.87	92.69	92.7567	0.7
L7	92.78	92.72	92.74	92.75	92.61	92.49	92.6817	0.7

Figure 17. Results of characterisation study for $(D/H)_I$ in ERM-AE200c [L; sugar beet] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material.

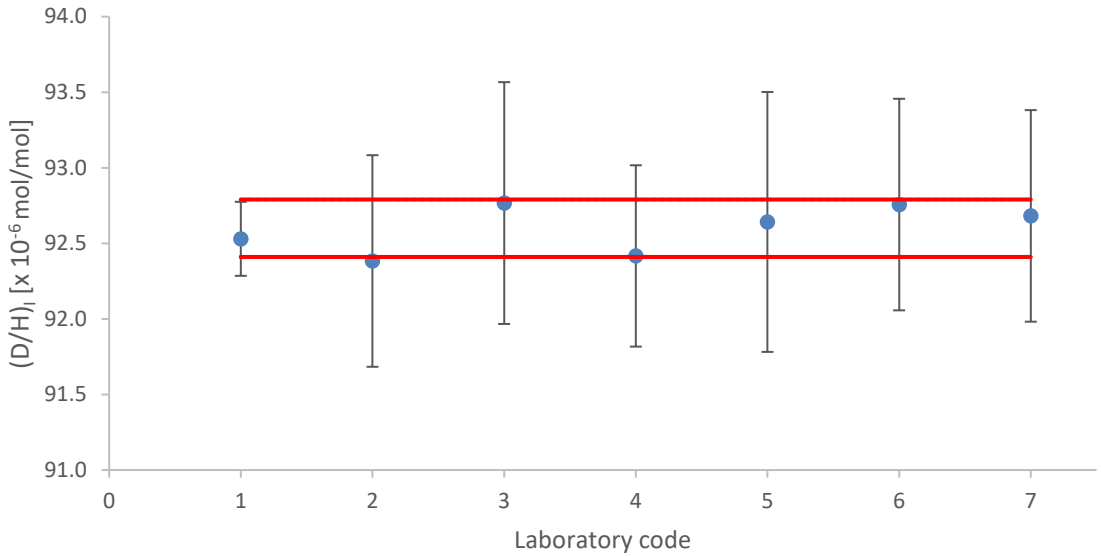


Table 20. Results of characterisation study for $(D/H)_{II}$ in ERM-AE200c [L; sugar beet]

Laboratory code	replicate 1 [x10^{-6}]	replicate 2 [x10^{-6}]	replicate 3 [x10^{-6}]	replicate 4 [x10^{-6}]	replicate 5 [x10^{-6}]	replicate 6 [x10^{-6}]	Mean [x10^{-6} mol/mol]	Expanded uncertainty [x10^{-6} mol/mol]
L1	125.61	125.2	125.29	125.4	125.53	125.39	125.403	0.32667
L2	125.74	125.58	125.54	125.26	125.25	125.62	125.498	1.3
L3	125.9	125.9	126	125.7	125.8	125.7	125.833	1.2
L4	125.4	125.7	125.3	125.4	125.5	125.7	125.500	1.2
L5	125.7	125.98	125.57	125.79	125.37	125.62	125.672	1.36
L6	125.83	125.91	125.89	125.79	126.03	125.79	125.873	1.9
L7	125.62	125.69	125.59	125.74	125.47	125.43	125.590	1.9

Figure 18. Results of characterisation study for $(D/H)_{II}$ in ERM- AE200c [L; sugar beet] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material.

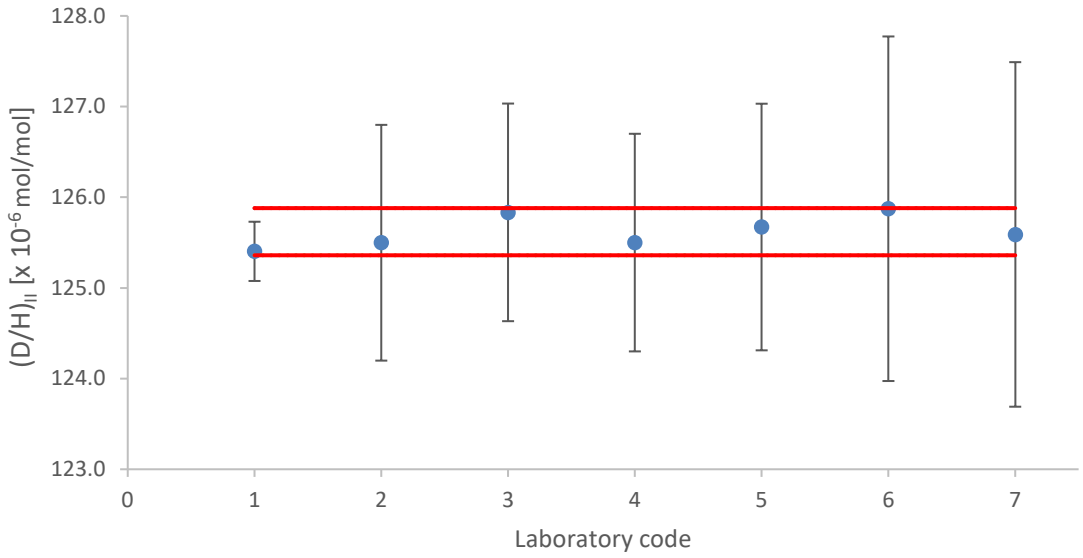


Table 21. Results of characterisation study for *R* in ERM-AE200c [L; sugar beet]

Laboratory code	replicate 1	replicate 2	replicate 3	replicate 4	replicate 5	replicate 6	Mean	Expanded uncertainty
L1	2.714	2.699	2.703	2.715	2.715	2.718	2.71067	0.01033
L2	2.7219	2.7204	2.7118	2.7166	2.7099	2.7207	2.71688	n.p.
L3	2.712	2.714	2.717	2.71	2.717	2.712	2.71367	0.012
L4	2.71	2.73	2.71	2.71	2.72	2.72	2.71667	0.02
L5	2.71	2.717	2.71	2.717	2.709	2.716	2.71317	n.p.
L6	2.713	2.713	2.716	2.714	2.714	2.714	2.71400	n.p.
L7	2.708	2.711	2.708	2.711	2.71	2.713	2.71017	n.p.

n.p. Not provided

Figure 19. Results of characterisation study for *R* in ERM- AE200c [L; sugar beet] with associated uncertainties as reported by participant laboratories. Red lines illustrate the certified value range for the material.

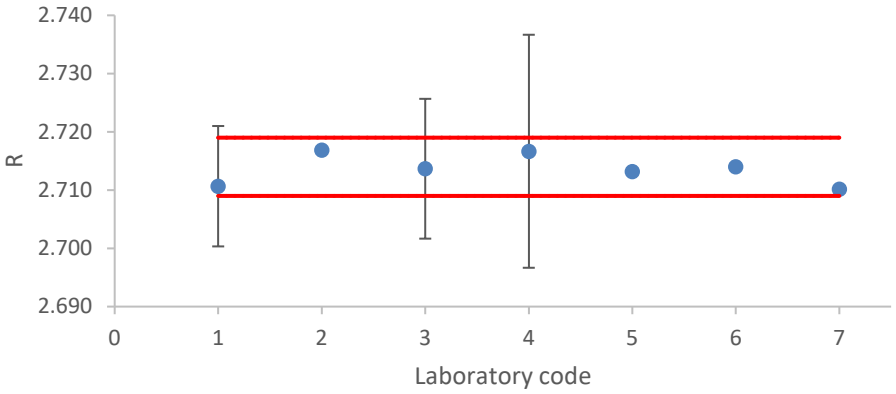
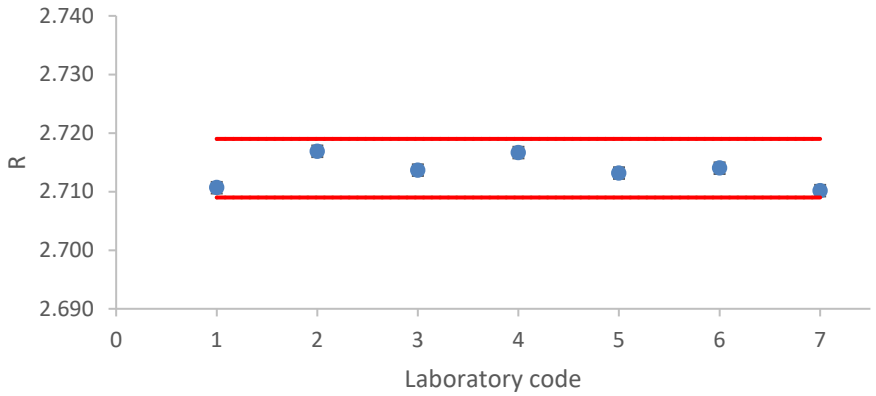


Figure 20. Results of characterisation study for *R* in ERM- AE200c [L; sugar beet]. Error bars represent standard deviations of data sets. Red lines illustrate the certified value range for the material.



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