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CERTIFICATION REPORT

The certification of the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point of biodiesel: ERM®- EF001

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The certification of the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point of biodiesel: ERM®- EF001

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Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper 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.

Summmary

This report describes the production of ERM-EF001, a biodiesel material certified for the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point. The material was produced following ISO Guide 34:2009 [1].

A rapeseed oil fatty acid methyl ester with the addition of an antioxidant (butylhydroxytoluene) was selected as the base material. It was provided by a biodiesel producer located in Germany. The material was filled in amber glass ampoules. To keep the material homogenous throughout the filling it was gently bubbled with argon.

Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006 [2]. The minimum sample intake is defined by the required sample volume stipulated in the respective documentary standard.

The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025 [3]. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [4] and include uncertainties related to possible inhomogeneity, and instability and to characterisation.

The material is intended for the quality control or assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in amber glass ampoules containing 27 mL of biodiesel closed under argon atmosphere.

The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium. The following values were assigned:

	Certified value [5]	Uncertainty [7]	Unit
Ester content [1]	98.9	1.7	% (m/m) [4]
Linolenic acid methyl ester content [1]	8.82	0.16	% (m/m) [4]
Monoglyceride content [2]			% (m/m) [4]
Diglyceride content [2]			% (m/m) [4]
Triglyceride content [2]			% (m/m) [4]
Total glycerol content [2]	0.187	0.009	% (m/m) [4]
Water content [3]	0.0205	0.0024	% (m/m) [4]

SUPERSEDED
see addenda (overleaf)

1) As defined by EN 14103:2011

2) As defined by EN 14105:2011

3) As defined by EN ISO 12937:2000

4) As called in EN14103:2011, EN 14105:2011, and EN ISO 12937:2000, which is equivalent to 10^{-2} g/g

5) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI).

6) The value corresponds to the limit of quantification (LOQ) of the standard method EN 14105:2011. The mass fraction of triglycerides in ERM-EF001 is below the stated value with a 95 % level of confidence. The value is traceable to the International System of Units (SI).

7) The uncertainty of the certified value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

	Certified value [7]	Uncertainty [8]	Unit
Density (at 15 °C) [1]	883.20	0.04	kg/m^3
Viscosity (at 40 °C) [2]	4.465	0.005	mm^2/s
Oxidation stability (at 110 °C) [3]			h
Acid value [4]			mg KOH/g
Iodine value [5]			g iodine/100 g
Flash point [6]	181	14 [9]	°C

1) As defined by EN ISO 12185:1996

2) As defined by EN ISO 3104:1996

3) As defined by EN 14112:2003

4) As defined by EN 14104:2003

5) As defined by EN 14111:2003

6) As defined by EN ISO 3679:2004

7) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI).

8) The uncertainty of the certified value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

9) The uncertainty of the certified value is the expanded uncertainty with a coverage factor k = 2.8 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

ADDENDUM TO:

The certification of the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point of biodiesel: ERM®- EF001

Certification report

EUR 26711 EN

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Directorate F – Health, Consumers and Reference Materials

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Summary

This addendum to the certification report EUR 26711 EN [1] is concerning the update of the certificate of the certified biodiesel reference material ERM-EF001, whose properties are defined by measurement procedures that were partly outdated and adapted to newly revised measurement procedures. It describes the measures taken either to confirm the validity of certified values for editorially changed measurement procedures, or to certify properties with measurement procedures that were subject to fundamental technical changes or were newly published.

New certified values and uncertainties were assigned for the ester content, the linolenic acid methyl ester content, viscosity, and the iodine value. All properties were characterised by an interlaboratory comparison of laboratories of demonstrated competence using the newly revised measurement procedures and adhering to ISO/IEC 17025:2017 [2]. Technically invalid results were removed but 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 [3] and ISO Guide 35:2017 [4] and include uncertainties related to possible inhomogeneity and instability as reported in EUR 26711 EN [1] and uncertainties related to characterisation reported in this addendum.

Old measurement procedures on the certificate were replaced by new measurement procedures in the case of purely editorial modifications, with the assigned certified values and their uncertainties remaining unchanged. This applied to the oxidation stability, the flash point and the methanol content.

Before release of the updated certificate, the project was subjected to an internal peer-review.

The following values were assigned (implemented changes given in bold):

	Certified value [10]	Uncertainty [11]	Unit
Ester content [1]	**97.4**	**0.6**	[% (m/m)] [9]
Linolenic acid methyl ester content [1]	**8.52**	**0.09**	[% (m/m)] [9]
Ester content [2]	98.9	1.7	[% (m/m)] [9]
Linolenic acid methyl ester content [2]	8.82	0.16	[% (m/m)] [9]
Density (at 15 °C) [3]	883.20	0.04	[kg/m^3]
Viscosity (at 40 °C) [4]	**4.474**	**0.006**	[mm^2/s]
Oxidation stability (at 110 °C) [5]	9.8	0.5	[h]
Iodine value [6]	112	4	[g iodine/100 g]
Iodine value [7]	**107.3**	**1.9**	[g iodine/100 g]
Flash point [8]	181	14 [12]	[°C]

1) As defined by **EN 14103:2020**
2) As defined by EN 14103:2011
3) As defined by EN ISO 12185:1996
4) As defined by **EN ISO 3104:2020**
5) As defined by **EN 15751:2014** and **EN 14112:2020**
6) As defined by EN 14111:2003
7) As defined by **EN 16300:2012**
8) As defined by **EN ISO 3679:2015**
9) As called in EN 14103:2011 and **EN 14103:2020**, which is equivalent to 10^{-2} g/g
10) 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. The certified value and its uncertainty are traceable to the International System of Units (SI).
11) 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.
12) The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2.8$ corresponding to a level of confidence of 95 %, estimated in accordance with ISO 17034:2016 and ISO Guide 35:2017.

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Glossary

ANOVA	Analysis of variance
c_{CRM}	Certified value
CEN	European Committee for Standardization
c_{meas}	Mean measured value
CRM	Certified reference material
EN	European norm (standard)
ERM®	Trademark owned by the European Commission; used by the JRC for reference materials
FAME	Fatty acid methyl ester
GC	Gas chromatography
GUM	Guide to the Expression of Uncertainty in Measurement
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
n	Number of replicate analysis per unit
p	Number of technically valid datasets
rel	Index denoting relative figures (uncertainties etc.)
RSD	Relative standard deviation
r	Repeatability limit
R	Reproducibility limit
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SI	International System of Units
s_L	Standard deviation between laboratories
s_r	Repeatability standard deviation
s_R	Reproducibility standard deviation
s	Standard deviation
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
t	Two-tailed Student t value at the 95 % confidence level
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; 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_{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_{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_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
Δ_{meas}	Absolute difference between mean measured value and the certified value
VMR	Verification measurement results

1 Introduction

1.1 Background

In 2014, ERM-EF001, a biodiesel material based on 100 % rapeseed oil fatty acid methyl ester, was certified for selected parameters of EN 14214:2012 [5]. The certified properties are operationally defined measurands and can only be obtained by following the measurement procedures specified on the corresponding certificate of the certified reference material (CRM), i.e.

- the ester and linolenic acid methyl ester content as defined by EN 14103: 2011 [6];
- density as defined by EN ISO 12185:1996 [7];
- viscosity as defined by EN ISO 3104:1996 [8];
- oxidation stability as defined by EN 14112:2003 [9];
- iodine value as defined by EN 14111:2003 [10];
- flash point as defined by EN ISO 3679:2004 [11];
- methanol as defined by EN 14110:2003 (indicative value) [12].

These measurement procedures can always be subject to revision. Two of the measurement procedures remained unchanged since the release of ERM-EF001, i.e.

- density as defined by EN ISO 12185:1996 [7];
- iodine value as defined by EN 14111:2003 [10].

The other measurement procedures were either revised, i.e.

- the ester and linolenic acid methyl ester content as defined by EN 14103: 2020 [13];
- viscosity as defined by EN ISO 3104:2020 [14];
- oxidation stability as defined by EN 14112:2020 [15];
- flash point as defined by EN ISO 3679:2015 [16];
- methanol as defined by EN 14110:2019 [17];

or new measurement procedures related to some of the certified properties were published during the lifetime of the original project, or immediately thereafter, i.e.

- oxidation stability as defined by EN 15751:2014 [18];
- iodine value as defined by EN 16300:2012 [19].

The modifications in a new revision can often only be editorial, but sometimes a measurement procedure can also undergo fundamental technical changes that can have an impact on the measurement results. In both cases, the certificate should be updated to the new measurement

procedures in order to keep the material fit for purpose, whereby the update process requires different measures depending on the nature of the modification.

If there is only an editorial change, it should be checked that measurement results obtained with the newly revised measurement procedure are in agreement with the certified value as defined by the previous edition. If this is confirmed, the old measurement procedure on the certificate can be replaced by the new measurement procedure, without changing the certified value and its uncertainty.

In case of a fundamental technical change, a completely new certified value must be assigned, with the material being re-characterised in an interlaboratory comparison using the newly revised measurement procedure.

This addendum describes the measurements and evaluations carried out

- for the confirmation of the validity of the certified values for editorially changed measurement procedures,
- and for the certification of properties with measurement procedures that were subject to fundamental technical changes or were newly published.

1.2 Outline of the CRM project

The production of a CRM as defined in ISO 17034 [3] is a project comprising planning, processing of the material, homogeneity and stability testing, characterisation and assigning of the property values and finally distribution and post-certification monitoring to control stability. ERM-EF001, a biodiesel material certified for selected parameters of EN 14214 [5], was released in 2014 following the above steps.

Some of the originally certified properties were retracted in 2018 as a result of the post certification monitoring to control stability, namely monoglyceride content, diglyceride content, total glycerol content, water content, and acid value. For this reason, it was decided to retract the triglyceride content as well from the current certificate, since its relevance is no longer given by the withdrawal of the other glycerides. All these properties will not be discussed further in this addendum.

The current project is regarding the update of the certificate of the existing ERM-EF001. Many processes, usually part of the production of a CRM, are therefore not dealt with here, only the measures taken to adapt ERM-EF001 to the newly revised measurement procedures are addressed (Figure 1).

In order to be able to classify the extent of the modifications made in a newly revised measurement procedures, these were first compared editorially with the former editions.

The changes found were then discussed with an expert laboratory to confirm, from a practical point of view, whether the modifications made in the measurement procedure could have any impact on the final measurement result or are only of an editorial nature without affecting the measurement result.

In case of a purely editorial change, the newly revised measurement procedure was assessed by a series of verification measurements performed by an expert laboratory. The verification measurement results obtained with the newly revised measurement procedure should not differ from the certified value as defined by the previous edition. If this was confirmed, the outdated measurement procedure on the certificate was replaced by the new measurement procedure, without changing the assigned certified value and its uncertainty.

In case of a fundamental technical modification, a completely new certified value was assigned, with the material being re-characterised in an interlaboratory comparison using the newly revised measurement procedure. Uncertainties from this new characterisation study were combined with the uncertainties from homogeneity and stability studies from the original project in 2014. Finally, certified values as defined by the newly revised measurement procedures and uncertainties were implemented in the certificate.

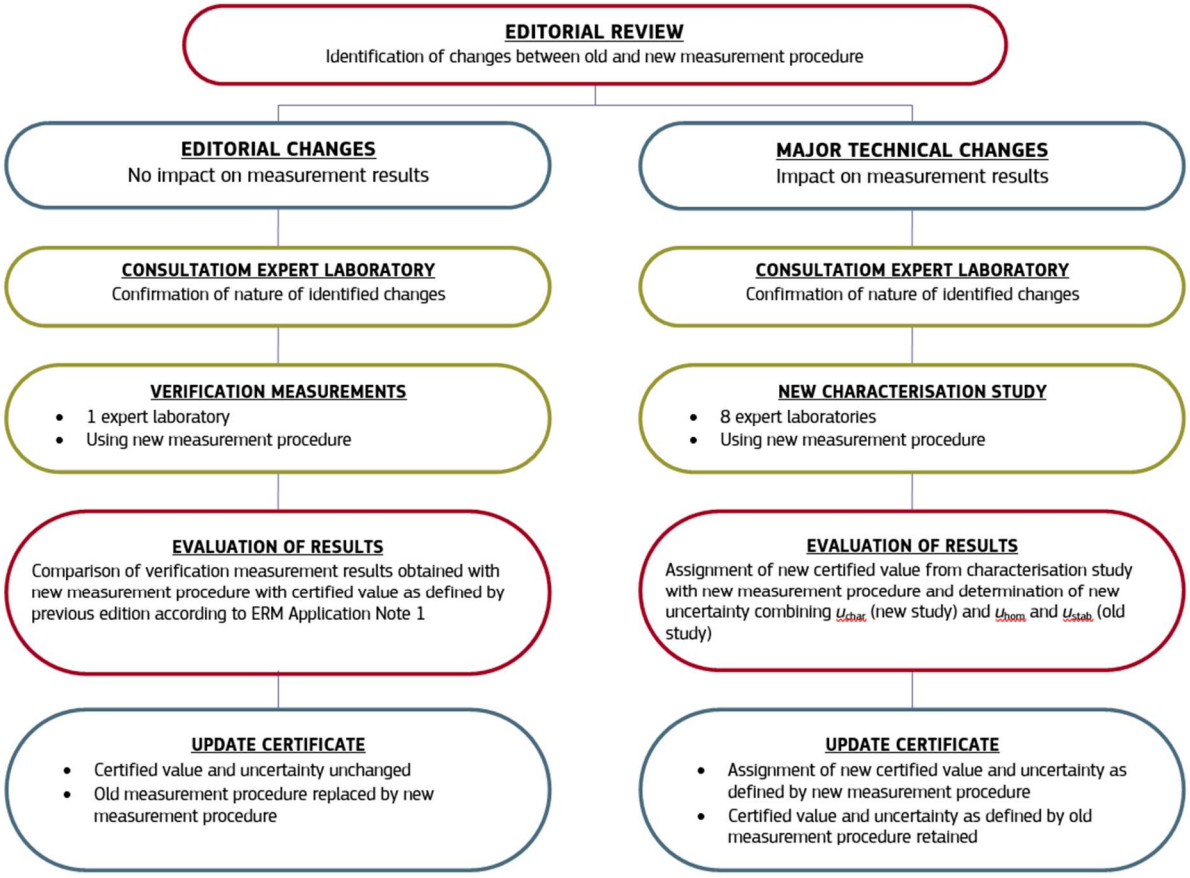

Figure 1: Process to update the certificate of ERM-EF001 to newly revised measurement procedures

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) [20].

The current project, including the outcome of the review process of the measurement procedures, the evaluation of the obtained measurement data from the verification study for editorially changes measurement procedures, the characterisation study for technically changed measurement procedures and the assignment of certified values and uncertainties, was subjected to an internal peer-review.

2 Participants

2.1 Project management and data evaluation

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

2.2 Editorial review of measurement procedures

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE

2.3 Verification measurements

EESTI KESKKONNAUURINGUTE KESKUS OÜ (Estonian Environmental Research Centre), Tallinn, EE
(measurements under the scope of ISO/IEC 17025:2017 accreditation EAK L008)

2.4 Characterisation measurements

ASG Analytik-Service AG, Neusäss, DE
(measurements under the scope of ISO/IEC 17025:2017 accreditation D-PL-11334-01-00)

EESTI KESKKONNAUURINGUTE KESKUS OÜ (Estonian Environmental Research Centre), Tallinn, EE
(measurements under the scope of ISO/IEC 17025:2017 accreditation EAK L008)

INNOVHUB - Stazioni Sperimentali per l'Industria, Milan, IT

INTERTEK BELGIUM NV, Antwerp, BE
(measurements partially under the scope of ISO/IEC 17025 accreditation BELAC; No. 105-TEST)

ITERG - Département Analyse & Expertise, Canéjan, FR

NAITEC - Fundación I+D Automoción y Mecatrónica, Noain, ES

SGS ESPAÑOLA DE CONTROL, S.A., Barcelona, ES
(measurements under the scope of ISO/IEC 17025:2017 accreditation ENAC 14/LE249)

VÚRUP, a.s., Bratislava, SK
(measurements under the scope of ISO/IEC 17025:2017 accreditation SNAS No. S-119)

All laboratories are identified by a code (e.g. L01). The numbering is not in the alphabetical order presented above.

3 Editorial review of measurement procedures

To classify the extent of the modifications made in the newly revised measurement procedures, the new version of the measurement procedures were first compared with the former editions by the JRC (Geel, BE).

EN 14103:2020 [13] describes the determination of the mass percentage of total methyl esters of fatty acids and the mass percentage of linolenic acid methyl ester present in the sample, by gas chromatography (GC) according to a procedure using internal calibration (nonadecanoic acid methyl ester) (see Annex A Table A1). EN 14103:2020 [13] supersedes EN 14103:2011 [6]. In comparison with the previous edition, the following technical modifications have been made: a) note on natural nonadecanoic acid methyl ester added in the scope; b) new procedure to check nonadecanoic acid methyl ester purity, with new GC conditions, and reduction of the minimum GC purity (99.5 to 99.0 % (m/m)); c) calculation of results revised by incorporation of theoretical flame ionization detector correction factor, which gives a better accuracy of the calculated contents in case of presence of methyl esters with short chains; d) new interlaboratory study conducted and precision adopted; e) new sample chromatograms recorded and added; f) calculation of the pattern of fatty acid methyl esters (FAMEs) incorporated as informative Annex C; g) modification of the way of integration by taking all the peaks into consideration whereas in the previous edition all the peaks identified as FAMEs were taken into consideration; h) increase of the FAME sample test portion to 250 mg whereas in the previous edition the sample test portion was 100 mg; i) document revised editorially. It was concluded that the modifications have an impact on the measurement results.

EN ISO 12185:1996 specifies a measurement procedure for the determination of density. This measurement procedure remained unchanged since the release of ERM-EF001. Hence, no further action was taken.

EN ISO 3104:2020 [14] specifies a measurement procedure to measure the time for a fixed volume of liquid to flow under gravity through the glass capillary of a calibrated viscosimeter under a reproducible driving head and at a known and closely controlled temperature. The kinematic viscosity is the product of the measured flow time and the calibration constant of the viscosimeter (see Annex A Table A2). EN ISO 3104:2020 [14] supersedes EN ISO 3104:1996 [8]. In comparison with the previous edition, the following modifications have been made: (a) precision data have been updated to all actual fuels on the market. (NOTE: no changes for FAMEs), (b) biodiesel blends and paraffinic diesel have been included in the scope (NOTE: not relevant in this context), (c) the procedure description and allowance of automated techniques have been included (NOTE: Previous edition allowed already automated viscosimeters, i.e. automated viscometers, which have been shown to measure kinematic viscosity within the limits of precision given in clause 14, are acceptable alternatives.) It was concluded that none of these changes should have an impact on the measurement result for ERM-EF001.

EN 14112:2020 [15] specifies a measurement procedure for the determination of the oxidation stability of FAMEs at 110 °C, by means of measuring the induction period up to 48 h. EN 15751:2014 [18] describes a similar measurement procedure for oxidation stability determination of pure FAMEs and of blends of FAME with petroleum based diesel (see Annex A Table A3). In principle, EN 15751 is based on EN 14112, which was specifically adapted for the determination of oxidation stability of FAMEs. At the time of development the measurement procedure was applicable for FAME fuel according to EN 14214 [5], but questions remained on the accuracy

towards blends of FAME and diesel fuel. The goal was to have one single measurement procedure for FAME fuel, diesel/FAME blends and pure diesel fuels. Although the modifications cover FAME fuel and diesel/FAME blends, the European Committee for Standardization (CEN/TC 307) decided that it was better to retain EN 14112 for methyl esters and publish a separate standard for all automotive fuel and heating oil applications, as the use of 'diesel and diesel blends' falls out the scope of CEN/TC 307. EN 14112:2020 [15] supersedes EN 14112:2003 [9]. In comparison with the previous edition, the following modifications have been made: (a) change of Figure 2, removal of dimension between air inlet and heating block; (b) introduction removed, (c) document revised editorially. It was concluded that none of these changes should have an impact on the measurement result and that EN 15751 is equivalent to EN 14112 for this purpose.

EN 14111:2003 specifies a measurement procedure for the determination of the iodine value. This measurement procedure remained unchanged since the release of ERM-EF001. Hence, no further action was taken.

EN ISO 3679:2015 [16] is used to determine whether a product will or will not flash at a specified temperature (flash no-flash Procedure A) or the flash point of a sample (Procedure B) (see Annex A Table A4). EN ISO 3679:2015 [16] supersedes EN ISO 3679:2004 [11]. In comparison with the previous edition, the following modifications have been made: (a) incorporation of ISO 3680 flash point technique into flash/no flash technique as a separate procedure due to the fact that many apparatus on the market combine both tests (NOTE: the flash no- flash procedure is not relevant for ERM-EF001; the certified property is the flash point of biodiesel); (b) title change (NOTE: not relevant), (c) revision of temperature measuring device requirements (NOTE: not relevant), (d) new precision covering both gas and electric ignition (NOTE: in fact only precision data for gas ignition given; previous edition gives only gas ignition). It was concluded that none of these changes should have an impact on the measurement result for ERM-EF001.

EN 14110:2019 [17] specifies a measurement procedure for the determination of the methanol content of FAME for use as diesel fuel and domestic heating fuel. The sample is heated at 80 °C in a hermetically sealed vial to allow desorption of contained methanol into the gas phase. When the equilibrium is reached a defined part of the gas phase is injected into a GC, where methanol is detected with a flame ionization detector (see Annex A Table A5). The amount of methanol can be determined either by internal calibration (procedure A) or by external calibration (procedure B). EN 14110:2019 [17] supersedes EN 14110:2003 [12]. In comparison with the previous edition, the following modifications have been made: (a) addition of formula (1) - resolution between methanol and 2-propanol, (b) correction of the formula to calculate the methanol content based on external calibration, (c) addition of Clause 2 - Normative References, (d) addition of Clause 7 - Sampling. It was concluded that none of these changes should have an impact on the measurement result for ERM-EF001.

The identified changes were discussed with an expert laboratory holding an ISO/IEC 17025 accreditation for the concerned measurements, to assess whether the modifications made in the measurement procedure could have any impact on the final measurement result or are just editorial in nature.

A fundamental technical change was confirmed for EN 14103:2020 [13]. The application of the newly revised measurement procedure will have an impact on the measurement results of the ester and linolenic acid methyl ester content. Hence, it was decided that new certified values and uncertainties are assigned, with the material being re-characterised by an interlaboratory

comparison of laboratories of demonstrated competence using the newly revised measurement procedure (Section 5).

A purely editorial change was confirmed for viscosity (EN ISO 3104:2020 [14]), the oxidation stability (EN 14112:2020 [15] and EN 15751:2014 [18]), flash point (EN ISO 3679:2015 [16]), and methanol content (EN 14110:2019 [17]). To confirm this assessment, it was decided to test these properties by another expert laboratory using the newly revised measurement procedures (verification measurements).

Additionally, it was decided to include a newly published measurement procedure for the iodine value (EN 16300:2012 [19]), which, according to the expert laboratory, is being used more and more in practice (see Annex A Table A6). This European Standard specifies a calculation procedure for the determination of the iodine value. A new certified value and uncertainty will be assigned, with the material being characterised by an interlaboratory comparison of laboratories of demonstrated competence using EN 16300:2012 (Section 5).

4 Verification measurements

4.1 Study setup

The verification measurements were performed by the Estonian Environmental Research Centre (Tallinn, EE) using the newly revised measurement procedures (Table 1).

Table 1: Measurement procedures used for certified values of ERM-EF001 and for verification measurements

Property	Measurement procedures used for certified values	Measurement procedures used for verification measurements
Viscosity	EN ISO 3104:1996 [8]	EN ISO 3104:2020 [14]
Oxidation stability	EN 14112:2003 [9]	EN 15751:2014 [18], equivalent to EN 14112:2020 [15]
Flash point	EN ISO 3679:2004 [11]	EN ISO 3679:2015 [16]
Methanol content (indicative value)	EN 14110:2003 [12]	EN 14110:2019 [17]

The different sample intakes required for the individual measurands resulted in a different number of units being made available for the measurements. The laboratory received

- two units of ERM-EF001 for the measurements of the oxidation stability and was requested to provide six independent results, three per unit,
- three units of ERM-EF001 for the measurements of the flash point and was requested to provide six independent results, two per unit,
- and six units of ERM-EF001 for each, the methanol content and viscosity and was requested to provide six independent results, one per unit.

All measurements, apart from viscosity, were performed under intermediate precision conditions (different working days) due to the required analysis times.

4.2 Evaluation of verification measurement results

The individual verification measurement results (VMR) obtained with the newly revised measurement procedures are displayed in Table 2.

Table 2: Datasets as reported for each property

Property	Viscosity [mm^2/s]	Oxidation stability [h]	Flash point [°C]	Methanol content [% (m/m)]
Replicate 1	4.4710	10.70	174.9	0.04432
Replicate 2	4.4719	10.71	174.8	0.04314
Replicate 3	4.4727	10.40	175.1	0.04703
Replicate 4	4.4733	10.83	175.4	0.05379
Replicate 5	4.4750	10.56	176.7	0.05431
Replicate 6	4.4710	10.44	176.6	0.05294

The assessment of the agreement between verification measurement results obtained with the newly revised measurement procedure and the certified value as defined by the previous edition was carried out according to the procedure described in the ERM Application Note 1 [21].

The difference between the verification measurements results and the certified value is compared with its uncertainty, i.e. the combined uncertainty of the two values, where the difference between the verification measurements results and the certified value of ERM-EF001, Δ_{meas}, is calculated as

$$\Delta_{meas} = |c_{meas} - c_{CRM}| \qquad \textbf{Equation 1}$$

c_{meas} mean measured value obtained with the newly revised measurement procedure
c_{CRM} certified value from ERM-EF001 obtained with the previous edition

The uncertainty of Δ_{meas} is calculated as:

$$U_{\Delta} = k \cdot \sqrt{u_{meas}^2 + u_{CRM}^2} \qquad \textbf{Equation 2}$$

U_{Δ} expanded combined uncertainty of the verification measurement results and the certified value
u_{meas} standard uncertainty of verification measurement results derived from the newly revised measurement procedure
u_{CRM} uncertainty of certified value
k coverage factor of 2 corresponding to a confidence level of approximately 95 %

The expanded uncertainty of the verification measurement results (U_{meas}) is derived from the respective revised measurement procedures. They give information on expected performance figures, i.e. repeatability and reproducibility limits (Table 3).

A repeatability limit, *r*, is the value of the maximum absolute difference between two single test results obtained under repeatability conditions that can be expected at a certain probability (usually 95 %). A reproducibility limit, *R*, is similarly defined for test results obtained under reproducibility conditions [22]. A repeatability limit is calculated from:

$$r = t \cdot \sqrt{2} \cdot s_r \qquad \textbf{Equation 3}$$

where t (1.96) is the two-tailed Student t value at the 95 % confidence level and s_r is the repeatability standard deviation.

Similarly, the reproducibility limit is calculated from:

$$R = t \cdot \sqrt{2} \cdot s_R \qquad \textbf{Equation 4}$$

where s_R is the reproducibility standard deviation.

The final expanded measurement uncertainty (U_{meas}) for the verification measurements was estimated using n=6 for the replicate measurements

$$U_{meas} = 2 \cdot \sqrt{s_L^2 + \frac{s_r^2}{n}} \qquad \textbf{Equation 5}$$

and the performance figures of the measurement procedure where the standard deviation between laboratories (s_L) is calculated as follows

$$s_L = \sqrt{s_R^2 - s_r^2} \qquad \textbf{Equation 6}$$

Table 3: Performance figures as laid down in respective measurement procedures and estimated expanded measurement uncertainties thereof

Measurand	Unit	*r*	*R*	U_{meas}
Viscosity as defined by EN ISO 3104:2020	[mm^2/s]	0.010	0.021	0.013
Viscosity as defined by EN ISO 3104:1994	[mm^2/s]	0.005	0.029	0.020
Oxidation stability as defined by EN 15751:2014	[h]	0.7	2.4	1.7
Oxidation stability as defined by EN 14112:2003	[h]	1.0	2.8	1.9
Flash point as defined by EN ISO 3679:2015	[°C]	1.9	15.0	10.6
Flash point as defined by EN ISO 3679:2004	[°C]	1.9	15.0	10.6
Methanol as defined by EN 14110:2019	[% (/m/m)]	0.004	0.014	0.010
Methanol as defined by EN 14110:2003	[% (/m/m)]	0.003	0.012	0.008

If the absolute difference between the values obtained from the verification measurements and the certified value is equal or smaller than the expanded combined uncertainty of the values from the verification measurements and the certified value, $\Delta_{meas} \leq U_{\Delta}$, then there is no significant difference between the verification measurement results obtained with the newly revised measurement procedure and the certified value as defined by the previous edition. This has been proven for all properties (Table 4).

Table 4: Comparison of verification measurement results with certified values using ERM Application Note 1 [21]

Property	Viscosity	Oxidation stability	Flash point	Methanol content
Unit	[mm^2/s}	[h]	[°C]	[% (m/m)]
c_{CRM}	4.465	9.8	181	0.041
U_{CRM}	0.005	0.5	14	0.016
c_{meas} [1)]	4.472	10.6	176	0.049
U_{meas}	0.013	1.7	11	0.010
Δ_{meas}	0.007	0.8	5	0.008
U_{Δ}	0.014	1.7	18	0.019
$\Delta_{meas} \leq U_{\Delta}$	YES	YES	YES	YES

Graphical depictions of the verification measurement results obtained with the newly revised measurement procedures and the results of the individual laboratories obtained in the characterisation study of ERM-EF001 are given in Figures 2 to 5.

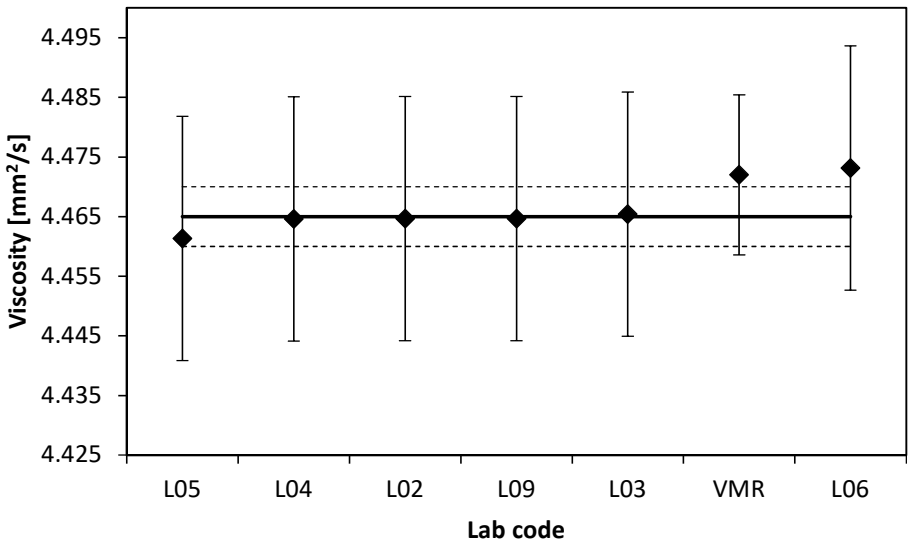

Figure 2: Results for viscosity (continuous line: certified value as defined by EN ISO 3104:1994; dashed line: expanded uncertainty of certified value with k = 2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN ISO 3104:1994 for L02 to L09, and EN ISO 3104:2020 for VMR)

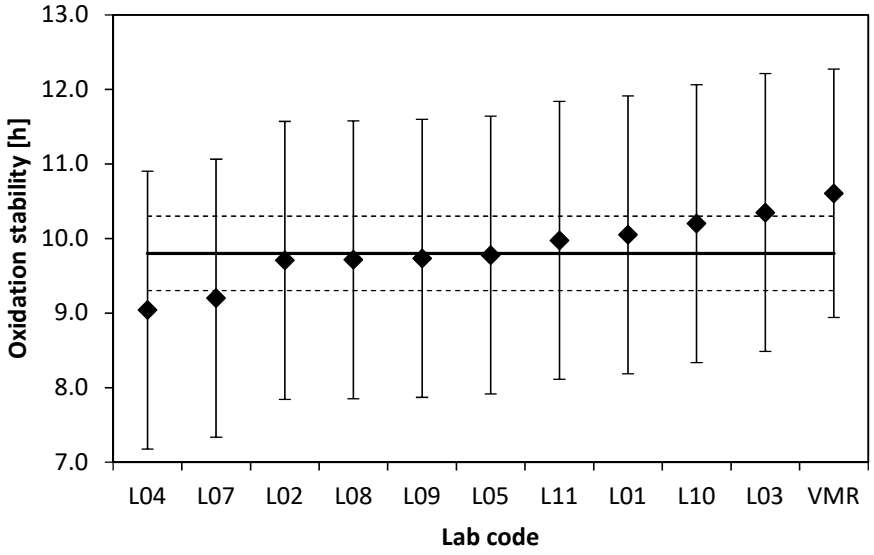

Figure 3: Results for the oxidation stability (continuous line: certified value as defined by EN 14112:2003; dashed line: expanded uncertainty of certified value with k = 2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN 14112:2003 for L01 to L11, and EN 15751:2014 for VMR)

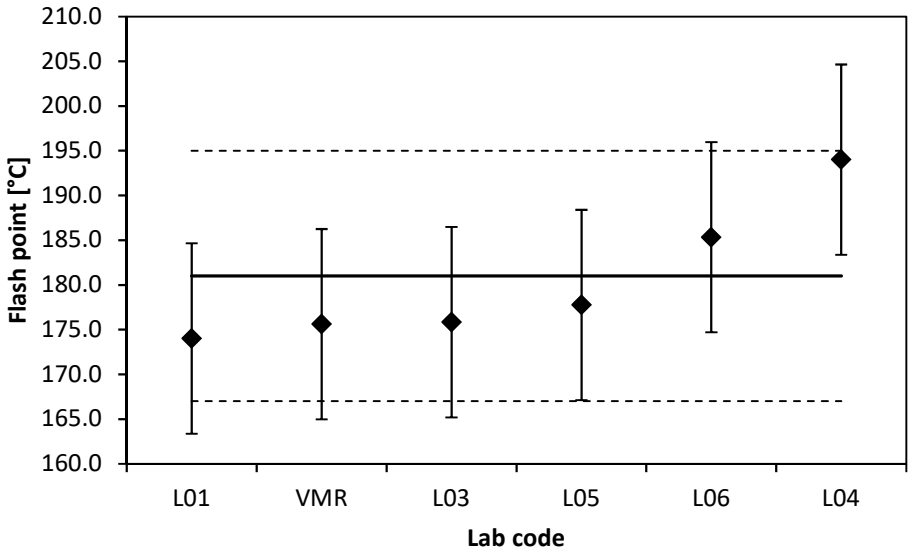

Figure 4: Results for flash point (continuous line: certified value as defined by EN ISO 3679:2004; dashed line: expanded uncertainty of certified value with k = 2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN ISO 3679:2004 for L01 to L06, and EN ISO 3679:2015 for VMR)

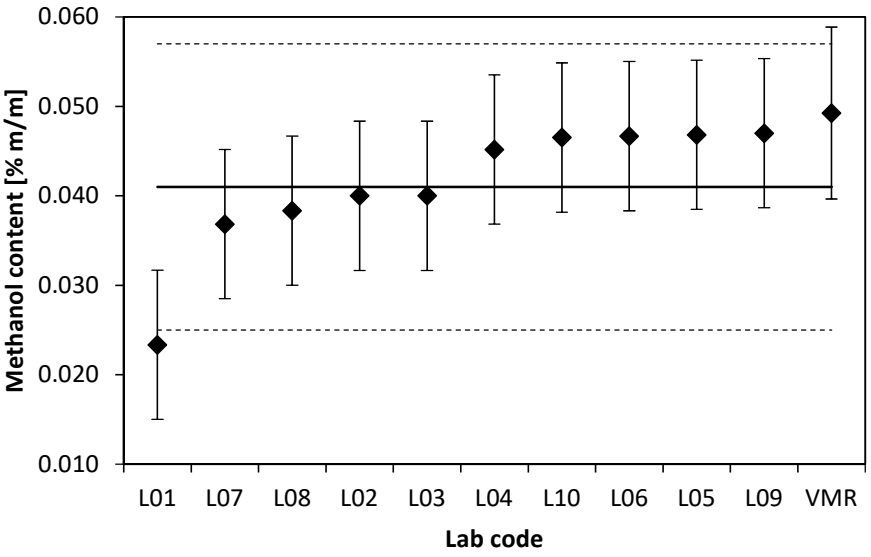

Figure 5: Results for methanol content (continuous line: certified value as defined by EN 14110:2003; dashed line: expanded uncertainty of certified value with k = 2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN 14110:2003 for L01 to L10 and EN 14110:2019 for VMR)

For all properties, the verification measurement results obtained with the newly revised measurement procedure did not differ from the certified value as defined by the previous edition. Hence, the old measurement procedures on the certificate are replaced by the latest editions, without changing the assigned certified values and their uncertainties (Table 5).

Table 5: Resulting updates to the certificate for editorially changed measurement procedures

	Certified value [5]	Uncertainty [6]	Unit
Oxidation stability (at 110 °C) [1]	9.8	0.5	[h]
Flash point [2]	181	14	[°C]
	Indicative value [7]	Uncertainty [8]	Unit
Methanol content [3]	0.041	0.016	[% (m/m)] [4]

1) As defined by **EN 15751:2015** and **EN 14112:2020**
2) As defined by **EN ISO 3679:2015**
3) As defined by **EN 14110:2019**
4) As called in **EN 14110:2020**, which is equivalent to 10^{-2} g/g
5) 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. The certified value and its uncertainty are traceable to the International System of Units (SI).
6) 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.
7) Indicative values are values where either the uncertainty is deemed too large or where too few independent datasets are available to allow certification and are therefore less reliable than certified values. Great caution should be used when using these values. The given value is an unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The indicative value and its uncertainty are traceable to the International System of Units (SI).
8) The uncertainty of the indicative value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO 17034:2016 and ISO Guide 35:2017.

Although the changes in the newly revised measurement procedure for viscosity were deemed to have no impact on the final measurement result, a different approach was chosen for its update on the certificate. Based on stability monitoring data received, which were gathered at the same time as the verification measurement results, it was decided to subject this property to a completely new characterisation and assign a new certified value and uncertainty (Section 5). The stability monitoring showed a trend towards increasing viscosity. If this trend were to continue in the next stability monitoring, viscosity would have to be withdrawn from the certificate, whereas a completely new re-characterisation will allow a longer use of the certified property.

5 Characterisation

The material characterisation is the process of determining the property values of a reference material.

The outcome of the editorial review (Section 3) was to re-characterise the ester and linolenic acid methyl ester content according to EN 14103:2020 [13].

In addition, viscosity was included for a complete re-characterisation using EN ISO 3104:2020 [14], although the changes in the newly revised measurement procedure were deemed to have no impact on the final measurement result (see Section 3.3). The decision to perform a complete re-characterisation was based on observing changes in the certified value for viscosity during stability monitoring.

Moreover, the iodine value according to EN 16300:2012 [19] was included. EN 16300:2012 [19] specifies a calculation procedure for the determination of the iodine value. The calculation procedure uses as data entry the results from the gas chromatography determination according to EN 14103 of individual fatty acid methyl esters for the determination of the iodine value. It is important to recognise that the latest version of EN 14103 is to be used for the determination of individual FAME components. The measurement procedure is not intended as a replacement for EN 14111 [10].

The re-characterisation was based on an interlaboratory comparison of expert laboratories, i.e. the properties of the material were determined in different laboratories to demonstrate the absence of a measurement bias. Due to the nature of the measurands all participants used the same measurement procedures 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.

5.1 Selection of participants

Eight 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. Laboratory proficiency in the field of biodiesel measurements was demonstrated by all laboratories through their successful participation in the interlaboratory comparison for the initial characterisation of ERM-EF001. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 [2] was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

5.2 Study setup

Each laboratory received three units of ERM-EF001 for the measurements of the ester content, the linolenic acid methyl ester content and iodine value and was requested to provide six independent results, two per unit. Furthermore, each laboratory received six units of ERM-EF001 for the measurements of viscosity and was requested to provide six independent results, one per unit.

The sample preparations and measurements had to be done on three days to ensure intermediate precision conditions. An independent calibration was performed for each result whenever possible.

Laboratories were not requested to submit measurement uncertainties. Instead, the performance figures specified in the documentary standards were used, which give information on expected repeatability and reproducibility limits (see Section 4.2).

5.3 Measurement procedures used

All laboratories used the same measurement procedures for the selected measurands, i.e.

- ester content as defined by EN 14103:2020 [13];
- linolenic acid methyl ester content as defined by EN 14103:2020 [13];
- viscosity at 40 °C as defined by EN ISO 3104:2020 [14];
- iodine value as defined by EN 16300:2012 [19].

5.4 Evaluation of results

The characterisation study resulted in eight datasets for the ester content, linolenic acid methyl ester content, and iodine value and seven datasets for viscosity. All individual results of the participating laboratories, grouped per measurand, are displayed in tabular and graphical form in Annex B.

5.4.1 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 preparations and measurements performed on three days
- method performance, i.e. agreement of the measurement results with performance figures of the measurement procedure (see Section 4.2 and Table 6)
    - Datasets were rejected when the absolute difference between two independent test results from the same unit exceeded the repeatability limit (r) as laid down in the measurement procedure
    - Datasets were rejected when the absolute difference between two independent test results from two different units exceeded the reproducibility limit (R) as laid down in the measurement procedure.

Table 6: Performance figures as laid down in respective measurement procedures and estimated expanded measurement uncertainties thereof

Measurand	Unit	r	R	U_{meas}
Ester content	[% (m/m)]	1.65	2.45	1.38
Linolenic acid methyl ester content	[% (m/m)]	0.11	0.23	0.15
Viscosity at 40 °C	mm^2/s	0.010	0.021	0.013
Iodine value	[g iodine/100 g]	0.87	6.81	4.83

All laboratories complied with the instructions and were strictly following the measurement procedures. Method performance for most of the laboratories was in agreement with the repeatability and reproducibility limits, despite the fact that the measurements were performed on three days. Based on the above criteria, the following datasets were rejected as not technically valid (Table 7).

Table 7: Datasets that showed non-compliance with the instructions given and technical specifications, and action taken

Measurand	Lab code	Description of problem	Action taken
Ester content	L06	Technical problem with GC	Not used for evaluation
Linolenic acid methyl ester content	L06	Technical problem with GC	Not used for evaluation
Viscosity	L07	Reproducibility limit not met	Not used for evaluation
Iodine value	L06; L07	L06: Technical problem with GC; L07: Repeatability limit not met	Not used for evaluation

Laboratory 07 did not meet the repeatability limit for the iodine value and the reproducibility limit for viscosity. As the laboratory confirmed that this was not a transcription error, the datasets were rejected.

The datasets for the ester content, the linolenic acid methyl ester content, and iodine value from laboratory L06 were not used for the evaluation as the laboratory reported a technical issue while using the GC that caused incorrect results. The lab retracted the results.

5.4.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using 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-EF001. *p*: number of technically valid datasets

Measurand	*p*	Outliers		Normally distributed	Statistical parameters				
		Means	Variances		Unit	Mean	*s*	$s_{between}$	s_{within}
Ester content	7	none	none	yes	[% (m/m)]	97.387	0.603	0.587	0.333
Linolenic acid methyl ester	7	none	none	yes	[% (m/m)]	8.515	0.100	0.099	0.039
Viscosity	6	none	yes	yes	[mm^2/s]	4.4739	0.0065	0.0064	0.0024
Iodine value	6	none	none	yes	[g iodine/100 g]	107.289	0.905	0.896	0.317

For all parameters the laboratory means follow normal distributions. None of the data contains outlying means.

The statistical evaluation flags laboratory 3 as outlying variance for viscosity while its mean result for this measurand still agrees with the other data. Laboratory 3 was using a manual glass viscometers (procedure A) whereas the others used a glass capillary viscometers in an automated assembly (procedure B), both specified in the measurement procedure. Finally, all datasets were retained, as all results still agree well with the repeatability and reproducibility requirements of the respective measurement procedure.

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

Table 9: Uncertainties of characterisation for ERM-EF001. *p*: number of technically valid datasets

Measurand	*p*	Unit	Mean	*s*	u_{char}
Ester content	7	[% (m/m)]	97.387	0.603	0.228
Linolenic acid methyl ester content	7	[% (m/m)]	8.515	0.100	0.038
Viscosity	6	[mm^2/s]	4.4739	0.0065	0.0027
Iodine value	6	[g iodine/100 g]	107.289	0.905	0.369

6 Value Assignment

Certified values are values that fulfil the highest standards of accuracy. Procedures at JRC Directorate F recommend pooling of at least six datasets to assign certified values. Full uncertainty budgets in accordance with ISO 17034 [3] and ISO Guide 35 [4] were established.

New certified values were assigned for the ester content, linolenic acid methyl ester content, viscosity, and iodine value.

6.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 8 were assigned as certified values for the ester and linolenic acid methyl ester content according to EN 14103:2020 [13], viscosity at 40 °C according to EN ISO 3104:2020 [14], and the iodine value according to EN 16300:2012 [19].

The assigned uncertainty consists of uncertainties relating to characterisation (u_{char}) reported in this addendum, whereas potential between-unit inhomogeneity (u_{bb}), and potential degradation during transport (u_{sts}), and long-term storage (u_{lts}) derive from EUR 26711 EN [1]. 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:

$$U_{CRM, rel} = k \cdot \sqrt{u^2_{bb, rel} + u^2_{sts, rel} + u^2_{lts, rel} + u^2_{char, rel}} \qquad \textbf{Equation 7}$$

- u_{char} was estimated as described in Section 5 of this addendum.
- u_{bb} was estimated as described in EUR 26711 EN [1] in Section 4.
- u_{sts} and u_{lts} were estimated as described in EUR 26711 EN [1] in Section 5.

The choice of the coverage (k) factor was based on the number of effective degrees of freedom as calculated using the Welch-Satterthwaite equation [20]. Applying this equation, the effective degrees of freedom shown in Table 10 were obtained.

Table 10: Effective degrees of freedom calculated using the Welch-Satterthwaite equation

Certified property	Effective degrees of freedom
Ester content	10
Linolenic acid methyl ester content	9
Iodine value	33
Viscosity (at 40 °C)	8

The JRC's procedures for assigning uncertainties to certified values stipulate that for more than five effective degrees of freedom a coverage (k) factor of 2 can be chosen. Therefore, a k-factor of 2 was applied to obtain the expanded uncertainties. The certified values and their uncertainties are summarised in Table 11.

Table 11: Certified values and their uncertainties for ERM-EF001

Certified property	Unit	Certified value	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [1)]
Ester content	[% (m/m)]	97.4	0.234	0.057	0.001	0.178	0.60	0.6
Linolenic acid methyl ester content	[% (m/m)]	8.52	0.444	0.068	0.001	0.208	1.0	0.09
Viscosity (at 40 °C)	[mm^2/s]	4.474	0.0593	0.0143	0.00019	0.028	0.134	0.006
Iodine value	[g iodine/100 g]	107.3	0.344	0.478	0.005	0.657	1.77	1.9

[1)] Expanded (k = 2) and rounded uncertainties; uncertainties are always rounded up [23] and in a way that the rounding error corresponds to 3 % to 30 % of the uncertainty

7 Metrological traceability

7.1 Metrological traceability

Identity – Editorially changed measurement procedures

Certified and indicative values and their uncertainties remained unchanged for the oxidation stability, the flash point and the methanol content. The verification measurements for editorially changed measurement procedures confirmed the validity of the assigned certified values and their uncertainties for the oxidation stability as defined by EN 15751:2014 [18], which is equivalent to EN 14112:2020 [15], the flash point as defined by EN ISO 3679:2015 [16], and the assigned indicative value for the methanol content as defined by EN 14110:2019 [17]. Consequently, the new identity statements are as follows:

Oxidation stability is an operationally defined measurand and can only be obtained by following the measurement procedures specified in EN 15751:2014 [18] and EN 14112:2020 [15].

Flash point is an operationally defined measurand and can only be obtained by following the measurement procedure specified in EN ISO 3679:2015 [16].

Methanol content is an operationally defined measurand and can only be obtained by following the measurement procedure specified in EN 14110:2019 [17].

A new certified value and uncertainty was assigned for viscosity although the changes in the newly revised measurement procedure were deemed to have no impact on the final measurement result. The update was prompted by stability issues that required the assignment of a new certified value. In this case, the certified value as defined by EN ISO 3104:1996 [8] is no longer valid and withdrawn from the certificate. Consequently, the new identity statement is as follows:

Viscosity is an operationally defined measurand and can only be obtained by following the measurement procedure specified in EN ISO 3104:2020 [16].

Identity – Technically changed measurement procedures

New certified values and uncertainties were assigned for technically changed measurement procedures, i.e. ester and linolenic acid methyl ester content as defined by 14103:2020 [13], and iodine value as defined by EN 16300:2012 [19]. The identity statements for the new certified values and uncertainties are as follows:

Ester and linolenic acid methyl ester content, and iodine value are operationally defined measurands and can only be obtained by following the measurement procedures specified in EN 14103:2020 [13] and EN 16300:2012 [19].

In addition, the certified values and uncertainties as defined by their corresponding old measurement procedures are retained on the certificate to be used independently from the new measurement procedure.

Quantity value - Technically changed measurement procedures

Traceability of the obtained results is based on the traceability of all relevant input factors. Investigation of the measurement procedure and measurement details of the individual results show that all relevant input parameters of each technically accepted dataset have been properly calibrated. All technically accepted datasets are therefore traceable to the same reference, namely the SI. 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 the SI, the assigned quantity values themselves are traceable to the SI as well.

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9 References

1 M. Ulberth-Buchgraber, V. Morales, J. Charoud-Got, H. Emteborg, A. Held, The certification of the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point of biodiesel: ERM®- EF001, EUR 26711, European Commission, Luxembourg, 2018

2 ISO/IEC 17025:2017, General requirements for the competence of testing and calibration laboratories, International Organization for Standardization, Geneva, Switzerland

3 ISO 17034:2016, General requirements for the competence of reference material producers, International Organization for Standardization, Geneva, Switzerland

4 ISO Guide 35:2017, Reference materials – Guidance for characterization and assessment of homogeneity and stability, International Organization for Standardization, Geneva, Switzerland

5 EN 14214:2012, Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods. European Committee for Standardization, Brussels, Belgium

6 EN 14103:2011, Fat and oil derivates - Fatty acid methyl esters (FAME) - Determination of ester and linolenic acid methyl ester contents. European Committee for Standardization, Brussels, Belgium

7 EN ISO 12185:1996, Crude petroleum and petroleum products -Determination of density - Oscillating U-tube method. European Committee for Standardization, Brussels, Belgium

8 EN ISO 3104:1996, Petroleum products - Transparent and opaque liquids - Determination of kinematic viscosity and calculation of dynamic viscosity. European Committee for Standardization, Brussels, Belgium

9 EN 14112:2003, Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of oxidation stability (accelerated oxidation test). European Committee for Standardization, Brussels, Belgium

10 EN 14111:2003, Oil and fat derivatives - Fatty Acid Methyl Esters (FAME) - Determination of iodine value. European Committee for Standardization, Brussels, Belgium

11 EN ISO 3679:2004, Determination of flash point - Rapid equilibrium closed cup method. European Committee for Standardization, Brussels, Belgium

12 EN 14110:2003, Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of methanol content. European Committee for Standardization, Brussels, Belgium

13 EN 14103:2020, Fat and oil derivatives - Fatty acid methyl esters (FAME) - Determination of ester and linolenic acid methyl ester contents. European Committee for Standardization, Brussels, Belgium

14 EN ISO 3104:2020, Petroleum products - Transparent and opaque liquids - Determination of kinematic viscosity and calculation of dynamic viscosity. European Committee for Standardization, Brussels, Belgium

15 EN 14112:2020, Fat and oil derivatives - Fatty acid methyl esters (FAME) - Determination of oxidation stability (accelerated oxidation test). European Committee for Standardization, Brussels, Belgium

16 EN ISO 3679:2015, Determination of flash no-flash and flash point - Rapid equilibrium closed cup method. European Committee for Standardization, Brussels, Belgium

17 EN 14110:2019, Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of methanol content. European Committee for Standardization, Brussels, Belgium

18 EN 15751:2014, Automotive fuels - Fatty acid methyl ester (FAME) fuel and blends with diesel fuel - Determination of oxidation stability by accelerated oxidation method. European Committee for Standardization, Brussels, Belgium

19 EN 16300:2012, Automotive fuels - Determination of iodine value in fatty acid methyl esters (FAME) - Calculation method from gas chromatographic data. European Committee for Standardization, Brussels, Belgium

20 ISO/IEC Guide 98-3:2008, Uncertainty of measurement – Part 3: Guide to the Expression of Uncertainty in Measurement (GUM 1995), International Organization for Standardization, Geneva, Switzerland

21 T.P.J. Linsinger, ERM Application Note 1: Comparison of a measurement result with the certified value, https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes (last accessed on 03/05/2022)

22 ISO 5725-1:1994, Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definition, International Organization for Standardization, Geneva, Switzerland

23 DIN 1333:1992-02, Zahlenangaben, Deutsches Institut für Normung e.V., Berlin, Germany

Annexes

Annex A: Scope of measurement procedures used for the verification measurements and the characterisation study

Table A1: Measurement procedure for the ester and linolenic acid methyl ester contents

Standard Reference	**EN 14103:2020**
Technical Body	CEN/TC 307 - Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis
Title	Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of **ester and linolenic acid methyl ester contents**
Scope	The purpose of this document is to describe a procedure for the determination of the ester content in fatty acid methyl esters (FAME) intended for incorporation into diesel oil. It also allows determining the linolenic acid methyl ester content. It allows verifying that the ester content of FAME is greater than 90 % (m/m) and that the linolenic acid methyl ester content is between 1 % (m/m) and 15 % (m/m). The precision was established using FAMEs with an ester content of 95 % (m/m) and 100 % (m/m) only, thus covering the range of the limit value. The method is also suitable outside of this range; however, precision for lower concentrations is subject to further work. This method is suitable for FAME which contains methyl esters between C6 and C24. NOTE 1 For the purposes of this document, the term "% (m/m)" is used to represent the mass fractions. This method was elaborated for FAME samples from usual raw material. For FAME sample from unidentified raw material, a solution of the test sample is prepared without any internal standard addition, in order to verify the absence of natural nonadecanoic acid methyl ester or other unknown substances co-eluting with the IS. NOTE 2 The calculation method of the pattern of fatty acid methyl esters is given in Annex C.

Table A2: Measurement procedure for viscosity

Standard Reference	**EN ISO 3104:2020**
Technical Body	ISO/TC 28 Petroleum and related products, fuels and lubricants from natural or synthetic sources
Title	Petroleum products — Transparent and opaque liquids — Determination of **kinematic viscosity** and calculation of dynamic viscosity
Scope	This document specifies Procedure A, using manual glass viscometers, and Procedure B, using glass capillary viscometers in an automated assembly, for the determination of the kinematic viscosity, v, of liquid petroleum products, both transparent and opaque, by measuring the time for a volume of liquid to flow under gravity through a calibrated glass capillary viscometer. The dynamic viscosity, n, is obtained by multiplying the measured kinematic viscosity by the density, ρ, of the liquid. The range of kinematic viscosities covered in this test method is from 0.2 mm^2/s to 300 000 mm^2/s over the temperature range 20 °C to +150 °C. NOTE The result obtained from this document is dependent upon the behaviour of the sample and is intended for application to liquids for which primarily the shear stress and shear rates are proportional (Newtonian flow behaviour). If, however, the viscosity varies significantly with the rate of shear, different results can be obtained from viscometers of different capillary diameters. The procedure and precision values for residual fuel oils, which under some conditions exhibit non-Newtonian behaviour, have been included.

Table A3: Measurement procedure for the oxidation stability

Standard Reference	**EN 15751:2014**
Technical Body	CEN/TC 19 Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin
Title	Automotive fuels - Fatty acid methyl ester (FAME) fuel and blends with diesel fuel - Determination of **oxidation stability** by accelerated oxidation method
Scope	This European Standard specifies a test method for the determination of the oxidation stability of fuels for diesel engines, by means of measuring the induction period of the fuel up to 48 h. The method is applicable to fatty acid methyl esters (FAME) intended for the use as pure biofuel or as a blending component for diesel fuels, and to blends of FAME with diesel fuel containing 2 % (V/V) of FAME at minimum. NOTE 1 EN 14112 [1] describes a similar test method for oxidation stability determination of pure fatty acid methyl esters (see the Introduction to this European Standard). NOTE 2 For induction periods higher than 48 h the precision is not covered by the precision statement of this method. The limit values of the relevant fuel standards are well within the scope of this test method. NOTE 3 The presence of cetane improver can reduce the oxidation stability determined by this test method. Limited studies with EHN (2-ethyl hexyl nitrate) indicated, however, that the stability is reduced to an extent which is within the reproducibility of the test method. NOTE 4 For the purposes of this European Standard, the term "% (V/V)" is used to represent the volume fraction (φ) of a material.

Table A4: Measurement procedure for flash point

Standard Reference	**EN ISO 3679:2015**
Technical Body	ISO/TC 28 Petroleum and related products, fuels and lubricants from natural or synthetic sources
Title	Determination of flash no-flash and **flash point** — Rapid equilibrium closed cup method
Scope	ISO 3679:2015 specifies procedures for flash point tests, within the temperature range of -30 °C to 300 °C, for paints, including water-borne paints, varnishes, binders for paints and varnishes, adhesives, solvents, petroleum, and related products. The procedures are used to determine whether a product will or will not flash at a specified temperature (flash no-flash Procedure A) or the flash point of a sample (Procedure B). When used in conjunction with a flash detector, ISO 3679:2015 is also suitable to determine the flash point of fatty acid methyl esters (FAME).

Table A5: Measurement procedure for methanol content

Standard Reference	**EN 14110:2019**
Technical Body	CEN/TC 307 - Oilseeds, vegetables and animal fats and oils and their by-products - Methods of sampling and analysis
Title	Fat and oil derivatives - Fatty Acid Methyl Esters - Determination of methanol content
Scope	This document specifies a method for the determination of the methanol content of fatty acid methyl esters (FAME) for use as diesel fuel and domestic heating fuel. The method is applicable to methanol contents between 0.01 % (m/m) and 0.5 % (m/m). The method is not applicable to mixtures of FAME containing other low boiling components. (NOTE For the purposes of this document, the terms "% (m/m)" and "% (V/V)" are used to represent respectively the mass fraction and the volume fraction) WARNING - The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all of the safety problems associated with its use. It is the responsibility of users of this document to take appropriate measures to ensure the safety and health of personnel prior to application of the standard, and fulfil statutory and regulatory requirements for this purpose.

Table A6: Measurement procedure for the iodine value

Standard Reference	**EN 16300:2012**
Technical Body	CEN/TC 19 Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin
Title	Automotive fuels - Determination of **iodine value** in fatty acid methyl esters (FAME) - Calculation method from gas chromatographic data
Scope	This European Standard specifies a calculation procedure for the determination of Iodine value ("CIV" - "calculated iodine value"), of fatty acid methyl esters (FAME) to be used either as automotive or heating fuel for diesel engines as specified in EN 14214 [2] or as an extender for automotive fuel for diesel engines as specified in EN 590 [4]. This procedure has originally been described in Annex B of EN 14214:2008 [2]. The calculation procedure is now specified for methyl esters between C14 and C24. The calculation procedure uses as data entry the results from the gas chromatography determination (GC) according to EN 14103 of individual fatty acid methyl esters and is based on AOCS recommended practice Cd 1c – 85 for the determination of the iodine value of edible oil from its fatty acid composition. It is important to recognise that the latest version of EN 14103 is to be used for the determination of individual FAME components. NOTE 1 Experience from the field and from several precision evaluation campaigns in Germany and elsewhere indicates that the results of the determination of iodine value by calculation specified here are very close to results obtained by titration with Wijs solvent according to EN 14111. Observed small differences were always found to be smaller than the reproducibility published in the actual EN 14111. For informative purposes only, but not for cases of dispute, EN 14331 may also be used to extract the FAME contents from FAME containing diesel fuels (like B5, B7, B30, etc.) and to use the contents of the individual FAME components from this method as data entry for the calculation specified in this European Standard. In principle, other fatty acid alkyl esters can also be analysed. However, neither the close correlation to the titration method EN 14111 has been verified nor is any precision information available for such an extension of application range. NOTE 2 For the purposes of this European Standard, the term "% (m/m)" is used to represent the mass fraction, μ, of a material.

Annex B: Results of the characterisation measurements

Table B1: Mass fraction of the ester content in ERM-EF001 as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L01	97.10	97.52	97.01	97.20	97.35	96.96	97.19	0.22
L02	98.3	98.4	97.8	98.1	98.1	97.9	98.10	0.23
L03	96.37	96.33	96.24	96.55	96.24	96.58	96.39	0.15
L04	97.5	97.4	96.7	96.9	97.8	97.4	97.3	0.42
L05	97.21	96.9	97.19	97.24	96.51	97.05	97.02	0.29
L07	97.5	98.6	98.6	97.3	98.2	97.6	98.0	0.59
L08	97.4	98.0	97.4	98.0	97.9	97.9	97.8	0.29
Results not used for value assignment								
L06	*94.6*	*95.1*	*93.2*	*93.9*	*93.4*	*93.4*	*93.9*	*0.81*

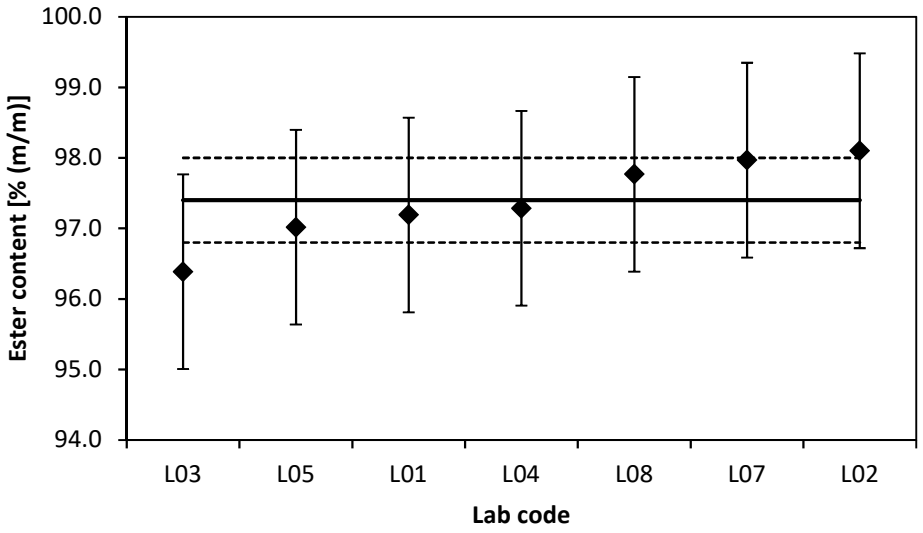


Figure B1: Results of the characterisation study for the mass fraction of the ester content in ERM-EF001 as defined by EN 14103:2020 (continuous line: certified value; dashed line: expanded uncertainty of certified value with k=2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN 14103:2020)

Table B2: Mass fraction of linolenic acid methyl ester content in ERM-EF001 as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L01	8.48	8.52	8.47	8.49	8.50	8.46	8.49	0.25
L02	8.7	8.7	8.6	8.7	8.6	8.6	8.7	0.63
L03	8.38	8.38	8.38	8.31	8.35	8.39	8.37	0.36
L04	8.5	8.5	8.4	8.4	8.5	8.5	8.5	0.61
L05	8.47	8.46	8.46	8.47	8.4	8.45	8.45	0.31
L07	8.55	8.61	8.64	8.53	8.6	8.55	8.58	0.50
L08	8.57	8.63	8.57	8.64	8.60	8.61	8.60	0.34
Results not used for value assignment								
L06	*7.4*	*7.3*	*7.2*	*7.2*	*7.1*	*7.0*	*7.2*	*1.96*

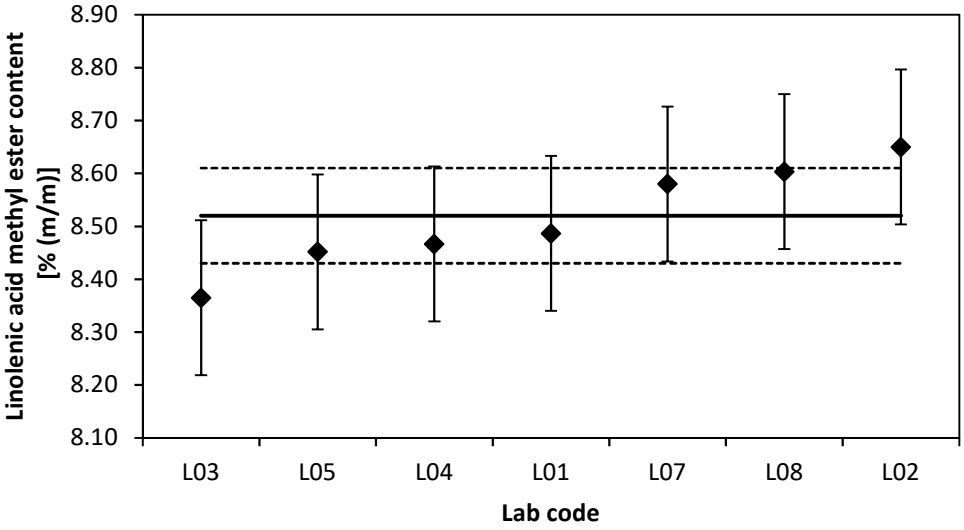


Figure B2: Results of the characterisation study for the mass fraction of the linolenic acid methyl ester content in ERM-EF001 as defined by EN 14103:2020 (continuous line: certified value; dashed line: expanded uncertainty of certified value with k=2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN 14103:2020)

Table B3: Viscosity of ERM-EF001 as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L01	4.474	4.479	4.479	4.475	4.473	4.473	4.476	0.06
L02	4.468	4.469	4.468	4.469	4.469	4.471	4.469	0.02
L03	4.48389	4.48839	4.48001	4.49215	4.48693	4.48281	4.48570	0.10
L04	4.471	4.472	4.472	4.471	4.471	4.472	4.472	0.01
L05	4.4674	4.4674	4.4674	4.4692	4.4678	4.4678	4.4678	0.02
L06	4.474	4.473	4.469	4.476	4.475	4.476	4.474	0.06
Results not used for value assignment								
L07	*4.466*	*4.47*	*4.528*	*4.507*	*4.534*	*4.47*	*4.496*	*0.692*

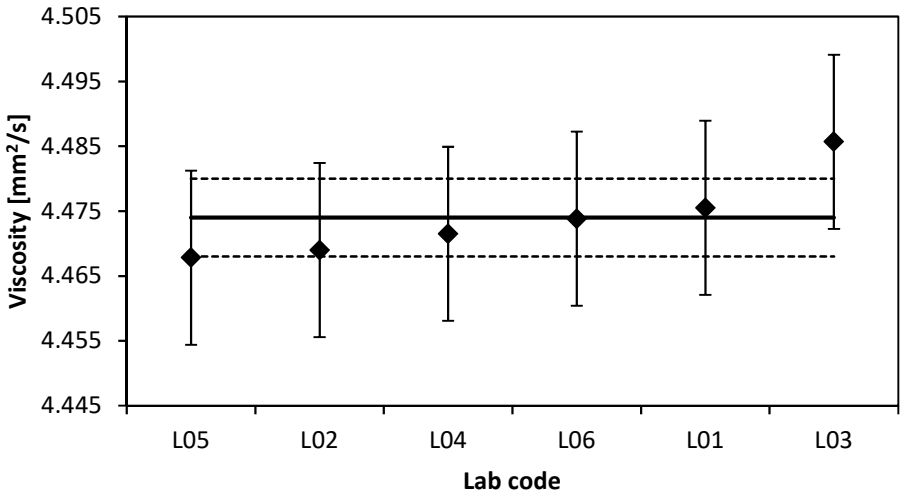


Figure B3: Results of the characterisation study for viscosity in ERM-EF001 as defined by EN ISO 3104:2020 (continuous line: certified value; dashed line: expanded uncertainty of certified value with k=2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN ISO 3104:2020)

Table B4: Iodine value of ERM-EF001 as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L01	107.2	107.6	107.1	107.2	107.4	107.0	107.3	0.20
L02	108.9	109.0	108.4	108.7	108.7	108.5	108.7	0.21
L03	106.5	106.6	106.4	106.5	106.2	106.8	106.5	0.19
L04	107.1	106.9	106.3	106.3	107.5	107	106.9	0.44
L05	106.6	106.5	106.6	106.6	105.8	106.4	106.4	0.29
L08	107.5	108.3	107.6	108.4	108.1	108.2	108.0	0.35
Results not used for value assignment								
L06	*104.1*	*104.5*	*103.6*	*102.8*	*103.0*	*102.6*	*103.4*	*0.74*
L07	*108.4*	*109.5*	*109.5*	*108.1*	*109.1*	*108.4*	*108.8*	*0.56*

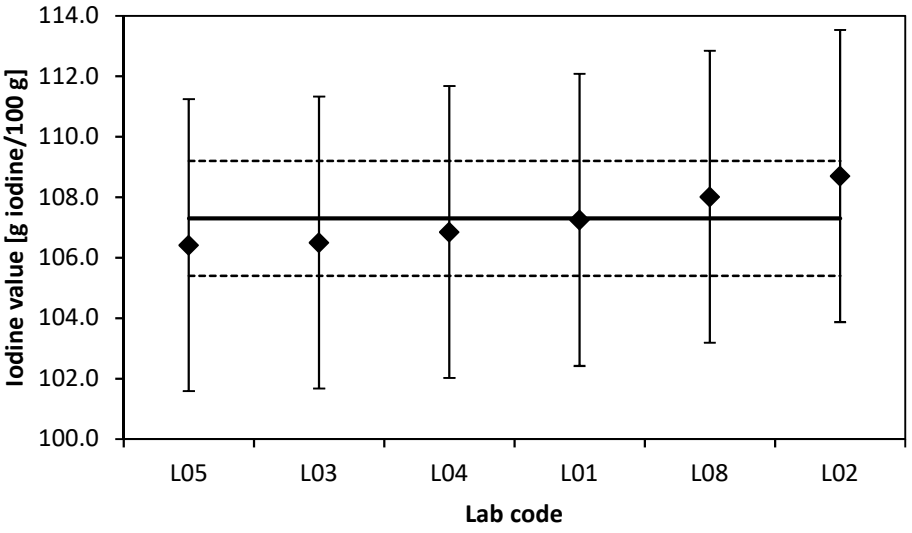


Figure B4: Results of the characterisation study for the iodine value in ERM-EF001 as defined by EN 16300:2012 (continuous line: certified value; dashed line: expanded uncertainty of certified value with k=2; error bars: expanded measurement uncertainty (U_{meas}) derived from EN 16300:2012)

ADDENDUM TO

The certification of the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point of biodiesel:

ERM®- EF001

Report

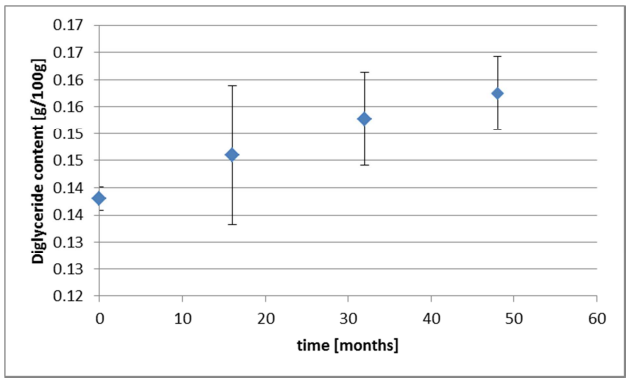
EUR 26711 EN - 2014

Introduction:

The original stability studies described in section 5.2 had shown an upward trend that was statistically significant on a 99 % confidence level for diglycerides. A subsequent 24-months stability study (not described in this report) confirmed this trend and also results from the stability monitoring in May 2016 gave results that were in agreement with the certified values, but confirmed these upward trend. As the extent of the changes is small, it was decided to re-assess the uncertainties to include the apparent change.

Long-term stability

Stability was assessed in a 48 month stability study with time points of 0, 16, 32 and 48 months. Test temperature was 18 °C, reference temperature was 4 °C. For each time/temperature combination, duplicate analyses were performed on 2 ampoules each, giving 4 data points per temperature/time combination. The figures below show for each time point average and its 95 % confidence interval.



The data were evaluated as described in section 5.2 of the certification report. No outliers were detected, but the slope for diglycerides was significant on a 99 % confidence level.

Therefore, the uncertainty of long term stability (u_{lts}) consists of two parts, one reflecting the observed change ($u_{deg;\ x=xshelf}$) and the second one the uncertainty of this change ($u_{b\ x=xshelf}$). $u_{deg;\ x=xshelf}$ is modelled as a rectangular distribution, so the observed change

over the chosen shelf life is divided by the square root of three as shown in the equations below.

$$u_{\mathrm{deg},x=x_{shelf}} = \frac{b \cdot x_{shelf}}{\sqrt{3}}$$

$u_{\mathrm{deg,\ x=x\ shelf}}$ .....uncertainty contribution due to degradation

b.....slope of the regression line

x_{shelf}.....chosen shelf life

$$u_{b,x=x_{shelf}} = \frac{s_{y.x}}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot x_{shelf}$$

$u_{\mathrm{b,\ x\ =x\ shelf}}$.....uncertainty due to lack of fit of the degradation at the time x_{shelf}

s_{yx} .....standard error of the estimate

x_{shelf}.....chosen shelf life

$$s_{y.x} = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n-2}}$$

y_i .....individual result *i* for time point x_i

$\hat{y}_i$ .....estimated result from the regression line at time-point x_i

Using these equations, following uncertainty is obtained for a shelf life of 48 months

Table 1: Results of the stability study on ERM-EF001

	Average ± s	Slope ± s	$u_{lts,\ 48\ months}$
Diglyceride content	(0.149 ± 0.009) % (m/m)	(0.00041 ± 0.00006) %(m/m) /month	7.86 %

Revised uncertainties

Inserting the revised data for u_{lts} into Table 12 of the certification report, the following expanded uncertainty is obtained:

	Certified value	$u_{char,\ rel}$ [%]	$u_{bb,\ rel}$ [%]	$u_{sts,\ rel}$ [%]	$u_{lts,\ rel}$ [%]	$U_{CRM,\ rel}$ [%]	U_{CRM}
Diglyceride content	0.136 % (m/m)	4.43	1.29	0.016	7.86	18.2	0.025 % (m/m)

The new certified value is therefore

Diglyceride content: 0.136 ± 0.025 % (m/m)

Geel, September 2016

ADDENDUM TO

The certification of the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point of biodiesel:

ERM®- EF001

Report

EUR 26711 EN - 2014

Retraction of the acid value

Stability tests in April 2017 indicated instability of the acid value. The certified value for this parameter was therefore retracted.

Geel, May 2017

Retraction of the certified values for the mass fraction of monoglycerides, diglycerides, total glycerol and water

Stability tests in autumn 2018 indicated instability of the mass fractions of monoglycerides, diglycerides and water. Although the measured values were still within the certified ranges, the certified values for these parameters were retracted as a preventive measure. No change was observed for the mass fraction of total glycerol, but as this value is calculated from the ones for mono- and diglycerides, this value was retracted too.

Geel, December 2018

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Glossary

a	Intercept in the equation of linear regression $y = a + bx$
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
b	Slope in the equation of linear regression $y = a + bx$
CEN	European Committee for Standardization
CI	Confidence interval
CRM	Certified reference material
EN	European norm (standard)
ERM®	Trademark of European Reference Materials
FS	Feasibility study
GUM	Guide to the Expression of Uncertainty in Measurements
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
JRC	Joint Research Centre
k	Coverage factor
LOD	Limit of detection
LOQ	Limit of quantification
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
n.a.	Not applicable
n.c.	Not calculated
QC	Quality control
RM	Reference material
RSD	Relative standard deviation
r	Repeatability limit
R	Reproducibility limit
s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an
SI	International System of Units
s_L	Standard deviation between laboratories
s_{meas}	Standard deviation of measurement data; an additional index "rel" is

s_r	Repetability standard deviation
s_R	Reproducibility standard deviation
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
t_i	Time point for each replicate
$t_{\alpha,\,df}$	Critical *t*-value for a *t*-test, with a level of confidence of 1-α and df
t_{sl}	Set shelf life
t_{tt}	Transport time
u	standard uncertainty
U	expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity;
u_c	combined standard uncertainty; an additional index "rel" is added as
$u_{c,bb}$	Standard deviation of the results of the 20 individual samples in the
u_{cal}	Standard uncertainty of calibration
u_{char}	Standard uncertainty of the material characterisation; an additional index
u_{CRM}	Combined standard uncertainty of the certified value; an additional index
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is
u_Δ	Combined standard uncertainty of measurement result and certified
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel"
Δ_{meas}	Absolute difference between mean measured value and the certified
$\nu_{s,meas}$	Degrees of freedom for the determination of the standard deviation s_{meas}
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

The term biofuels refers to liquid or gaseous fuels for the transport or heating sectors that are predominantly produced from biomass. A variety of fuels can be produced from biomass resources, including liquid fuels, such as ethanol, methanol, biodiesel, and Fischer–Tropsch diesel, and gaseous fuels, such as hydrogen and methane. In Europe the most important biofuel is biodiesel, which is defined as the mono-alkyl esters of fatty acids derived from vegetable oils or animal fats.

Due to the increasing use of biofuels over the last years, technical standards defining the quality requirements for biofuels are of vital importance for its producers, suppliers and consumers for quality assurance. To this end, biofuel standards have been established in various countries and regions but until now, there has been no international consensus on the minimum technical specifications to ensure biofuel quality. As differing standards are a potential handicap to the free circulation of biofuels among the various regions, a need for further harmonisation of biofuels standards was identified in the White Paper on Internationally Compatible Biofuel Standards prepared by a Tripartite Task Force comprising Brazil, the European Union and the United States [5]. This document recommends to "support the development of internationally-accepted reference methods and certified reference materials for improving the accuracy of measurement results that underpin assessment of product quality, and help facilitate trade".

Moreover, there is an increasing demand to accurately measure the quality of biofuel products, particularly in view of the European directives promoting renewable energies [6] and setting out fuel quality requirements [7].The European standard for biodiesel to be used as automotive fuel was set in 2003 by the European Committee for Standardization (CEN). It is known under the European standard EN 14214:2012 [8]. This documentary standard is the basis for defining product specifications and measurement methods for biodiesel. While standard methods go a long way to support comparability of results, they cannot guarantee that each laboratory applies the standard correctly. Therefore, laboratories need to be able to check the performance of their methods. This is also true for standardised methods, the use of which does not per se guarantee reliable results. Certified reference materials (CRMs) are needed to give laboratories the possibility to demonstrate their method proficiency and proper working of their instruments.

ERM-EF001 is certified for selected parameters of EN 14214:2012 [8], i.e. the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value, and flash point. An indicative value is given for the methanol content.

The provision of ERM-EF001 increases the comparability of measurements between laboratories, thus proving the competence of analytical laboratories.

1.2 Choice of the material

EN 14214:2012 [8] defines biodiesel as fatty acid methyl esters in general. This documentary standard was developed on the basis of rapeseed-based biodiesel. Most information and data available are dealing with the practical experience gained in the use of rapeseed oil fatty acid methyl esters. Therefore, the chosen material is a commercial 100 % biodiesel produced from rapeseed oil. It is the predominant source of biodiesel in Europe. The material was provided by a biodiesel producer located in Germany.

1.3 Design of the project

The chosen parameters for this project were a selection of those listed in 14214:2012 [8]. A few parameters had to be excluded for practical reasons, as their required sample intakes would have exceeded the 27 mL that was filled per unit (cold filter plugging point, total contamination, copper strip corrosion, cetane number, and sulfated ash content). For a few parameters the concentration level present in the material was expected to be rather low (polyunsaturated fatty acid methyl esters, sodium, potassium, calcium, magnesium, phosphorus, and sulfur), not allowing reliable measurements thereof. In total, 15 parameters were investigated, covering both chemical and physical properties (Table 1). The homogeneity and stability of the material was evaluated through studies involving measurement of all certified parameters using the documentary standards as listed in Table 1. The certified values were established by an intercomparison of different laboratories using all the same measurement methods for each parameter (Table 1).

Table 1: Selected parameters and corresponding documentary standards for measurements

Parameter	Documentary standard
Ester content	EN 14103:2011 [9]
Linolenic acid methyl ester content	EN 14103:2011 [9]
Monoglyceride content	EN 14105:2011 [10]
Diglyceride content	EN 14105:2011 [10]
Triglyceride content	EN 14105:2011 [10]
Free glycerol content	EN 14105:2011 [10]
Total glycerol content	EN 14105:2011 [10]
Methanol content	EN 14110:2003 [11]
Water content	EN ISO 12937:2000 [12]
Density at 15 °C	EN ISO 12185:1996 [13]
Viscosity at 40 °C	EN ISO 3104:1996 [14]
Oxidation stability at 110 °C	EN 14112:2003 [15]
Acid value	EN 14104:2003 [16]
Iodine value	EN 14111:2003 [17]
Flash point	EN ISO 3679:2004 [18]

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.3 Homogeneity study

ASG Analytik-Service Gesellschaft mbH, Neusäss, DE
(measurements under the scope of ISO/IEC 17025 accreditation D-PL-11334-01-00)

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM; measurements partially under the scope of ISO/IEC 17025 accreditation BELAC No. 268-TEST*)

2.4 Stability study

ASG Analytik-Service Gesellschaft mbH, Neusäss, DE
(measurements under the scope of ISO/IEC 17025 accreditation D-PL-11334-01-00)

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM; measurements partially under the scope of ISO/IEC 17025 accreditation BELAC No. 268-TEST*)

2.5 Characterisation

ASG Analytik-Service Gesellschaft mbH, Neusäss, DE
(measurements under the scope of ISO/IEC 17025 accreditation D-PL-11334-01-00)

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM; measurements partially under the scope of ISO/IEC 17025 accreditation BELAC No. 268-TEST)

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(measurements partially under the scope of ISO/IEC 17025 accreditation ACCREDIA No. 0137)

INTERTEK BELGIUM NV, Antwerp, BE
(measurements partially under the scope of ISO/IEC 17025 accreditation BELAC; No. 105-TEST)

INTERTEK - Immingham, Immingham, UK
(measurements partially under the scope of ISO/IEC 17025 accreditation UKAS No. 4162)

ITS Testing Services (UK) Limited (Teesside Laboratory), Cleveland, UK
(measurements partially under the scope of ISO/IEC 17025 accreditation UKAS No. 4106)

ITERG, Pessac, FR

OŰ EESTI KESKKONNAUURINGUTE KESKUS (Estonian Environmental Research Centre), Tallinn, EE
(measurements under the scope of ISO/IEC 17025 accreditation EAK L008)

SGS ESPAÑOLA DE CONTROL, S.A.U., Barcelona, ES
(measurements under the scope of ISO/IEC 17025 accreditation ENAC 14/LE249 Rev.15)

VÚRUP, a.s., Bratislava, SK
(measurements under the scope of ISO/IEC 17025 accreditation SNAS No. S-119)

3 Material processing and process control

3.1 Origin of the starting material

A commercial unblended biodiesel, so called B100, based on rapeseed oil fatty acid methyl ester, with the addition of about 1 g/kg of the antioxidant butylhydroxytoluene (supplier information) was selected as base material and provided by ADM Research GmbH, Hamburg (DE). Ten 20 L plastic cans were delivered to IRMM, accompanied with a certificate of analysis, with the following values:

Table 2: Certificate of analysis as provided by biodiesel producer

Parameter	Unit	Result	Specification	Test method
Ester content	[% (m/m)]	98.2	min. 96.5	EN 14103
Linolenic acid methyl ester content	[% (m/m)]	9.0	max. 12	EN 14103
Monoglyceride content	[% (m/m)]	0.69	max. 0.80	EN 14105
Diglyceride content	[% (m/m)]	0.14	max. 0.20	EN 14105
Triglyceride content	[% (m/m)]	0.03	max. 0.20	EN 14105
Free glycerol content	[% (m/m)]	0.00	max. 0.02	EN 14105
Total glycerol content	[% (m/m)]	0.20	max. 0.25	EN 14105
Methanol content	[% (m/m)]	0.03	max. 0.20	EN 14110
Water content	[% (m/m)]	0.0174	max. 0.05	EN ISO 12937
Density at 15 °C	[kg/m^3]	883.1	875-900	EN ISO 12185
Viscosity at 40 °C	[mm^2/s]	4.5	3.5-5	EN ISO 3104
Oxidation stability at 110 °C	[h]	>8.0	min. 8	EN 14112
Acid value	[mg KOH/g]	0.19	max. 0.5	EN 14104
Iodine value	[g iodine/100 g]	111.7	max. 120	EN 14111
Flash point	[°C]	>120	min. 120	EN ISO 2719 [19]

3.2 Processing

Upon arrival at the IRMM the material was immediately stored at 4 °C until further treatment. One week before the ampouling, the material was moved from 4 °C to room temperature to stabilise it at this temperature. The contents of the ten plastic cans were combined by pouring it into one 200 L plastic drum over a 125 µm nylon filter. The material was mixed with an IKA Turbotron (Janke & Kunkel, Staufen, Germany) for 30 minutes. Principal means of stabilisation were the addition of an antioxidant (butylhydroxytoluene), which was identified as a viable means of improving oxidation stability by several working groups [20, 21, 22, 23], and creation of an inert atmosphere. For the latter, argon was gently bubbled through the material throughout the filling process. To remove most of the oxygen from the amber glass ampoules, they were (i) flushed with argon, (ii) filled with biodiesel, and (iii) flushed with argon over the headspace. Afterwards, the ampoules were flame-sealed. Ampouling was

performed on a ROTA automatic ampouling machine, model R910/PA (ROTA Verpackungstechnik GmbH & Co.KG, Wehr, DE). 30 mL amber glass ampoules were filled with 27 mL of biodiesel. In total, 6000 ampoules were filled, referring in this report to the term "unit".

3.3 Process control

After processing, 20 units were selected using a random stratified sampling scheme (see 4.1) and two replicate water measurements applying coulometric Karl Fischer titration were made on each unit. The water content did not show any trend in the filling sequence (95 % confidence level) and was below 0.03 % (m/m), which was the predefined quality criterion, indicating that the material was homogenously filled.

4 Homogeneity

A key requirement for any reference material (RM) is the equivalence between the various units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value. In contrast to that it is not relevant if this variation between units is significant compared to the analytical variation. Consequently, ISO Guide 34 [1] requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. For all parameters the minimum sample intake is defined by the required sample volume stipulated in the respective documentary standard.

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 uncertainty. The number of selected units for each parameter corresponds to approximately the cubic root of the total number of the produced units. Three different study designs were applied.

For the ester content, linolenic acid methyl ester content, monoglyceride content, diglyceride content, triglyceride content, free glycerol content, total glycerol content, density, oxidation stability, iodine value, and flash point the following study design was used. For each parameter, 20 units were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into 20 groups (with a similar number of units) and one unit was selected randomly from each group. Two independent samples were taken from each selected unit, and analysed by using the respective standard methods of EN 14214:2012 (Table 1).

For the methanol content and water content a slightly different design was used. For each parameter, 20 units were selected using a random stratified sampling scheme as described above. However, for both of them, four independent samples were taken from each selected unit, due to their higher volatility that could result in a higher method standard deviation.

A different design was used for the measurements of the acid value and viscosity, as the required sample intakes for a single analysis allows only for one analysis per unit. As different units can be only measured once, the variability between results contains both repeatability and real between-unit variation. To obtain an assessment of the repeatability standard deviation of the laboratory, it was decided to pool several units (20 units), mix them and perform replicate measurements (20 replicates). Between-unit measurements were done on the 20 individual units, and method repeatability was determined by performing 20

independent measurements using the pooled sample. Consequently, for each parameter, 40 units were selected using a random stratified sampling scheme. To this end, the batch was divided into 20 groups (with a similar number of units) and two units were selected randomly from each group.

All measurements were done in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. The results are shown as graphs in Annex A.

All measurements, apart from density, viscosity and acid value were performed under intermediate precision conditions (different days). Consequently, day-to-day effects can occur that could mask the between-bottle variation. Therefore, it had to be checked first if there is a significant difference between the day means using a t-test at a 95 % confidence level or ANOVA for the measurements spread over more than two days. Significant day to day effects were present for the ester content, monoglyceride content, diglyceride content, triglyceride content, free glycerol content, total glycerol content, methanol content, oxidation stability, iodine value and flash point. A correction was applied by dividing every data point by the respective day mean in order to limit day-to-day effects in the between bottle uncertainty evaluation.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling sequence or the analytical sequence were visible for the ester content, linolenic acid methyl ester content, monoglyceride content, diglyceride content, triglyceride content, free glycerol content, total glycerol content, water content, oxidation stability, acid value, and iodine value.

Significant (95 % confidence level) trends in the analytical sequence were visible for density and viscosity, pointing at instability of the analytical systems. The correction of biases, even if they are statistically not significant, was found to combine the smallest uncertainty with the highest probability to cover the true value [24]. Correction of trends is therefore expected to improve the sensitivity of the subsequent statistical analysis through a reduction in analytical variation without masking potential between-unit heterogeneities. As the analytical sequences and the bottle numbers were not correlated for density and viscosity, trends significant on at least a 95 % confidence level were corrected as shown below:

$$corrected\ \ result = measured\ \ \ result - b \cdot i \qquad \textbf{Equation 1}$$

b **=** slope of the linear regression

i **=** position of the result in the analytical sequence

Filling trends were detected for methanol content, density and flash point at a 95 % confidence level. In these cases the uncertainty was assessed in a different way, using the half-width of a rectangular distribution between the highest and lowest unit average, as explained below.

All datasets (analytical trend-corrected datasets for density and viscosity) were tested for consistency using Grubbs outlier tests on a confidence level of 99 % on the individual results and the unit means. Some outlying individual results and outlying unit means were detected. Since no technical reason for the outliers could be found, all the data were retained for statistical analysis.

Quantification of between-unit inhomogeneity was accomplished by analysis of variance (ANOVA), which can separate 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 are representative for the whole unit.

Evaluation by ANOVA requires unit means which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. Distribution of the unit means 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 visually checked 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 3.

Table 3: Results of the statistical evaluation of the homogeneity studies at 99 % confidence level

Parameter	Trends [2]		Outliers		Distribution	
	Analytical sequence	Filling sequence	Individual results	Bottle means	Individual results	Bottle means
Ester content [1]	no	no	no	no	unimodal	unimodal
Linolenic acid methyl ester content	no	no	no	no	unimodal	unimodal
Monoglyceride content [1]	no	no	1-statistical reason (retained)	1-statistical reason (retained)	unimodal	unimodal
Diglyceride content [1]	no	no	1-statistical reason (retained)	1-statistical reason (retained)	unimodal	unimodal
Triglyceride content [1]	no	no	no	no	unimodal	unimodal
Free glycerol content [1]	no	no	1-statistical reason (retained)	no	unimodal	unimodal
Total glycerol content [1]	no	no	1-statistical reason (retained)	1-statistical reason (retained)	unimodal	unimodal
Methanol content [1]	no	yes	no	no	unimodal	unimodal
Water content	no	no	no	no	unimodal	unimodal
Density at 15 °C	yes	yes [3]	no	no	unimodal	unimodal
Viscosity at 40 °C	yes	n.a. [4]	no	-	unimodal	unimodal
Oxidation stability at 110 °C [1]	no	no	no	no	unimodal	unimodal
Acid value	no	n.a. [4]	no	-	unimodal	unimodal
Iodine value [1]	no	no	no	no	unimodal	unimodal
Flash point [1]	no	yes	no	no	unimodal	unimodal

[1] Statistical evaluation done using day-to-day corrected data, due to non-repeatability conditions
[2] Day-to-day corrected data used
[3] After correction of analytical trend
[4] n.a.: not applicable due to different study design: the required sample intakes for a single analysis allows only for one analysis per unit. As different units can be only measured once, no bottle means are available.

One has to bear in mind that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and 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 negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower 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.* [25]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

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

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \textbf{Equation 2}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \textbf{Equation 3}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}\sqrt[4]{\frac{2}{v_{MSwithin}}}}}{\bar{y}} \qquad \textbf{Equation 4}$$

MS_{within} mean square within a unit from an ANOVA

$MS_{between}$ mean squares between-unit from an ANOVA

$\bar{y}$ mean of all results of the homogeneity study

n mean number of replicates per unit

$v_{MSwithin}$ degrees of freedom of MS_{within}

Due to the different study design used for the acid value and viscosity the applied evaluation approach differed. To obtain the standard deviation between units (s_{bb}) the standard deviation from the 20 individual units ($u_{c,bb}$) must be corrected for the pure measurement standard deviation (s_{meas}) coming from the pooled sample as shown in equation 5 [26].

$$s_{bb,rel} = \frac{\sqrt{u^2_{c,bb} - s^2_{meas}}}{\bar{y}} \qquad \textbf{Equation 5}$$

As in both cases $u_{c,bb}$ was smaller than s_{meas} the inhomogeneity that can be hidden by method repeatability is defined as follows

$$u^*_{bb,rel} = \frac{s_{meas}\sqrt[4]{\frac{2}{v_{s,meas}}}}{\bar{y}} \qquad \textbf{Equation 6}$$

A different approach was adopted for the monoglyceride content, diglyceride content and total glycerol content for which outlying unit means were detected. In these cases between-unit inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit mean, and the rectangular standard uncertainty of homogeneity was estimated by:

$$u_{rec} = \frac{\left|outlier - \bar{y}\right|}{\sqrt{3} \cdot \bar{y}} \qquad \textbf{Equation 7}$$

$\bar{y}$ mean of all results of the homogeneity study

For each parameter the outlying unit mean is detected on the same unit and is only deviating 2 % from the overall mean. Moreover, it should also be mentioned that the outlying unit means are a result of presence of outlying individual values and do not necessarily reflect the real distribution of these elements in the material.

When a trend in the filling sequence was significant at least at a 95 % confidence level, the uncertainty was assessed in a different way. This applies for methanol content, density, and flash point. Here, u_{rec} was estimated using a rectangular distribution between the highest and lowest unit mean. The corrected uncertainty in those cases where there was a significant trend in the filling sequence is given in:

$$u_{rec} = \frac{\left|highest\ mean - lowest\ mean\right|}{2 \cdot \sqrt{3} \cdot \bar{y}} \qquad \textbf{Equation 8}$$

The results of the evaluation of the between-unit variation are summarised in Table 4. The resulting values from the above equations were converted into relative uncertainties.

Table 4: Results of the homogeneity studies

Parameter	$s_{wb,rel}$	$s_{bb,rel}$	$u^*_{bb,rel}$	$u_{rec,rel}$	$u_{bb,rel}$
Ester content	0.142	n.c. [1)]	0.057	n.a. [2)]	0.057
Linolenic acid methyl ester content	0.171	n.c. [1)]	0.068	n.a. [2)]	0.068
Monoglyceride content	0.94	0.195	0.38	1.32	1.32
Diglyceride content	1.18	n.c. [1)]	0.47	1.29	1.29
Triglyceride content	3.13	n.c. [1)]	1.25	n.a. [2)]	1.25
Free glycerol content	6.10	n.c. [1)]	2.43	n.a. [2)]	2.43
Total glycerol content	0.96	n.c. [1)]	0.38	1.21	1.21
Methanol content	4.81	n.c. [1)]	1.03	2.34	2.34
Water content	3.97	1.81	0.85	n.a. [2)]	1.81
Density at 15 °C	0.00032	0.00035	0.00013	0.00046	0.00046
Viscosity at 40 °C	0.0251	n.c. [1)]	0.0143	n.a. [2)]	0.0143
Oxidation stability at 110 °C	0.70	0.115	0.28	n.a. [2)]	0.28
Acid value	1.20	n.c. [1)]	0.68	n.a. [2)]	0.68
Iodine value	0.76	0.48	0.30	n.a. [2)]	0.48
Flash point	0.54	0.54	0.213	0.79	0.79

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

The homogeneity study showed no outlying unit means or trends in the filling sequence for the ester content, linolenic acid methyl ester content, triglyceride content, free glycerol content, water content, viscosity, oxidation stability, acid value and iodine value. Therefore the between-unit standard deviation can be used as 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.

Outlying unit means were found for the monoglyceride content, diglyceride content and total glycerol content. However, taking these extreme values into account, the inhomogeneity as quantified as u_{rec} is still sufficiently small to make the material useful. Therefore, u_{rec} was used as estimate of u_{bb}.

For the methanol content, density and flash point trends in the filling sequence were detected. In these cases u_{rec}, calculated using the half-width of a rectangular distribution between the highest and lowest unit average, was used as estimate of $u_{bb.}$

4.2 Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the certified value within its stated uncertainty. The minimum sample intake is defined by the required sample volume stipulated in the respective documentary standard (Table 1).

5 Stability

Time, temperature, light and the presence of oxygen were regarded as the most relevant influences on stability of the material. Principal means of stabilisation were the addition of an antioxidant (butylhydroxytoluene), and creation of an inert atmosphere by flushing argon into the containment just before and after filling, removing the oxygen present, and by bubbling the material with argon throughout the filling. The influence of ultraviolet or visible radiation was minimised by the choice of the containment which eliminates most of the incoming light. In addition, materials are stored and dispatched in the dark, thus eliminating practically the possibility of degradation by light. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as conditions for dispatch to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C could be reached and stability under these conditions must be demonstrated if transport at ambient temperature will be applied.

The stability studies were carried out using an isochronous design [27]. In that approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples are analysed simultaneously in the shortest time interval possible.

Information on the short-term stability and long-term stability was already available from a previously performed feasibility study at IRMM [28, 29] and the BIOREMA project [30, 31]. In both projects a rapeseed oil fatty acid methyl ester material, similar to ERM-EF001, was investigated extensively. For this reason, stability studies were organised mainly to confirm that ERM-EF001 behaves similar to the previously tested ones. The outcome of both projects is summarised in Table 5.

Table 5: Summary of outcome for individual stability studies performed in the feasibility study (FS) and the BIOREMA project

Significance of the trend on a 99 % confidence level				
Measurand	**FS**	**BIOREMA**	**FS**	**BIOREMA**
	4 °C for 4 weeks	**4 °C for 4 weeks**	**4 °C for 12 months**	**4 °C for 6 months**
Ester content	no	no	no	no
Linolenic acid methyl ester content	no	no	no	no
Monoglyceride content	no	no	no	no
Diglyceride content	no	no	no	no
Triglyceride content	no	no	no	no
Free glycerol content	no	no	no	no
Total glycerol content	no	no	no	no
Methanol content	no	no	no	no
Water content	no	no	no	no
Density at 15 °C	no	no	no	no
Viscosity at 40 °C	no	no	no	no
Oxidation stability	no	no	no	no
Acid value	no	no	no	no
Iodine value	no	no	no	no
Flash point	no	yes	no	no
Measurand	**18 °C for 4 weeks**	**18 °C for 4 weeks**	**18 °C for 12 months**	**18 °C for 6 months**
Ester content	no	no	no	no
Linolenic acid methyl ester content	no	no	no	no
Monoglyceride content	no	no	no	no
Diglyceride content	no	no	no	no
Triglyceride content	no	no	no	no
Free glycerol content	no	no	no	no
Total glycerol content	no	no	no	no
Methanol content	no	no	no	no
Water content	no	no	no	no
Density at 15 °C	no	no	no	no
Viscosity at 40 °C	no	no	no	no
Oxidation stability	no	no	no	no
Acid value	no	no	no	no
Iodine value	no	no	no	no
Flash point	no	no	no	no
Measurand	**60 °C for 4 weeks**	**60 °C for 4 weeks**	**60 °C for 4 months**	**-**
Ester content	no	no	no	-
Linolenic acid methyl ester content	no	no	no	-
Monoglyceride content	no	no	no	-
Diglyceride content	no	no	yes	-
Triglyceride content	no	no	no	-
Free glycerol content	no	no	no	-
Total glycerol content	no	no	no	-
Methanol content	no	no	no	-
Water content	no	no	no	-
Density at 15 °C	-	yes	no	-
Viscosity at 40 °C	-	no	yes	-
Oxidation stability	yes	no	yes	-
Acid value	-	no	no	-
Iodine value	no	no	no	-
Flash point	no	no	no	-

In both projects, storage under extreme conditions at 60 °C was compared to storage at lower temperatures, i.e., 4 and 18 °C, during relatively short periods of time (1, 2, and 4 weeks). The outcome of the short-term stability studies showed that, at 4 and 18 °C for none of the parameters the slopes of the regression lines were significantly different from zero at a

99 % confidence level, with one exception, i.e. the flash point results obtained at 4 °C. As this outcome was not confirmed by the other stability studies at 4 °C, neither by stability studies at elevated temperatures, this was regarded as statistical artefact. At 60 °C the slopes were significantly different from zero for the oxidation stability (feasibility study), and density (BIOREMA project). Moreover, in the feasibility study storage under extreme conditions at 60 °C during a longer period of time (1, 2 and 4 months) was tested. The diglyceride content as well as viscosity showed some instability only after exposure to 60 °C for 4 months. As these are extreme conditions that would not be encountered under normal conditions, these parameters are still considered stable. Density showed an instability after storage at 60 °C for 4 weeks, but not after 4 months, therefore this was considered a statistical artefact and this parameter is also considered stable. For oxidation stability the slopes of the regression lines were significantly different from zero in two stability studies at 60 °C. This leads to the conclusion that the only parameter sensitive to a short (i.e. less than 4 weeks) exposure to extreme conditions (60 °C) would be the oxidation stability.

In both projects long-term stability was tested at 4 and 18 °C, but the testing time differed, i.e. 4, 8, and 12 months for the feasibility study and 2, 4, and 6 months for the BIOREMA project. For none of the parameters degradation was observed neither at 4 °C nor at 18 °C [28, 31].

Consequently for ERM-EF001, it was decided to limit the short-term stability studies to three parameters, i.e. the ester content (main component of biodiesel), linolenic acid methyl ester content (most vulnerable fatty acid methyl ester) and oxidation stability (most crucial parameter for stability) at 60 °C (1, 2, and 4 weeks), whereas the short-term stability study at 18 °C is covered by the long-term stability, executed for all parameters of interest at 18 °C (4, 8 and 12 months).

5.1 Short-term stability study

For the short-term stability study, units were stored at 60 °C for 0, 1, 2 and 4 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, two samples were measured for the ester content, linolenic acid methyl ester content and oxidation stability using EN 14103:2011 [9] and EN 14112:2003 [15], respectively. The measurements for the ester content and linolenic acid methyl ester content were performed under repeatability conditions, whereas the measurements for the oxidation stability were performed on three different working days due to the long time required for the measurements. All measurements were done in a randomised sequence to be able to separate a potential analytical drift from a trend over storage time.

The results were screened for outliers using the single and double Grubbs test and no outliers were detected on a 99 % confidence level.

Furthermore, the data were evaluated against storage time and regression lines of the ester content, the linolenic acid methyl ester content, and the oxidation stability versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to shipping conditions). For the ester content and linolenic acid methyl ester content, the slopes of the regression lines were not significantly different from zero (on 99 % confidence level). However, for the oxidation stability the slope of the regression line was significantly different from zero (on 99 % confidence level) at 60 °C. The results of the measurements are shown in Annex B.

Since a significant slope was observed for the oxidation stability, the material will be shipped under cooled conditions.

5.2 Long-term stability study

For the long-term stability study, units were stored at 18 °C for 0, 4, 8 and 12 months. The reference temperature was set to 4 °C. For all parameters, apart from the acid value and viscosity, two units per storage time were selected using a random stratified sampling scheme. From each unit, two samples were measured using the standard methods as given in Table 1. For the acid value and viscosity four units per storage time were selected using a random stratified sampling scheme, but only one measurement was done on each unit due to the higher sample amount needed.

The measurements were performed under repeatability conditions for the ester content, linolenic acid methyl ester content, monoglyceride content, diglyceride content, triglyceride content, free glycerol content, total glycerol content, methanol content, water content, density, viscosity and acid value. The measurements for the oxidation stability and flash point were performed on three different working days and the iodine value on two different working days. All measurements were done in a randomised sequence to be able to separate a potential analytical drift from a trend over storage time.

Significant (95 % confidence level) trends in the analytical sequence were visible for free glycerol and density, pointing at instability of the analytical systems. Hence, the data were corrected as described in Section 4.1 in Equation 1.

The results were screened for outliers using the single and double Grubbs test. Outlying results were only found for the acid value (Table 6). As no technical reason for the outliers could be found all data were retained for statistical analysis. A tentative removal of the outliers did not change the outcome of the trend test.

Furthermore, the data were plotted against storage time and linear regression lines of the determined parameters versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to storage conditions). For all parameters apart from the diglyceride content and methanol content, the slopes of the regression lines were not significantly different from zero (on 99 % confidence level) at 18 °C.

The results of the long term stability measurements are shown in Annex C. The results of the statistical evaluation of the long-term stability study are summarised in Table 6.

For all parameters, except diglyceride content and methanol content, no technically unexplained outliers were observed and none of the trends was statistically significant on a 99 % confidence level for any of the temperatures. A significant positive trend at 18 °C was found for the diglyceride content and methanol content. An increase in the diglyceride content should be reflected in a decrease of the triglyceride content, which is not the case. In the BIOREMA project and the feasibility study for none of these parameters degradation was observed neither at 4 °C nor at 18 °C [28, 31]. Moreover, by taking the standard deviation from the homogeneity study the whole range of the obtained values are covered. The same is true for the methanol content, however, no technical explanation could be found for the increase. Without additional evidence for their stability, their mass fractions are given with combined uncertainties with u_{lts} including potential degradation of the material. Consequently, the material can therefore be stored at 18 ± 5 °C. When additional information may become available as part of a two year long-term stability study, it may be possible to confirm stability.

Table 6: Results of the long-term stability tests

Parameter	Number of individual outlying results	Significance of the trend on a 99 % confidence level
Ester content	none	no
Linolenic acid methyl ester content	none	no
Monoglyceride content	none	no
Diglyceride content	none	yes
Triglyceride content	none	no
Free glycerol content	none	no
Total glycerol content	none	no
Methanol content	none	yes
Water content	none	no
Density at 15 °C	none	no
Viscosity at 40 °C	none	no
Oxidation stability at 110 °C	none	no
Acid value	1	no
Iodine value	none	no
Flash point	none	no

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can rule out degradation of materials completely, 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, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time".

Uncertainties of stability during dispatch and storage were estimated as described in [32] for each parameter. For this approach, the uncertainty of the linear regression line with a slope of zero is calculated. The uncertainty contribution u_{sts} and u_{lts} are calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt} \qquad \textbf{Equation 9}$$

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl} \qquad \textbf{Equation 10}$$

RSD *relative standard deviation of all results of the stability study*

ti *time elapsed at time point i*

$\bar{t}$ *mean of all ti*

t_{tt} chosen transport time (0.25 months at 18 ºC)

t_{sl} chosen shelf life (36 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 18 °C LTS study. The uncertainty describes the possible change during a dispatch at 18 °C lasting for 0.25 months (1 week).
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the 18 °C study. The uncertainty contribution describes the possible degradation during 36 months storage at 18 °C.

For two parameters (diglyceride content and methanol content), for which a significant positive trend was found, u_{lts} comprises two main contributions. A term due to the degradation mentioned in 5.2 corresponding to a bias (u_{b1}), calculated as a rectangular distribution of the slope (b). And a second term, which considers the uncertainty associated to such bias (u_{b2}) including potential degradation of the material are given. The u_{lts}, within the chosen shelf life of the material (t_{sl} = 36 months at 18 °C), is estimated as follows:

$$u_{\mathrm{lts,rel}} = \sqrt{u_{b1}^2 + u_{b2}^2} \cdot t_{sl} \qquad \textbf{Equation 11}$$

where,

$$u_{\mathrm{b1}} = \frac{b}{\sqrt{3}} \qquad \textbf{Equation 12}$$

$$u_{\mathrm{b2}} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \qquad \textbf{Equation 13}$$

The results of these evaluations are summarised in Table 7.

Table 7: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of 18 °C and 1 week, $u_{lts,rel}$ was calculated for a storage temperature of 18 °C and 3 years

Parameter	$u_{sts,\ rel}$ [%]	$u_{lts\ ,rel}$ [%]
Ester content	0.001	0.178
Linolenic acid methyl ester content	0.001	0.21
Monoglyceride content	0.007	1.04
Diglyceride content	0.016	2.29
Triglyceride content	0.024	3.41
Free glycerol content	0.038	4.27
Total glycerol content	0.007	0.98
Methanol content	0.091	13.04
Water content	0.027	3.96
Density at 15 °C	0.00001	0.00141
Viscosity at 40 °C	0.00019	0.028
Oxidation stability at 110 °C	0.014	1.97
Acid value	0.021	3.07
Iodine value	0.005	0.66
Flash point	0.011	1.63

After the certification campaign, the material will be subjected to IRMM's regular stability monitoring programme to control its further stability.

6 Characterisation

Because many of the parameters described in EN 14214:2012 [8] are operationally defined, certified values could only be obtained when a specific measurement protocol is strictly followed. In this case, the identity of the measurand would be defined by the applied standard method. Therefore, the material characterisation was based on an intercomparison of expert laboratories, i.e. the properties of the material were determined in different laboratories using all the same methods for the measurements (Table 8).

6.1 Selection of participants

For the characterisation exercise, between 6 to 11 laboratories were selected (Table 8) based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency for the respective parameters in the field of biodiesel measurements by submitting results for intercomparison exercises or method validation reports. Moreover, all admitted laboratories had proved their competence in the previously organised characterisation exercises for the feasibility study [29] and the BIOREMA project [30, 31]. 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

For every parameter, apart from viscosity and acid value, each laboratory received three units of ERM-EF001, and was requested to provide six independent results, two per unit. For both, viscosity and acid value, they received six units of ERM-EF001 and were requested to provide six independent results, one per unit. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The measurements had to be spread over at least three days to ensure intermediate precision conditions.

For all parameters, apart from the glyceride and methanol measurements, each participant received samples of the BIOREMA test material B [31] as a blinded quality control (QC) sample. Even so it is not a real CRM, it has been decided to use it as a QC sample, as the production of the material was planned and performed, where possible, in the same manner as for other RM production projects, following ISO Guide 34 [1] and ISO Guide 35 [2]. Uncertainties of the assigned values were calculated in compliance with the ‘Guide to the expression of uncertainty in measurement’ [4], and included contributions from homogeneity, stability during storage, and characterisation. For this project, the uncertainties of the assigned values for the BIOREMA test material B were adjusted to a shelf life of 48 months (initially 6 months), to cover the time after the BIOREMA project finished until the ERM-EF001 characterisation study.

Laboratories were not requested to submit measurement uncertainties, as the accuracy of the methods is described in the respective documentary standards. However, the laboratories were asked to follow strictly the standard test method protocols as provided in EN 14214:2012 [8].

6.3 Methods used

All laboratories used for the individual parameters the same measurement methods as given in Table 8. A summary of the individual measurement methods, giving their scopes and principles, is listed in Annex D.

These documentary standards give information on expected repeatability and reproducibility limits. A repeatability limit, *r*, is the value of the absolute difference between two single test results obtained under repeatability conditions that can be expected to be less than or equal to with a certain probability (usually 95 %). A reproducibility limit, *R*, is similarly defined for test results obtained under reproducibility conditions [33]. A repeatability limit is calculated from:

$r = t \times \sqrt{2} \times s_r$ **Equation 14**

where *t* (1.96) is the two-tailed Student *t* value at the 95 % confidence level and s_r is the repeatability standard deviation.

Similarly, the reproducibility limit is calculated from:

$R = t \times \sqrt{2} \times s_R$ **Equation 15**

where s_R is the reproducibility standard deviation.

As the standard deviation between laboratories (s_L) is [34]

$$s_L = \sqrt{s_R^2 - s_r^2}$$ **Equation 16**

and as the expanded measurement uncertainty (U_{meas}) of an average of *n* measurements is

$$U_{meas} = 2 \cdot \sqrt{s_L^2 + \frac{s_r^2}{n}}$$ **Equation 17**

expanded measurement uncertainties were estimated for *n*=6 replicates (Annex D, Table D2).

Table 8: Measurement methods used and number of participating laboratories

Parameter	Methods used	No. of participants
Ester content	EN 14103:2011	11
Linolenic acid methyl ester content	EN 14103:2011	11
Monoglyceride content	EN 14105:2011	11
Diglyceride content	EN 14105:2011	11
Triglyceride content	EN 14105:2011	11
Free glycerol content	EN 14105:2011	11
Total glycerol content	EN 14105:2011	11
Methanol content	EN 14110:2003	10
Water content	EN ISO 12937:2000	9
Density at 15 °C	EN ISO 12185:1996	9
Viscosity at 40 °C	EN ISO 3104:1996	9
Oxidation stability at 110 °C	EN 14112:2003	11
Acid value	EN 14104:2003	10
Iodine value	EN 14111:2003	10
Flash point	EN ISO 3679:2004	6

6.4 Evaluation of results

The characterisation campaign resulted in different numbers of submitted datasets for the individual parameters (Table 8). All individual results of the participants, grouped per parameter are displayed in tabular and graphical form in Annex E.

The results for the free glycerol content are only displayed in tabular form, as out of the 11 provided datasets, four laboratories reported that their measurements gave results below the limit of quantification (LOQ), i.e. less than 0.001 % (m/m). Therefore, it was decided that this parameter will not be further considered in this report and no certified value will be assigned.

For the triglyceride content the results are not presented in graphical form, too, as all laboratories reported values below the LOQ, i.e. less than 0.1 % (m/m).

The total glycerol content was recalculated for each laboratory using the provided formula as given in EN 14105:2011 [10], excluding the free glycerol and/or triglyceride fractions that were below the LOQs.

6.4.1 Technical evaluation

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

- compliance with the analysis protocol: sample preparations and measurements performed on three days.
- method performance (gross error check), i.e. agreement of measurement results with assigned values of the QC sample (BIOREMA test material B) (**ester content, linolenic acid methyl ester content, water content, oxidation stability, acid**

value, iodine value, flash point). Datasets were rejected when the QC results did not agree with the assigned values of the BIOREMA test material B according to ERM Application Note 1, using for the uncertainty of the measured value the measurement uncertainties (u_{meas}) derived from the respective documentary standards as given in Annex D, Table D2.

Based on the above criteria, the following datasets were rejected as not technically valid (Table 9).

All laboratories complied with the analysis protocol and were following the documentary standards. Some laboratories deviated from the sample intakes as specified in the respective documentary standards (acid value: laboratory 4, 6 and 10; water content: laboratory 7, 9, and 10; iodine value: laboratory 9). However, these changes were validated and the laboratories could demonstrate the equivalence between the modified method and the strict standard method. Results from such validated modifications are equivalent to results from strict adherence to the standard methods.

The results of laboratory 7 for the linolenic acid methyl ester content were not in agreement with the assigned value of the QC sample. Consequently both datasets, the ester content and the linolenic acid methyl ester content, were rejected, as they are measured with the same method (EN 14103) in a single run.

Moreover, the datasets of laboratory 7 for the water content and viscosity were not accepted, as the results of the QC sample did not agree with the actual assigned values. The laboratory confirmed that this was not a transcription error.

The flash point results of laboratory 10 were excluded, as they did not report any values for the QC sample.

Table 9: Datasets that showed non-compliances with the analysis protocol and technical specifications, and action taken

Parameter	Lab- code	Description of problem	Action taken
Ester content	7	QC measurements did not match the assigned value	not used for evaluation
Linolenic acid methyl ester content	7		not used for evaluation
Water content	7	QC measurements did not match the assigned value	not used for evaluation
Viscosity at 40 °C	7	QC measurements did not match the assigned value	not used for evaluation
Flash point	10	Failure to measure QC sample	not used for evaluation

6.4.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using 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 10.

Table 10: Statistical evaluation of the technically accepted datasets for ERM-EF001.

p: number of technically valid datasets

Parameter	*p*	Outliers		Normally	Statistical parameters				
		Means	Variances	distributed	Unit	Mean	*s*	$s_{between}$	s_{within}
Ester content	10	none	none	yes	[% (m/m)]	98.92	1.10	1.09	0.39
Linolenic acid methyl ester content	10	none	none	yes	[% (m/m)]	8.816	0.128	0.126	0.065
Monoglyceride content	11	none	yes (L10)	yes	[% (m/m)]	0.658	0.046	0.044	0.027
Diglyceride content	11	none	yes (L10)	yes	[% (m/m)]	0.1376	0.0188	0.0175	0.0083
Total glycerol content	11	none	yes (L10)	yes	[% (m/m)]	0.1892	0.0133	0.0128	0.0090
Methanol content	10	none	none	yes	[% (m/m)]	0.0411	0.0074	0.0073	0.0036
Water content	8	none	yes (L4)	yes	[% (m/m)]	0.02051	0.00181	0.00178	0.00081
Density at 15 °C	9	none	yes (L1, L6, L3, L4, L5)	yes	[kg/m^3]	883.199	0.028	0.026	0.025
Viscosity at 40 °C	8	none	yes (L1, L10)	yes	[mm^2/s]	4.4647	0.0059	0.0058	0.0024
Oxidation stability at 110 °C	11	none	yes (L6)	yes	[h]	9.87	0.49	0.43	0.56
Acid value	10	none	none	yes	[mg KOH/g]	0.1845	0.0149	0.0145	0.0081
Iodine value	10	none	none	yes	[g iodine/100 g]	112.2	2.0	2.0	1.0
Flash point	5	none	none	yes	[°C]	181.4	8.3	8.3	1.8

For all parameters the laboratory means follow normal distributions. None of the data contains outlying means. The statistical evaluation flags some laboratories as outlying variance for the monoglyceride content, diglyceride content, total glycerol content, water content, density, viscosity and oxidation stability while their mean results for these parameters still agree with the other data. As all laboratories used the same methods, this demonstrates that the proficiency of these laboratories in applying the respective method is worse than the one of the other laboratories. Therefore, the datasets of laboratory 10 for the monoglyceride, diglyceride and total glycerol content were removed from the calculation of the certified values and only considered confirmatory. The same was true for laboratory 4 (water content), laboratory 1 and 10 (viscosity), and laboratory 6 (oxidation stability). In case of density, five datasets were flagged as outlying variance. However, all datasets were retained, as the difference in variance is due to the given number of digits of the results. Moreover, all results still agree with the repeatability and reproducibility requirements of the respective documentary standards.

The uncertainty related to the characterisation (u_{char}) is estimated as the standard error of the mean of laboratory means ($s/\sqrt{p}$) (Table 11).

Table 11: Uncertainty of characterisation for ERM-EF001

Parameter	*p*	Unit	Mean	*s*	u_{char}
Ester content	10	[% (m/m)]	98.92	1.10	0.35
Linolenic acid methyl ester content	10	[% (m/m)]	8.815	0.128	0.041
Monoglyceride content	10	[% (m/m)]	0.650	0.039	0.0121
Diglyceride content	10	[% (m/m)]	0.1359	0.0191	0.0061
Triglyceride content	10	[% (m/m)]	<0.1 [1)]	n.a. [2)]	n.a. [2)]
Total glycerol content	10	[% (m/m)]	0.1866	0.011	0.0034
Methanol content	10	[% (m/m)]	0.0411	0.0074	0.00233
Water content	7	[% (m/m)]	0.02053	0.00195	0.00074
Density at 15 °C	9	[kg/m^3]	883.199	0.0277	0.0093
Viscosity at 40 °C	6	[mm^2/s]	4.46465	0.0040	0.00161
Oxidation stability at 110 °C	10	[h]	9.77	0.041	0.130
Acid value	10	[mg KOH/g]	0.1844	0.0149	0.0048
Iodine value	10	[g iodine/100 g]	112.2	1.94	0.62
Flash point	5	[°C]	181.4	8.3	3.7

[1)] The value corresponds to the limit of quantification (LOQ) of the standard method EN 14105:2011. The mass fraction of trigylcerides in ERM-EF001 is below the stated value with a 95 % level of confidence.
[2)] n.a.: not applicable

In case of the ester content and linolenic acid methyl ester content an additional uncertainty contribution was added, i.e. an uncertainty for the calibration (u_{cal}) as differences in the purity grade of the internal standards (C19:0) used were observed. In principle the documentary standard EN 14103:2011 [9] says that the internal standard used needs a purity grade of more than 99.5 %. Investigations using a longer GC temperature program than the one suggested in the standard method revealed that for some standards the determined purity was less than 98 %. Most probably, laboratories do not determine the lower purity grade as

their temperature program is too short. When strictly applying the standard method, the lower purity of the internal standard cannot be detected and the internal standard apparently complies with the requirement of the standard method. In order not to deviate from the standard no correction of the values is applied, rather an additional uncertainty contribution u_{cal} is introduced to cover the difference. To this end, the uncertainty is estimated using a rectangular distribution, i.e. half width of the difference between a maximum purity value from 100 % and a value which is in the range of the determined purity value having a lower purity grade (97.5 %), i.e. $u_{cal}=((100-97.5)/2)/\sqrt{3}$.

7 Value Assignment

Certified values are values that fulfil the highest standards of accuracy. Procedures at IRMM require generally pooling of not less than 6 datasets to assign certified values. In specific cases 5 datasets can be acceptable. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [4] were established.

Indicative values are values where either the uncertainty is deemed too large or where to few independent datasets were available to allow certification. Uncertainties are evaluated according to the same rules as for certified values.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 11 was assigned as certified value for ester content, linolenic acid methyl ester content, monoglyceride content, diglyceride content, total glycerol content, water content, density at 15 °C, viscosity at 40 °C, oxidation stability at 110 °C, acid value, iodine value, and flash point.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (Section 5). In case of the ester content and linolenic acid methyl ester content an additional uncertainty contribution was added for the calibration (u_{cal}) (Section 6.4.2). These different contributions were combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM,\ rel}$) with a coverage factor k as:

$$U_{CRM,rel} = k \cdot \sqrt{u_{char,rel}^2 + u_{cal,rel}^{2\ *} + u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2} \qquad \textbf{Equation 18}$$

- u_{char} was estimated as described in Section 6.
- u_{cal}* was estimated for the ester content and linolenic acid methyl ester content as described in Section 6.4.2.
- u_{bb} was estimated as described in Section 4.1.
- u_{sts} was estimated as described in section 5.3.
- u_{lts} was estimated as described in Section 5.3.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor k of 2 was applied, to obtain the expanded uncertainties.

In case of the flash point a certified value is assigned using only 5 datasets. To this end the different uncertainty contributions were combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM,\ rel}$) with a higher coverage factor k, i.e. 2.8, as the number of degrees of freedom is less than 5.

The certified values and their uncertainties are summarised in Table 12.

All results obtained in the intercomparison for the triglyceride content are below the LOQ of the standard method EN 14105:2011 [10]. The mass fraction of triglycerides in ERM-EF001 is therefore certified as <0.1 % (m/m) with a 95 % level of confidence.

For the iodine value, the difference between the mean value of laboratory 1 and the other results is not covered by the measurement uncertainties (U_{meas}) according to ERM Application Note 1 [36]. However, as the difference between the mean value of laboratory 1 and the other results is only small, it was decided to increase the uncertainty of the certified value to an extent that the results of laboratory 1 fulfils the condition of ERM Application Note 1 [36].

Table 12: Certified values and their uncertainties for ERM-EF001

Parameter	Unit	Certified value	$u_{cal, rel}$ [%]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [1)]
Ester content	[% (m/m)]	98.9	0.72	0.35	0.057	0.001	0.178	1.65	1.7
Linolenic acid methyl ester content	[% (m/m)]	8.82	0.72	0.46	0.068	0.001	0.21	1.77	0.16
Monoglyceride content	[% (m/m)]	0.65	-	1.86	1.32	0.007	1.04	5.02	0.04
Diglyceride content	[% (m/m)]	0.136	-	4.43	1.29	0.016	2.29	10.29	0.015
Triglyceride content	[% (m/m)]	<0.1 [2)]	-	-	-	-	-	-	-
Total glycerol content	[% (m/m)]	0.187	-	1.81	1.21	0.007	0.98	4.77	0.009
Water content	[% (m/m)]	0.0205	-	3.59	1.81	0.027	3.96	11.28	0.0024
Density at 15 °C	[kg/m^3]	883.20	-	0.0011	0.00046	0.00001	0.00141	0.0037	0.04
Viscosity at 40 °C	[mm^2/s]	4.465	-	0.037	0.0143	0.00019	0.028	0.096	0.005
Oxidation stability at 110 °C	[h]	9.8	-	1.34	0.28	0.014	1.97	4.78	0.5
Acid value	[mg KOH/g]	0.184	-	2.55	0.68	0.021	3.07	8.09	0.015
Iodine value	[g iodine/100 g]	112	-	0.55	0.49	0.005	0.66	2.75	4 [3)]
Flash point	[°C]	181	-	2.04	0.79	0.011	1.63	7.36	14 [4)]

[1)] Expanded (k = 2) and rounded uncertainty.
[2)] The value corresponds to the LOQ of the standard method EN 14105:2011. The mass fraction of triglycerides in ERM-EF001 is below the stated value with a 95 % level of confidence.
[3)] Increased to an extent that the result of laboratory 1 fulfils the condition laid down in ERM Application Note 1.
[4)] Expanded (k = 2.8) and rounded uncertainty.

7.2 Indicative values and their uncertainties

An indicative value was assigned for the mass fraction of the methanol content for several reasons. First of all, the difference between the mean value of laboratory 1 and the other results is not covered by the measurement uncertainty (U_{meas}) according to ERM Application Note 1 [36]. However, as the difference between the mean value of laboratory 1 and the other results is only small, it was decided to increase the uncertainty of the certified value to an extent that the results of laboratory 1 fulfils the condition of ERM Application Note 1 [36]. Moreover, the estimated final uncertainty was considered too large for the final use of the CRM. Long term stability uncertainty gives the highest contribution to the total uncertainty. However, as the methanol content was evaluated as all the other certified values, the results were regarded as sufficiently trustworthy to assign an indicative value. An indicative value may not be used as certified value. The uncertainty budget was set up as for the certified values and is listed together with the assigned value in Table 13.

Table 13: Indicative value and uncertainty for the mass fraction of the methanol content for ERM-EF001

Parameter	Unit	Indicative value	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [1)]
Methanol content	[% (m/m)]	0.041	5.68	2.34	0.091	13.04	28.82	0.016 [2)]

[1)] Expanded (k = 2) and rounded uncertainty.
[2)] Increased to an extent that the result of laboratory 1 fulfils the condition laid down in ERM Application Note 1.

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

All parameters are considered as method-defined measurands and can only be obtained by following the procedures specified in EN14214:2012 [8]. The assigned values are therefore operationally defined.

Quantity value

Traceability of the obtained results is based on the traceability of all relevant input factors. Instruments in individual laboratories were verified and calibrated with tools ensuring traceability to the International System of Units (SI). Consistency in the interlaboratory comparison demonstrates that all relevant input factors were covered. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

Many measurement procedures include one or more steps, which are selecting specific (or specific groups of) analytes from the sample for the subsequent steps of the whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all the analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions expressing this concept. For instance, the CSLI Guideline C-53A [] [35] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and, thus, is a crucial characteristic in case of the application of different measurement methods. When commutability of a CRM is not established in such cases, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

As the material comes from an industrial biodiesel producing plant, it is representative for other rapeseed based biodiesel samples and the analytical behaviour will be the same as for a routine rapeseed biodiesel sample. It is expected that the analytical behaviour will also not differ significantly from that of biodiesel of difference feedstock.

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

9.2 Storage conditions

The materials shall be stored at 18 °C ± 5 °C in the dark. Care shall be taken to avoid change of the moisture content once the units are open, as the material is hygroscopic.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened units.

9.3 Preparation and use of the material

The units shall be vigorously shaken by turning upside down for at least 2 min before opening to ensure material re-homogenisation.

9.4 Minimum sample intake

The minimum sample intake is defined by the required sample volume stipulated in the respective documentary standard [8].

9.5 Use of the certified value

The main purpose of this material is to assess method performance, i.e. for checking accuracy of analytical results/calibration. As any reference material, it can also be used for control charts or validation studies.

Comparing an analytical 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, www.erm-crm.org [36].

For assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine 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 ($u_{\Delta,}$) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_{meas} \leq U_{\Delta}$ no significant difference between the measurement result and the certified value, at a confidence level of about 95 % exists.

Use in quality control charts

The materials can be used for quality control charts. Different CRM-units will give the same result as inhomogeneity was included in the uncertainties of the certified values.

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11 References

1 ISO Guide 34, General requirements for the competence of reference materials producers, International Organization for Standardization, Geneva, Switzerland, 2009

2 ISO Guide 35, Reference materials – General and statistical principles for certification, International Organization for Standardization, Geneva, Switzerland, 2006

3 ISO/IEC 17025, General Requirements for the Competence of Testing and Calibration Laboratories; International Organization for Standardization, Geneva, Switzerland, 2005

4 ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement, (GUM 1995), International Organization for Standardization, Geneva, Switzerland, 2008

5 White Paper on International compatible Biofuel Standards, Tripartite Task Force: Brazil, European Union and United States of America, 31 December 2007. http://ec.europa.eu/energy/res/biofuels_standards/doc/white_paper_icbs_final.pdf

6 Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC, Official Journal of the European Union, 05.06.2009, L 140, 16-62

7 Directive 2009/30/EC of the European Parliament and of the Council of 23 April 2009 amending Directive 98/70/EC as regards the specification of petrol, diesel and gas-oil and introducing a mechanism to monitor and reduce greenhouse gas emissions and amending Council Directive 1999/32/EC as regards the specification of fuel used by inland waterway vessels and repealing Directive 93/12/EEC, Official Journal of the European Union, 05.06.2009, L 140, 88-113

8 EN 14214, Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods. European Committee for Standardization, Brussels, 2012

9 EN 14103, Fat and oil derivates - Fatty acid methyl esters (FAME) - Determination of ester and linolenic acid methyl ester contents. European Committee for Standardization, Brussels, 2011

10 EN 14105, Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of free and total glycerol and mono-, di-, triglyceride contents (Reference method). European Committee for Standardization, Brussels, 2011

11 EN 14110, Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of methanol content. European Committee for Standardization, Brussels, 2003

12 EN ISO 12937, Petroleum products - Determination of water - Coulometric Karl Fischer titration method. European Committee for Standardization, Brussels, 2000

13 EN ISO 12185, Crude petroleum and petroleum products -Determination of density - Oscillating U-tube method. European Committee for Standardization, Brussels, 1996

14 EN ISO 3104, Petroleum products - Transparent and opaque liquids - Determination of kinematic viscosity and calculation of dynamic viscosity. European Committee for Standardization, Brussels, 1996

15 EN 14112, Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of oxidation stability (accelerated oxidation test). European Committee for Standardization, Brussels, 2003

16 EN 14104, Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of acid value. European Committee for Standardization, Brussels, 2003

17 EN 14111, Oil and fat derivatives - Fatty Acid Methyl Esters (FAME) - Determination of iodine value. European Committee for Standardization, Brussels, 2003

18 EN ISO 3679, Determination of flash point - Rapid equilibrium closed cup method. European Committee for Standardization, Brussels, 2004

19 EN ISO 2719, Determination of flash point – Pensky-Martens closed cup method. European Committee for Standardization, Brussels, 2002

20 M. Canakci; A. Monyem; J. Van Gerpen, Accelerated oxidation processes in biodiesel, J. Trans. ASAE 42 (1999) 1565–1572

21 R.O. Dunn, Effect of oxidation under accelerated conditions on fuel properties of methyl soyate, J. Am. Oil Chem. Soc. 79 (2002) 910–915

22 M. Mittelbach, S. Schober, Storage stability of biodiesel, J. Am. Oil Chem. Soc. 80 (2003) 817–823

23 S. Schober, M. Mittelbach, The impact of antioxidants on biodiesel oxidation stability, Eur. J. Lipid Sci. Technol. 106 (2004) 382–389

24 G. O'Donnell, D. Brynn Hibbert, Treatment of bias in estimating measurement uncertainty, Analyst 130 (2005) 721-729

25 T.P.J. Linsinger, J. Pauwels, A.M.H. van der Veen, H. Schimmel, A. Lamberty, Homogeneity and stability of reference materials, Accred. Qual. Assur. 6 (2001) 20-25

26 J. Pauwels, A. Lamberty, H. Schimmel, Homogeneity testing of reference materials, Accred. Qual. Assur. 3 (1998) 51-55

27 A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem. 360 (1998) 359-361

28 M. Ulberth-Buchgraber, M. Potalivo, H. Emteborg, A. Held, Toward the development of certified reference materials for effective biodiesel testing. Part 1: Processing, homogeneity, and stability, Energy & Fuels 25 (2011) 4622-4629

29 M. Ulberth-Buchgraber, M. Potalivo, A. Held, Toward the development of certified reference materials for effective biodiesel testing. Part 2: Characterization and value assignment. Energy & Fuels 25 (2011) 4630-4637

30 A. Baldan, H. Ent, A.M.H. van der Veen, Adriaan, A. Held, M. Ulberth-Buchgraber, R.J. Daroda, V.S. da Cunha, S.A. Wise, M. Schantz, P.J. Brewer, R.J.C. Brown, G. O'Connor, The BIOREMA project—part 1: Towards international comparability for biofuel analysis. Accred. Qual. Assur. 18 (2013) 19–28

31 M. Ulberth-Buchgraber, M. Potalivo, A. Held, A. Baldan, A.M.H. van der Veen, H. Ent, V.S. Cunha, R.J. Daroda, B. Lang, M. Schantz, R. Hearn, R.J.C. Brown, P.J. Brewer, The BIOREMA project—Part 2: International interlaboratory comparison for biodiesel test methods. Accred Qual Assur 18 (2013) 29–39

32 T.P.J. Linsinger, J. Pauwels, A. Lamberty, H. Schimmel, A.M.H. van der Veen, L. Siekmann, Estimating the Uncertainty of Stability for Matrix CRMs, Fres. J. Anal. Chem. 370 (2001) 183-188

33 ISO 5725-1, Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definition, International Organization for Standardization, Geneva, Switzerland, 1994

34 ISO 5725-2, Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method, International Organization for Standardization, Geneva, Switzerland, 1994

35 H. Vesper, H. Emons, M. Gnezda, C. P. Jain, W. G. Miller, R. Rej, G. Schumann, J. Tate, L. Thienpont, J. E. Vaks, Characterization and Qualification of Commutable Reference Materials for Laboratory Medicine; Approved Guideline, CLSI document C53-A, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2010

36 T.P.J. Linsinger, ERM Application Note 1: Comparison of a measurement result with the certified value, www.erm-crm.org (last accessed on 17.01.2014)

Annexes

Annex A: Results of the homogeneity measurements

Annex B: Results of the short-term stability measurements

Annex C: Results of the long-term stability measurements

Annex D: Summary of methods used in the characterisation study

Annex E: Results of the characterisation measurements

Annex A: Results of the homogeneity measurements

Data points represent data as reported by the laboratories, unless indicated as "normalised" or "analytical trend corrected".

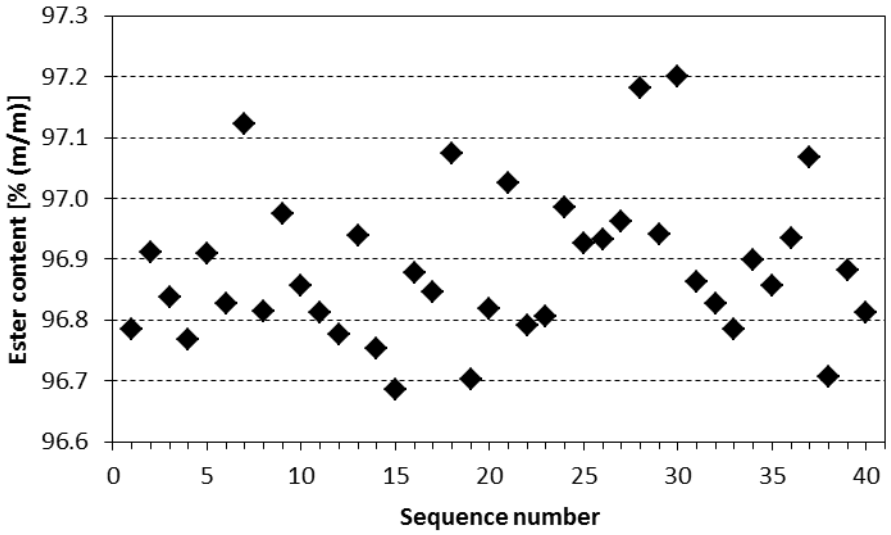

Figure A1: Individual measurement replicates for ester content, against sequence number.

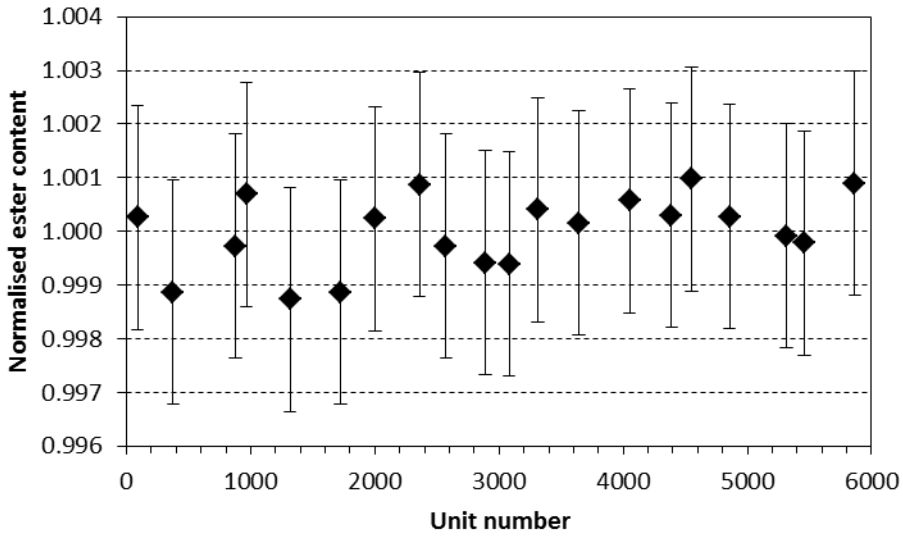

Figure A2: Normalised unit means for ester content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

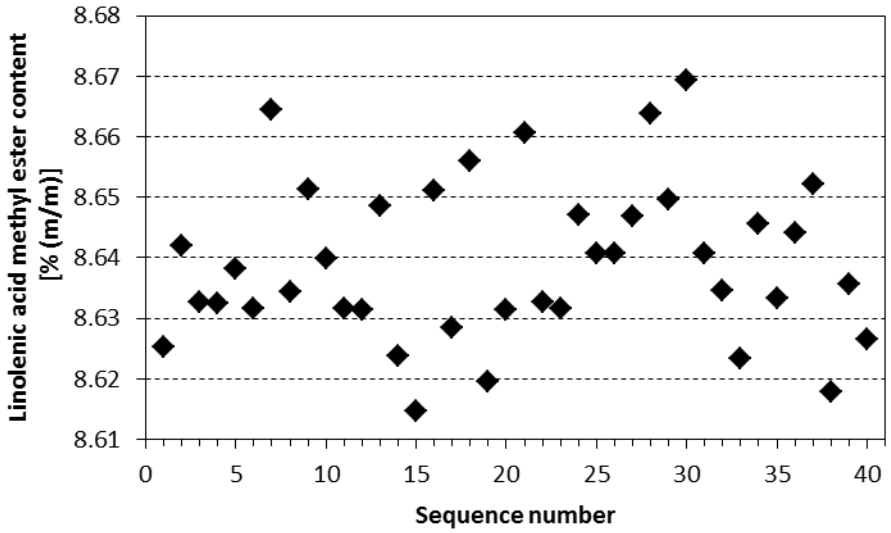

Figure A3: Individual measurement replicates for linolenic acid methyl ester content, against sequence number.

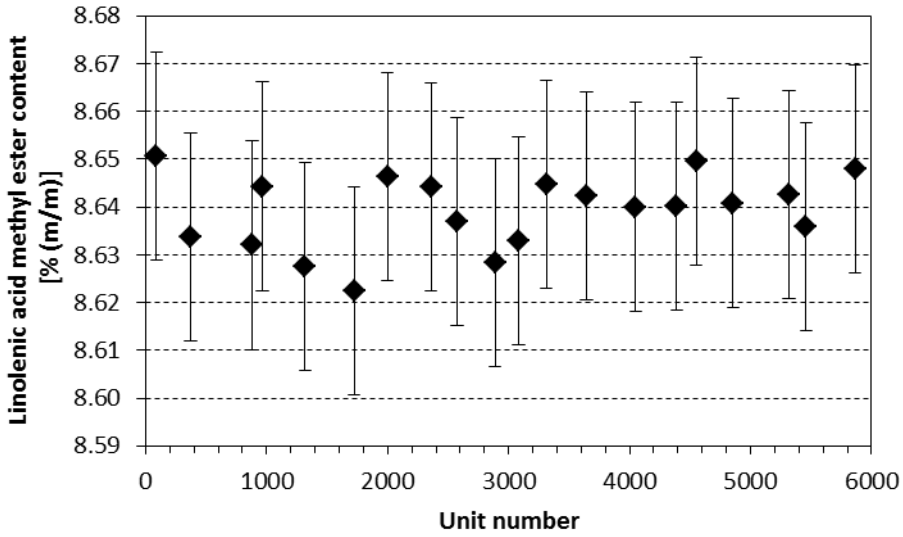

Figure A4: Unit means for linolenic acid methyl ester content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

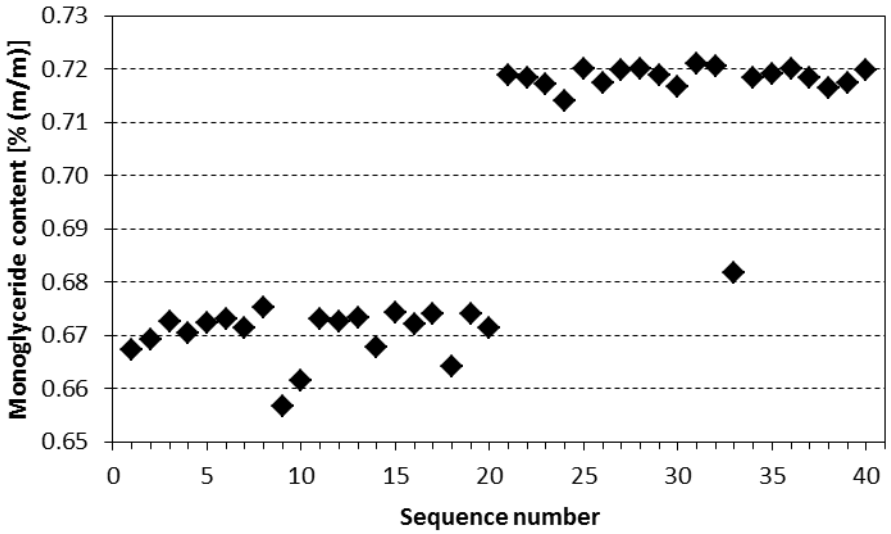

Figure A5: Individual measurement replicates for monoglyceride content, against sequence number.

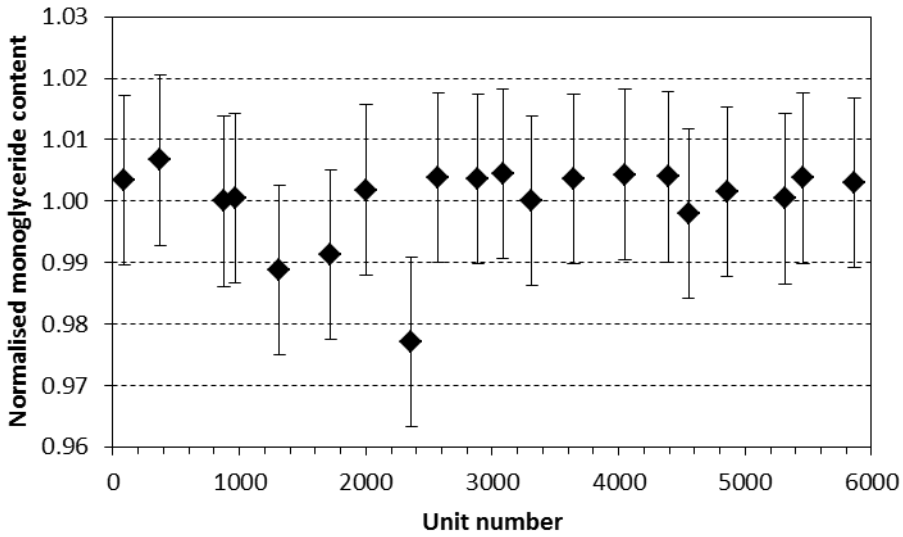

Figure A6: Normalised unit means for monoglyceride content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

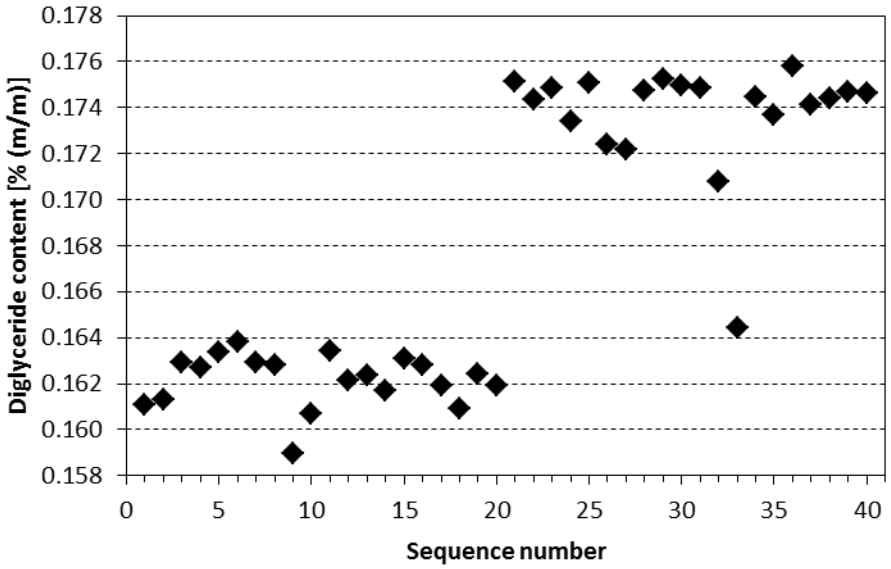

Figure A7: Individual measurement replicates for diglyceride content, against sequence number.

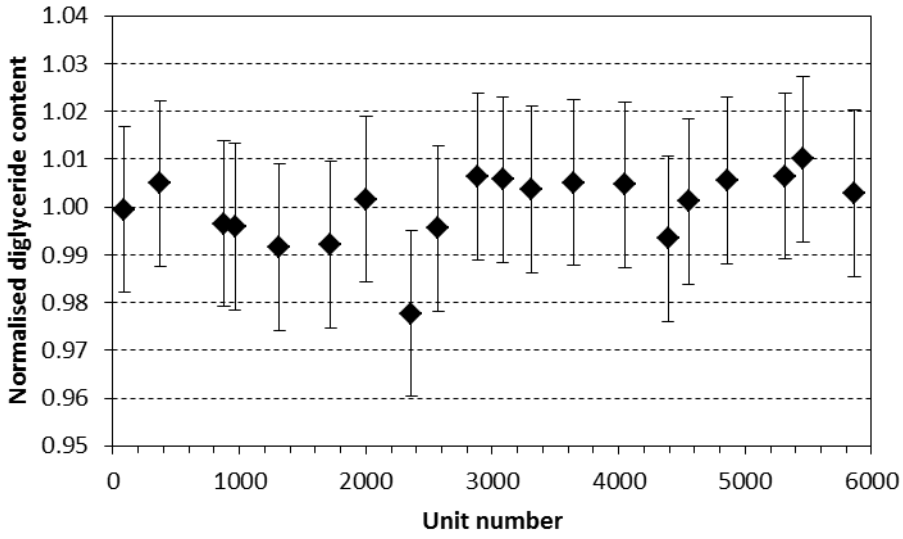

Figure A8: Normalised unit means for diglyceride content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

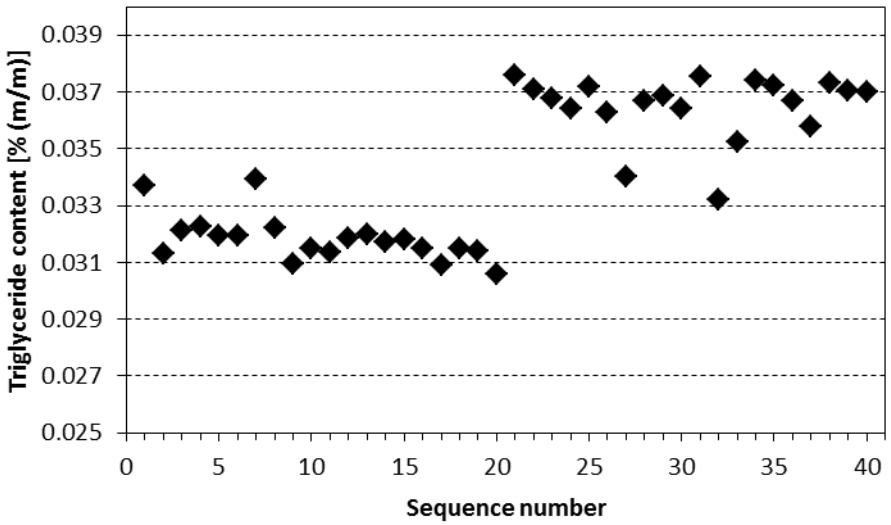

Figure A9: Individual measurement replicates for triglyceride content, against sequence number.

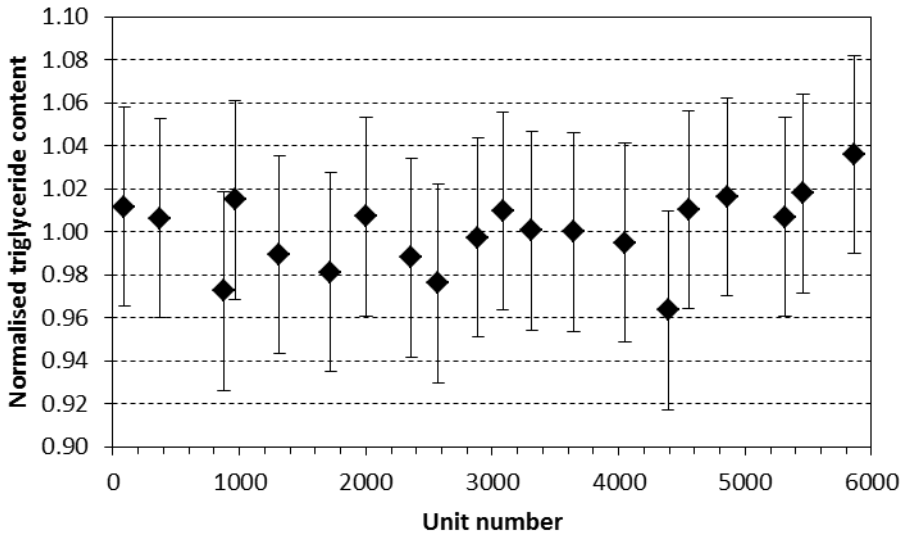

Figure A10: Normalised unit means for triglyceride content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

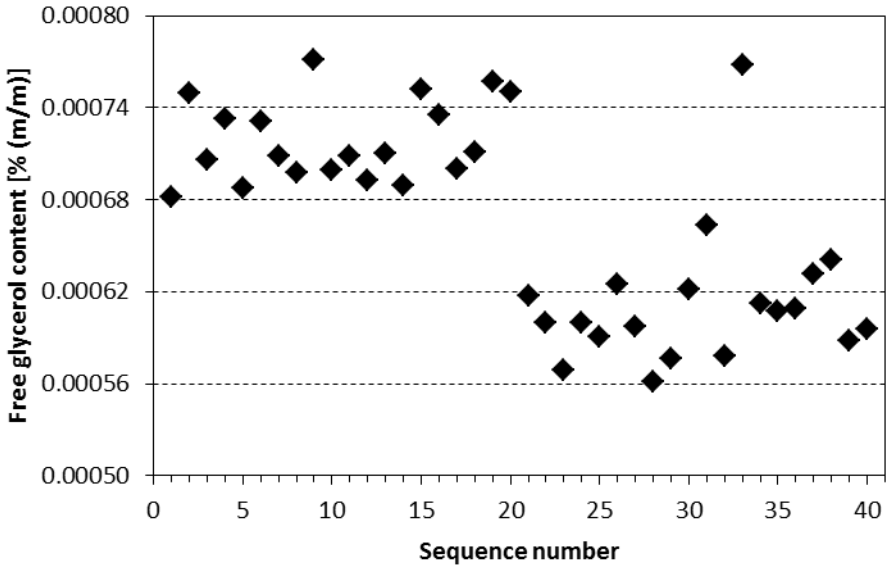

Figure A11: Individual measurement replicates for free glycerol content, against sequence number.

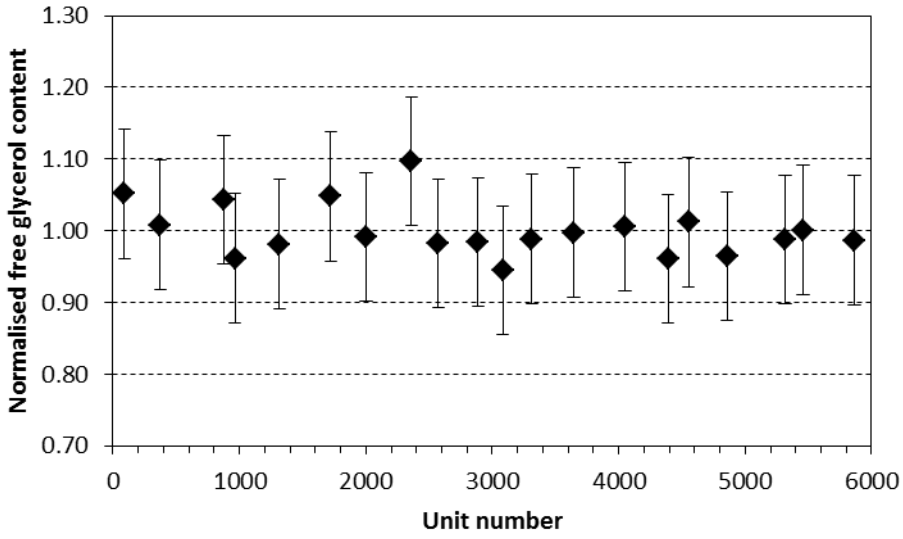

Figure A12: Normalised unit means for free glycerol content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

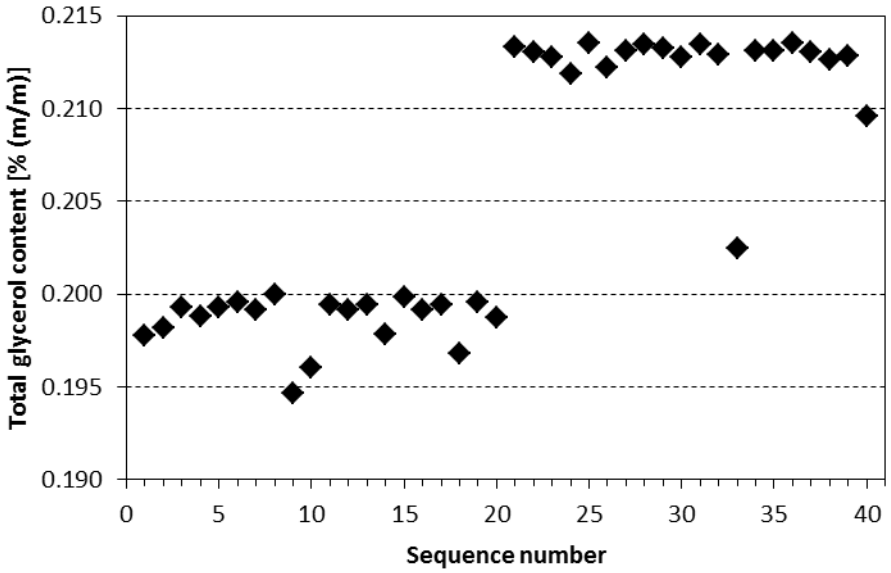

Figure A13: Individual measurement replicates for total glycerol content, against sequence number.

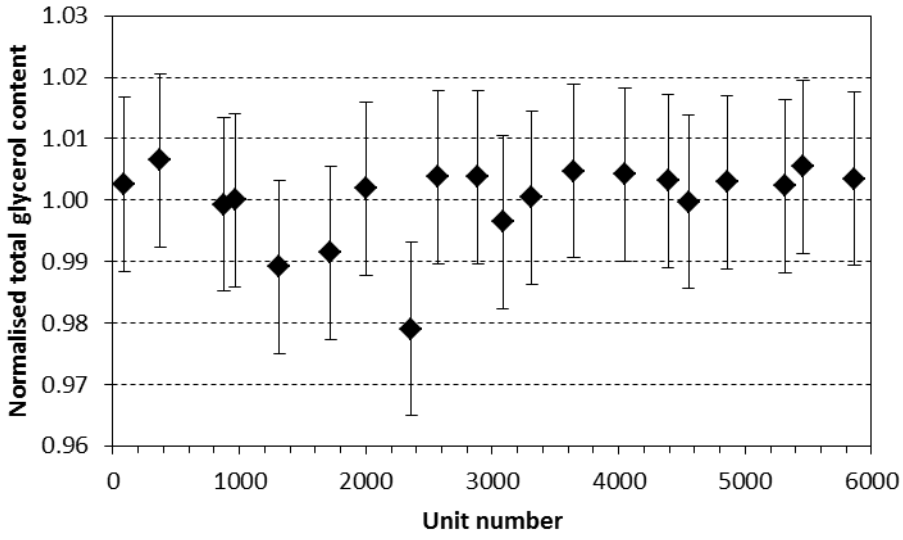

Figure A14: Normalised unit means for total glycerol content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

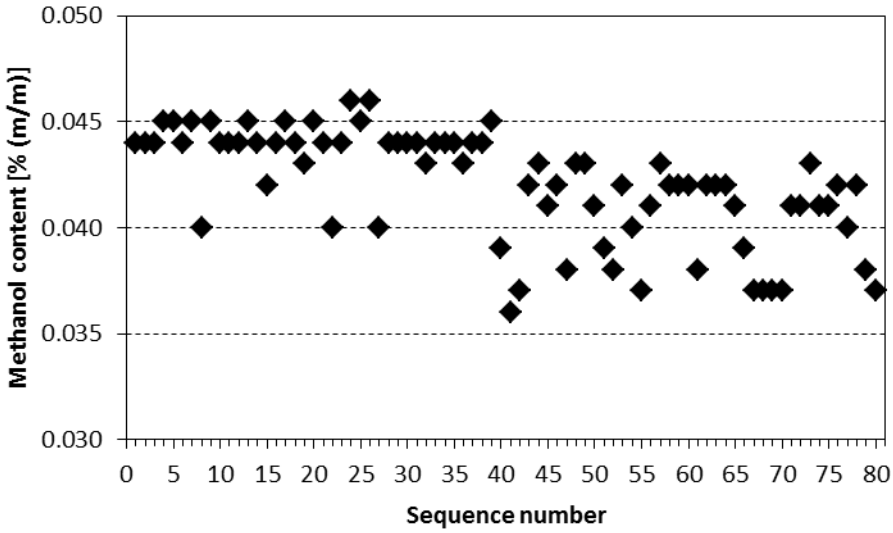

Figure A15: Individual measurement replicates for methanol content, against sequence number.

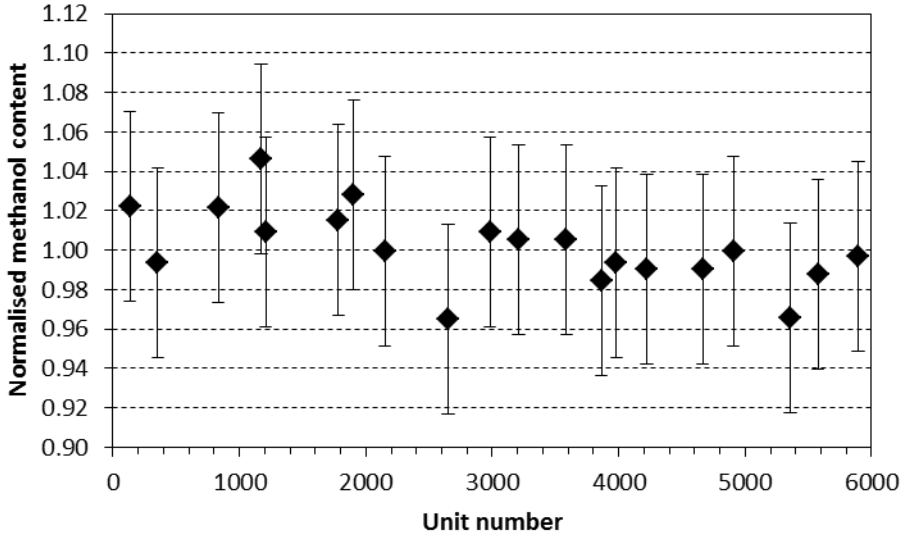

Figure A16: Normalised unit means for methanol content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

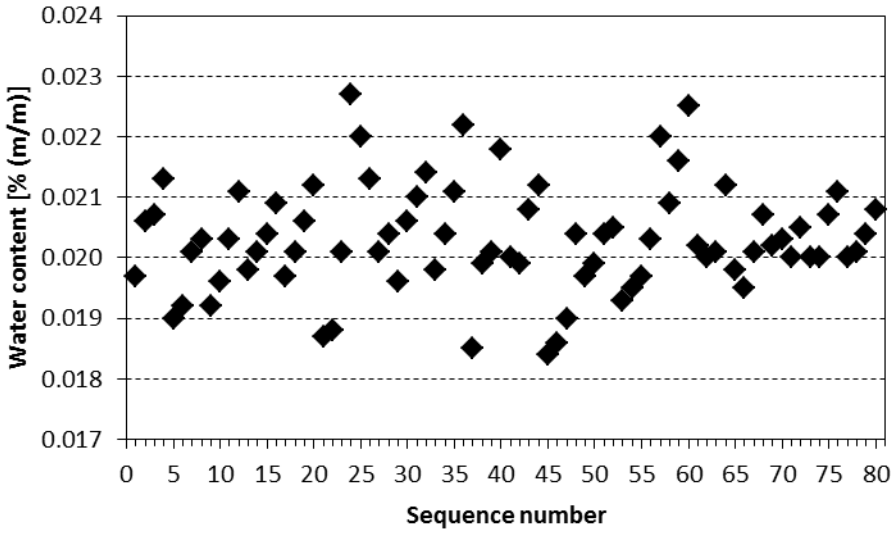

Figure A17: Individual measurement replicates for water content, against sequence number.

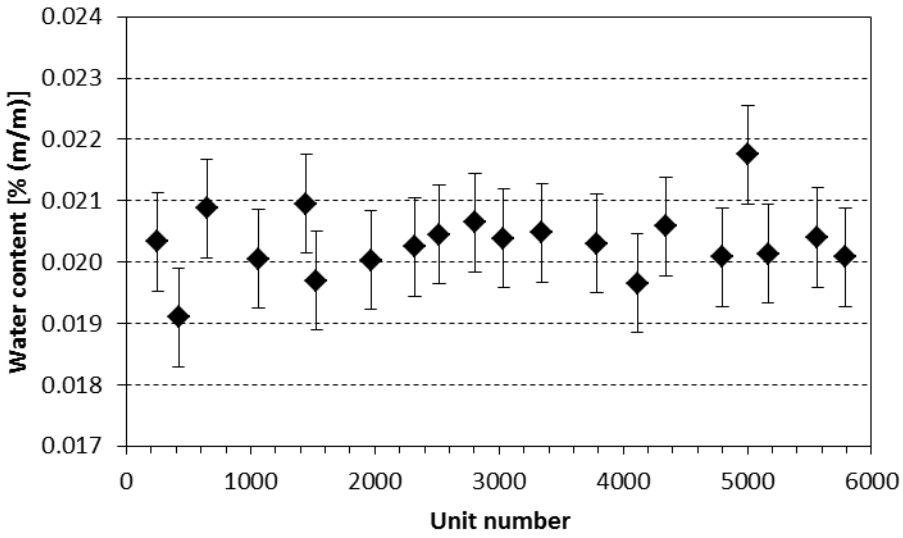

Figure A18: Unit means for water content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

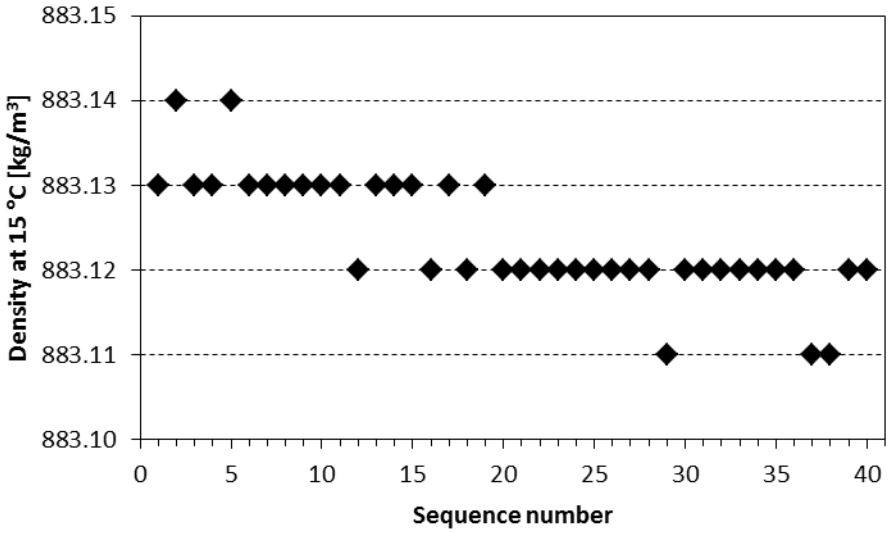

Figure A19: Individual measurement replicates for density at 15 °C, against sequence number.

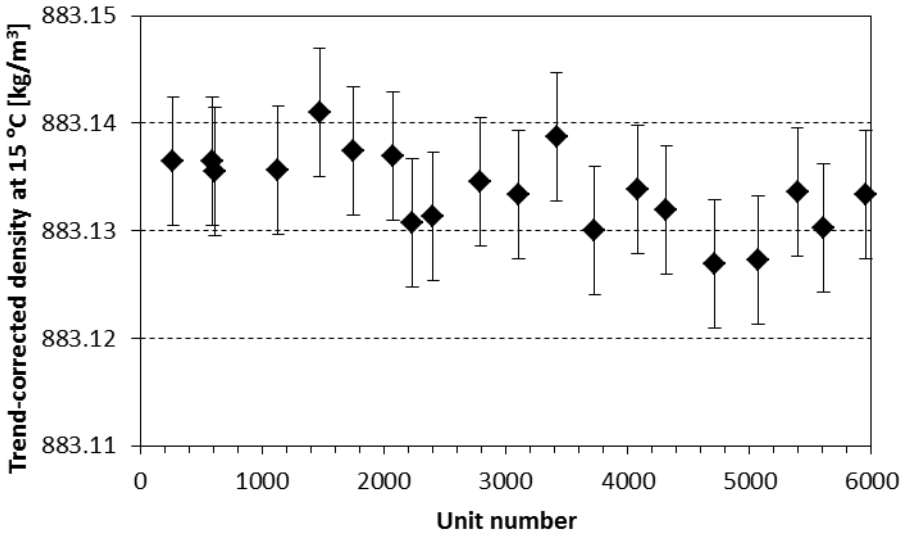

Figure A20: Analytical trend corrected unit means for density at 15 °C, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

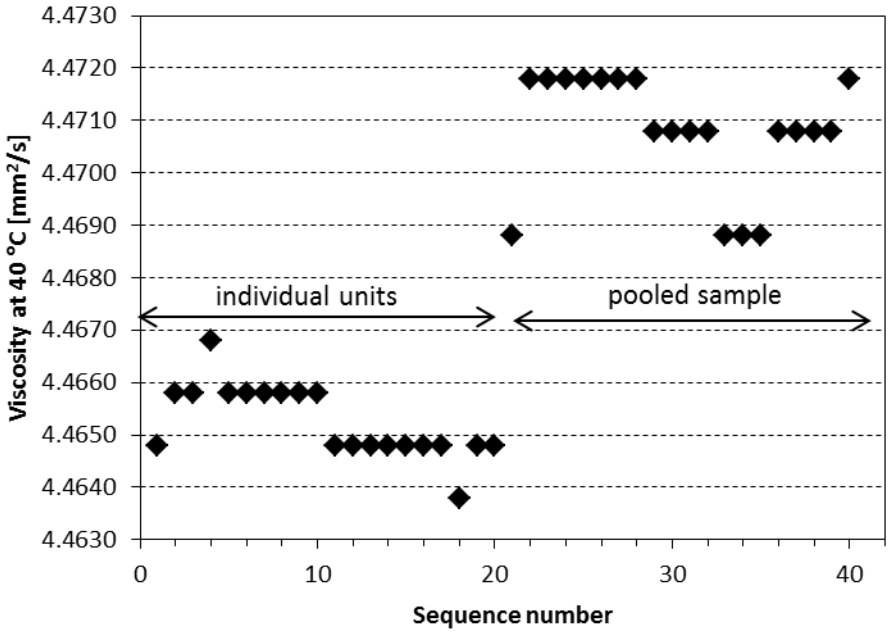

Figure A21: Individual measurement replicates for viscosity at 40 °C, against sequence number. (Sequence number: measurements on 20 individual units and 20 measurements from pooled sample)

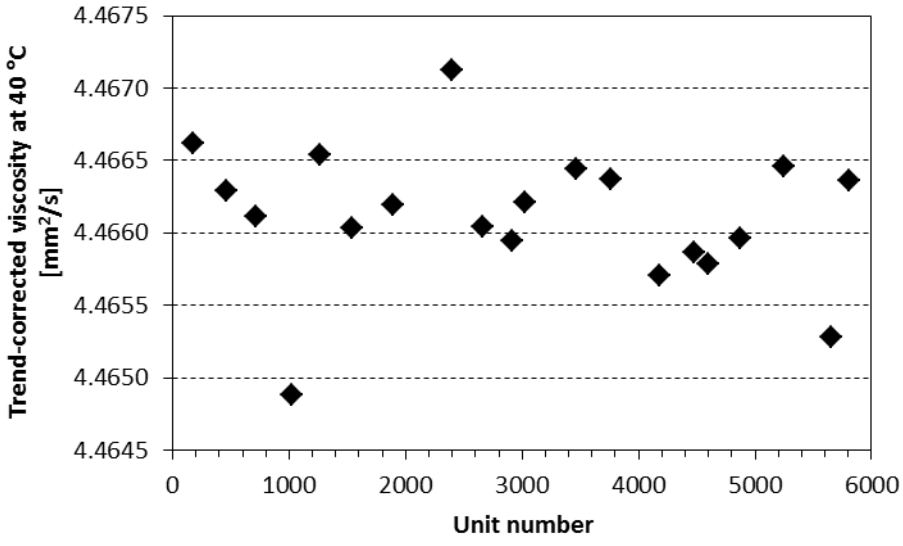

Figure A22: Analytical trend corrected unit means for viscosity at 40 °C, against unit number.

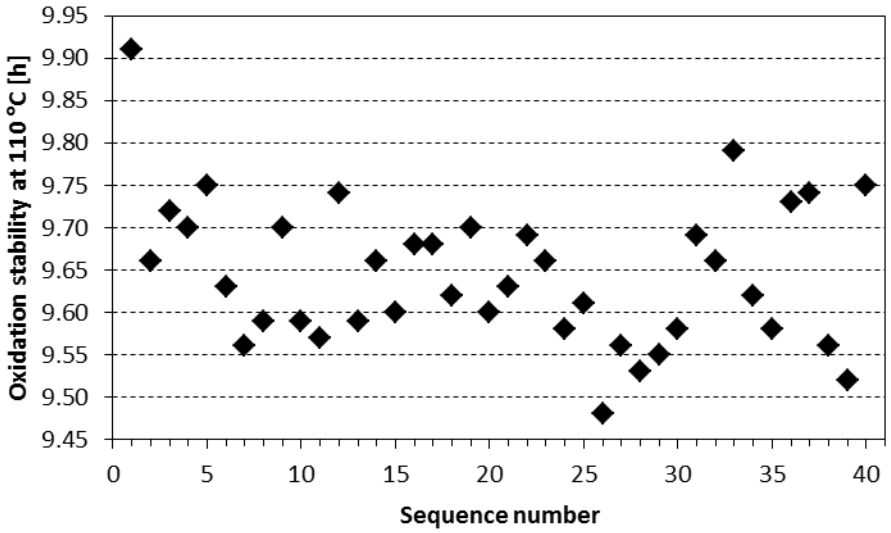

Figure A23: Individual measurement replicates for oxidation stability at 110 °C, against sequence number.

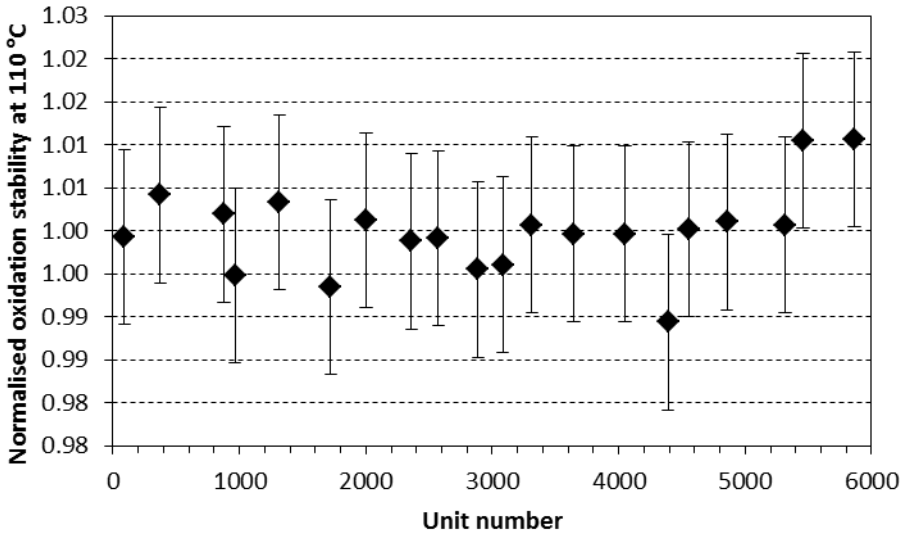

Figure A24: Normalised unit means for oxidation stability at 110 °C, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

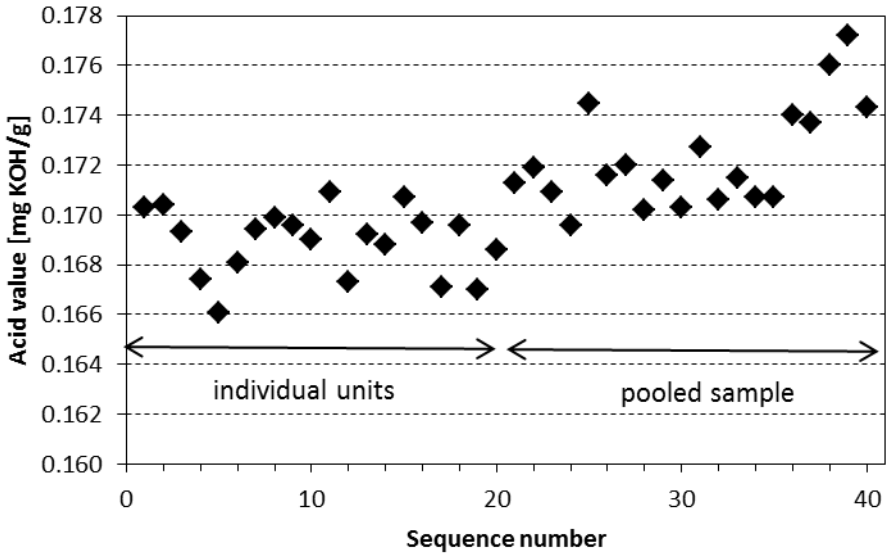

Figure A25: Individual measurement replicates for acid value, against sequence number (Sequence number: measurements on 20 individual units and 20 measurements from pooled sample).

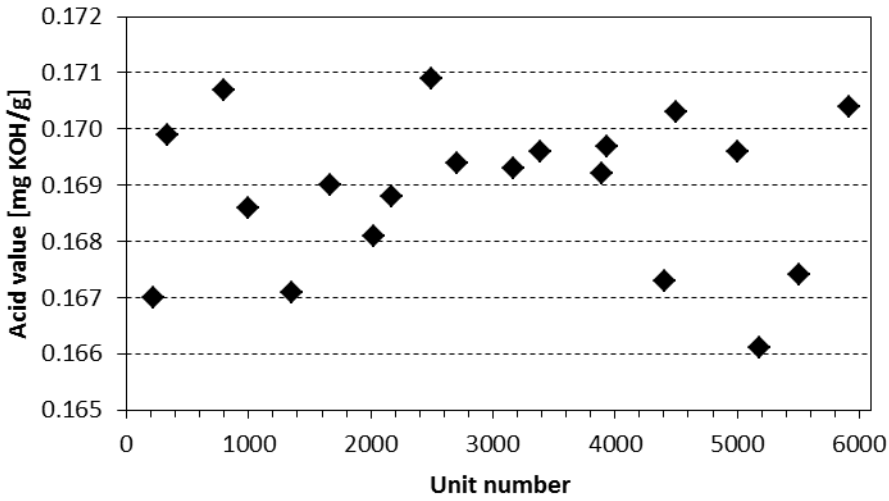

Figure A26: Unit means for acid value, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

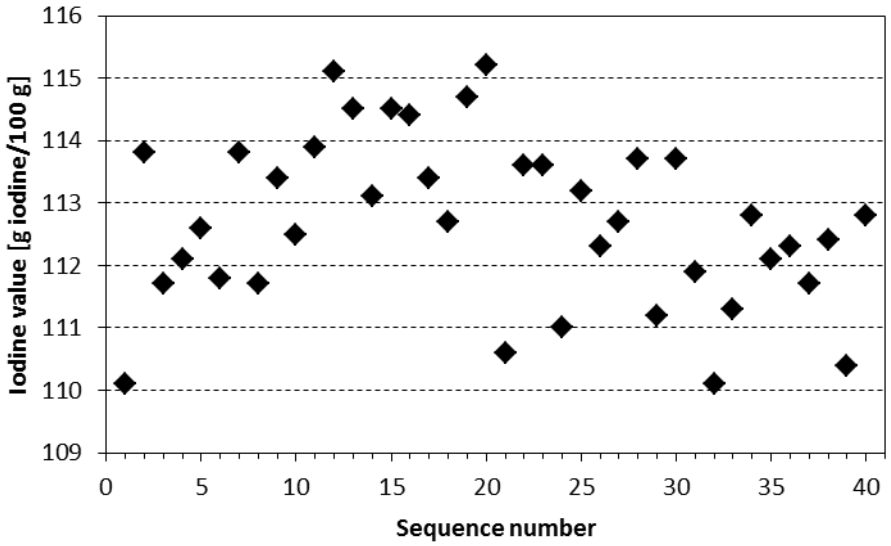

Figure A27: Individual measurement replicates for iodine value, against sequence number.

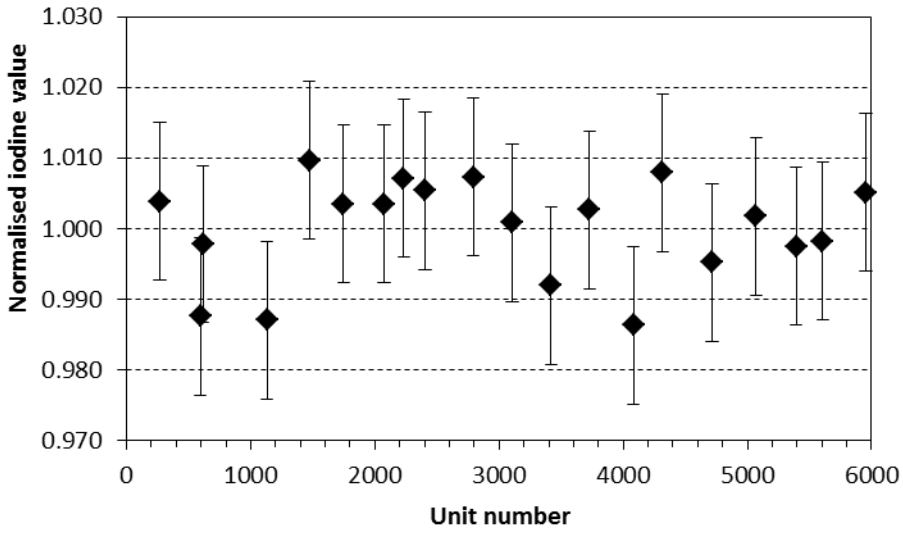

Figure A28: Normalised unit means for iodine value, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

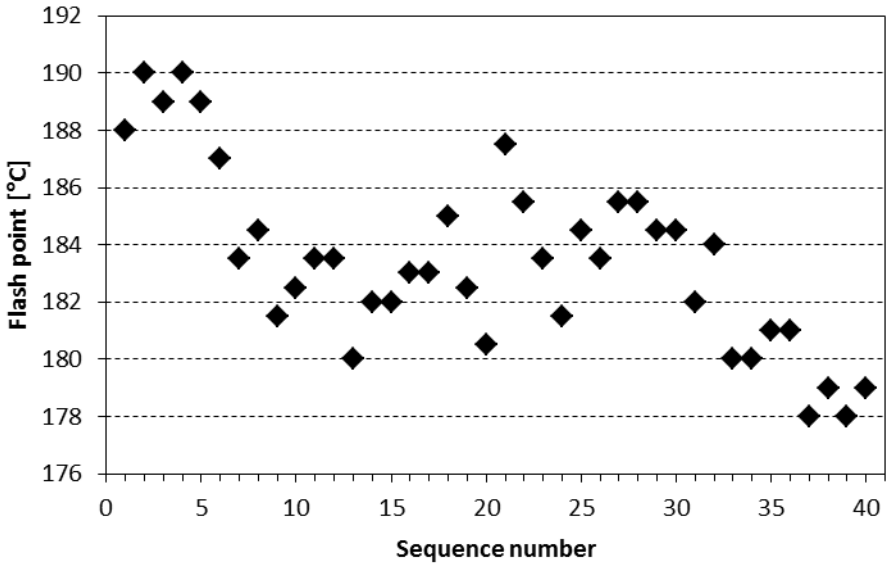

Figure A29: Individual measurement replicates for flash point, against sequence number.

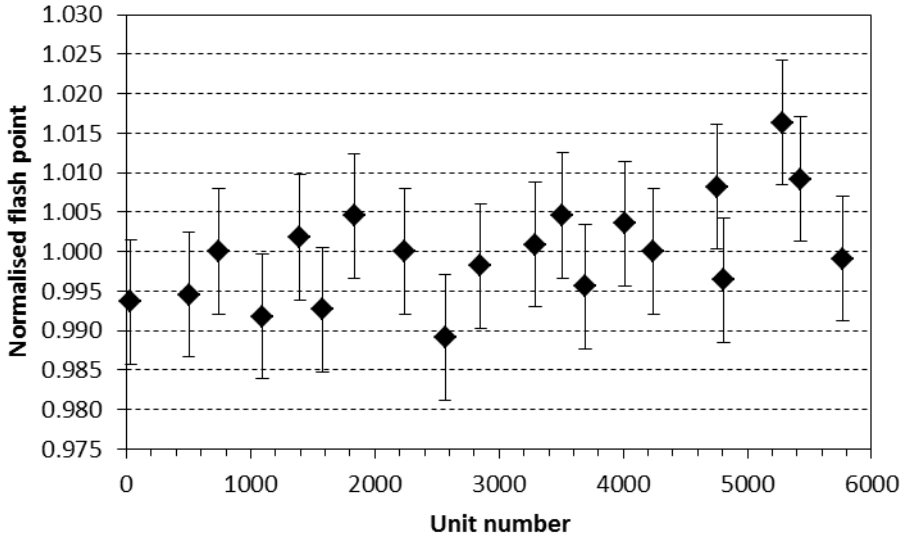

Figure A30: Normalised unit means for flash point, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

Annex B: Results of the short-term stability measurements

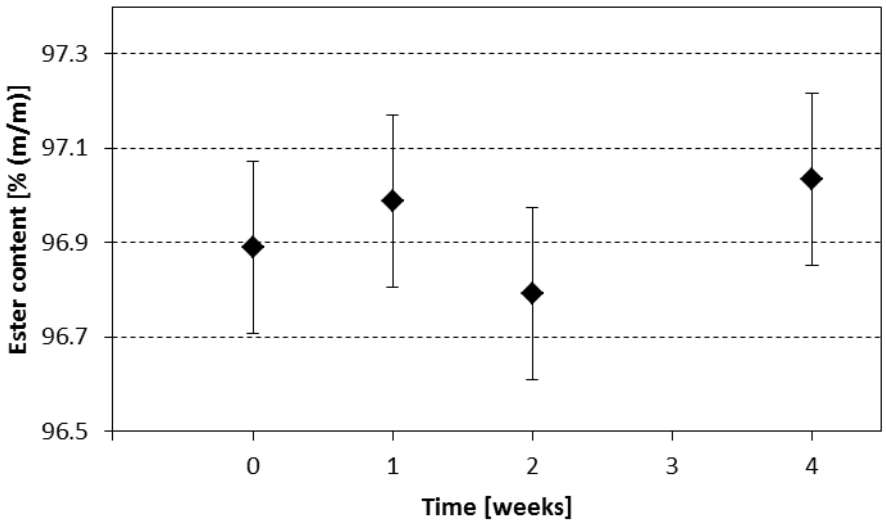

Figure B1: Ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

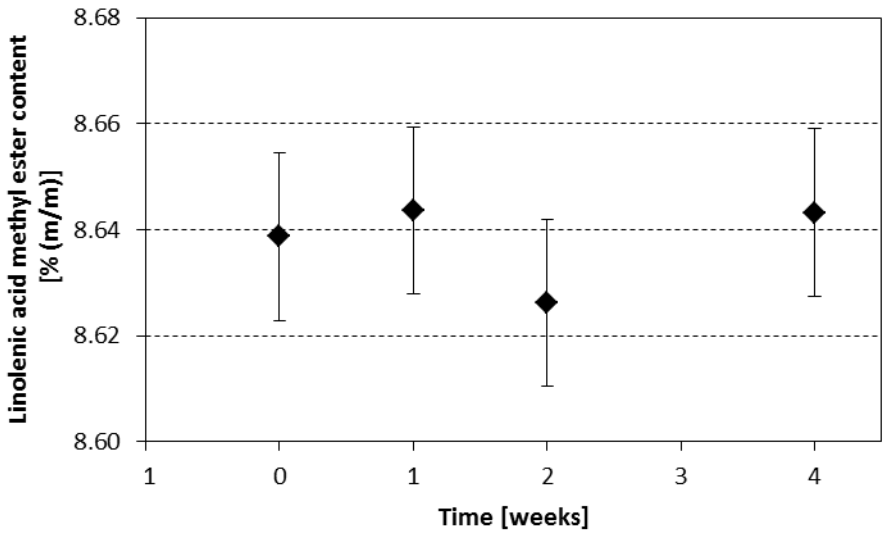

Figure B2: Linolenic acid methyl ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

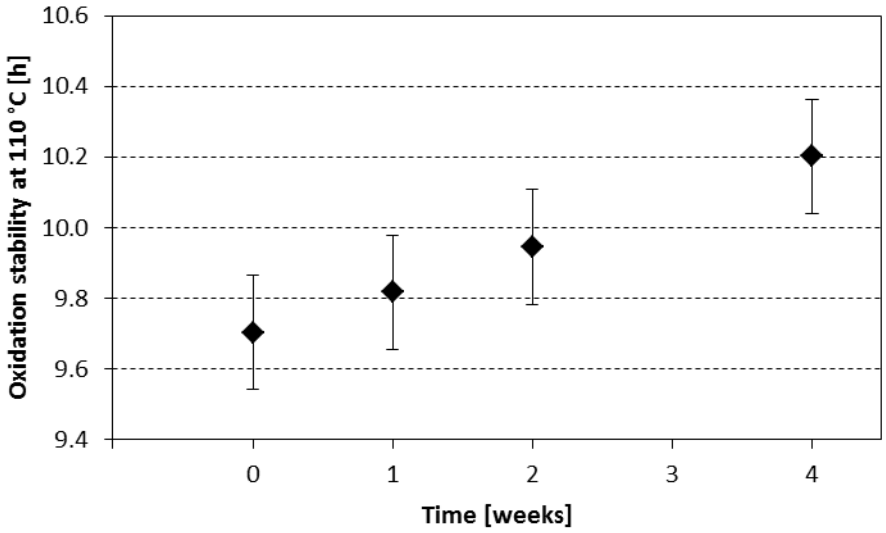

Figure B3: Oxidation stability means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

Annex C: Results of the long-term stability measurements

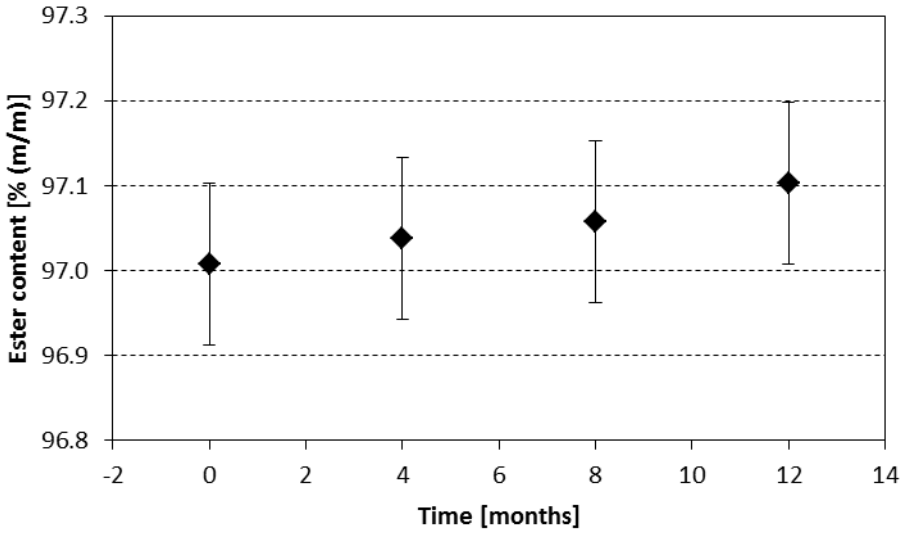

Figure C1: Ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

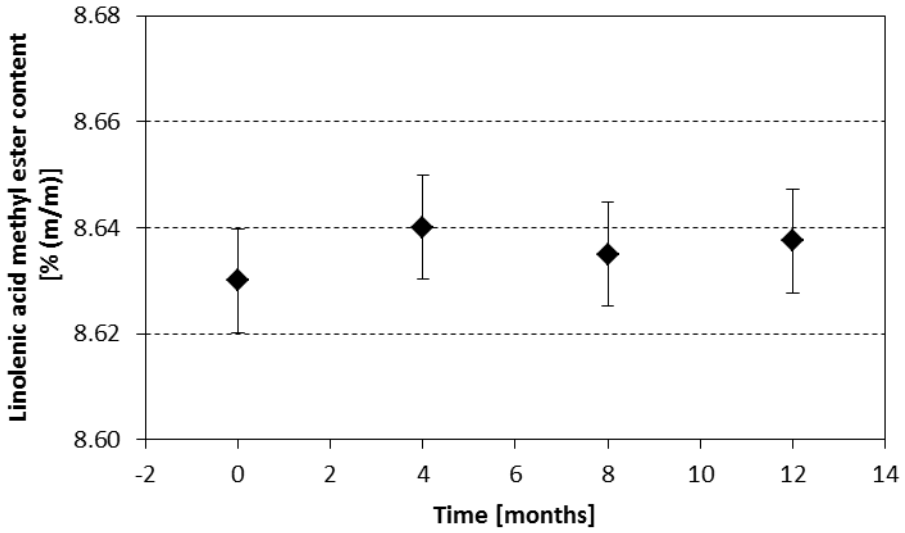

Figure C2: Linolenic acid methyl ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

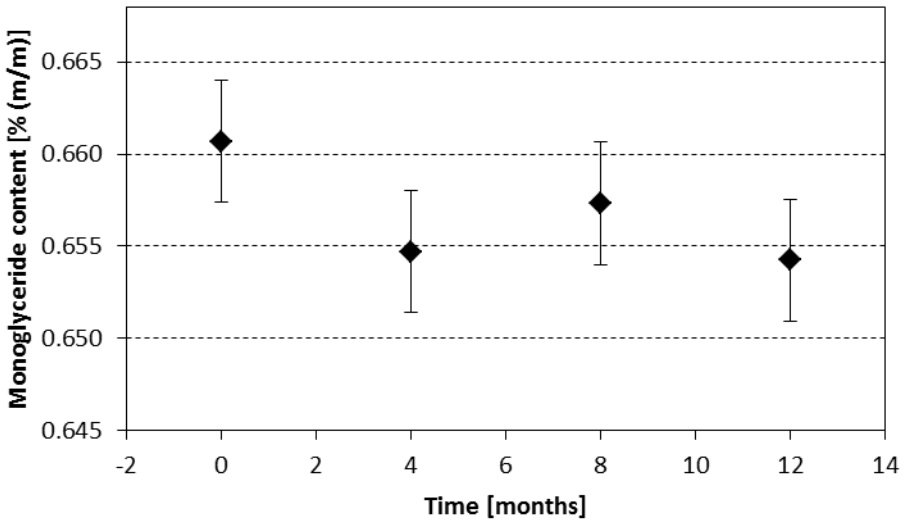

Figure C3: Monoglyceride content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

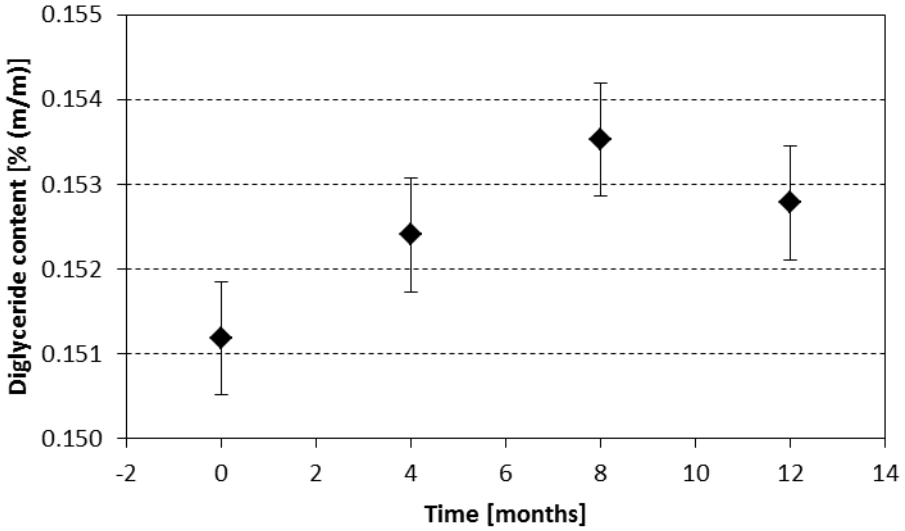

Figure C4: Diglyceride content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

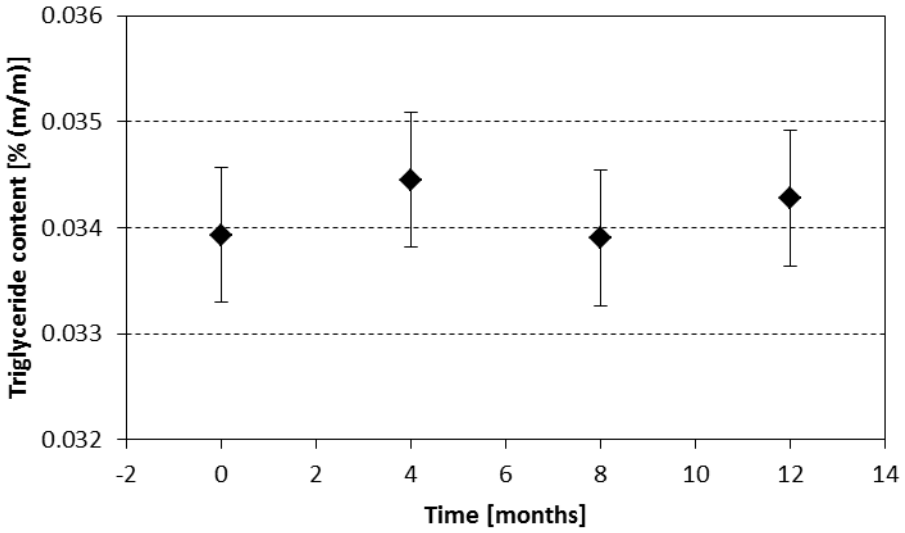

Figure C5: Triglyceride content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

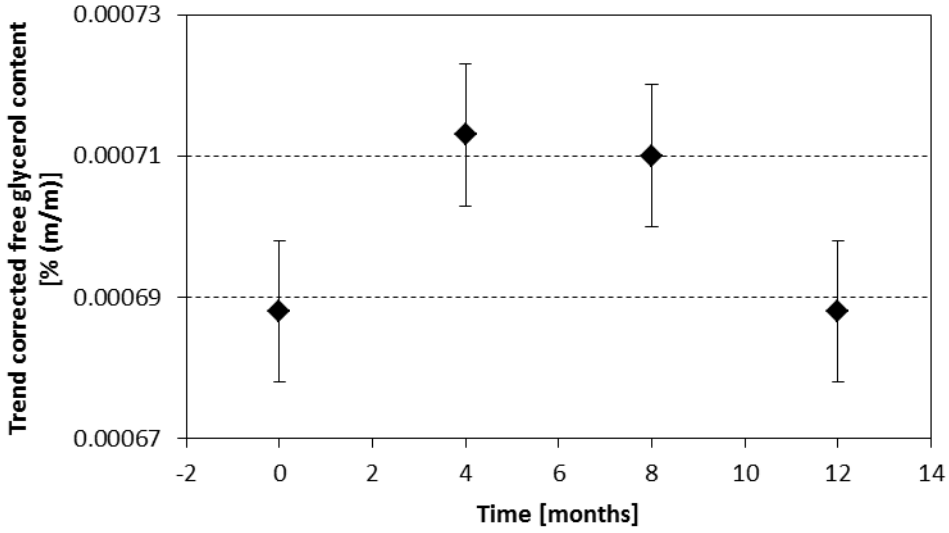

Figure C6: Analytical trend corrected free glycerol content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

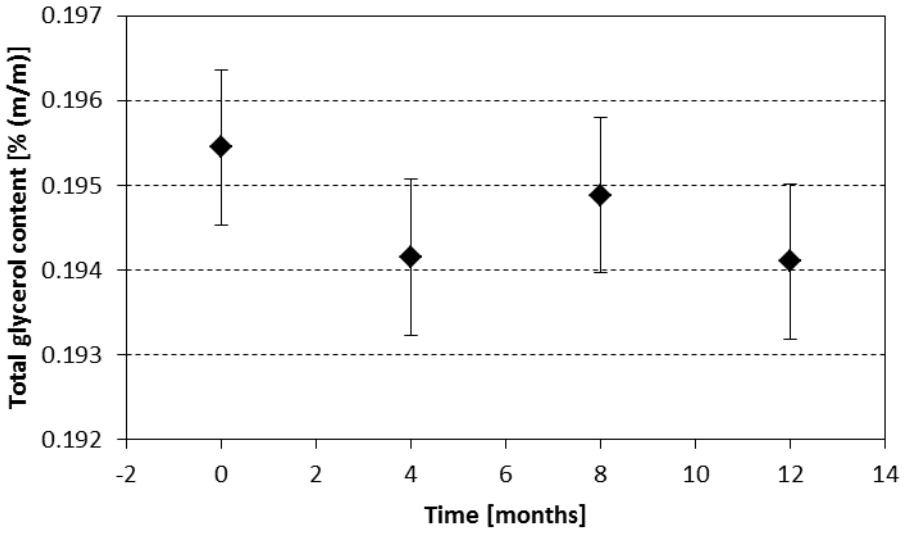

Figure C7: Total glycerol content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

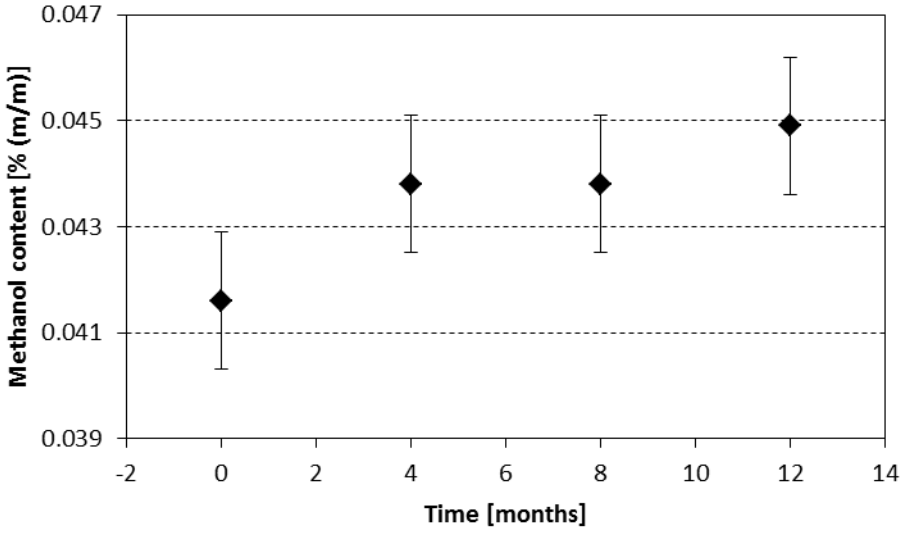

Figure C8: Methanol content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

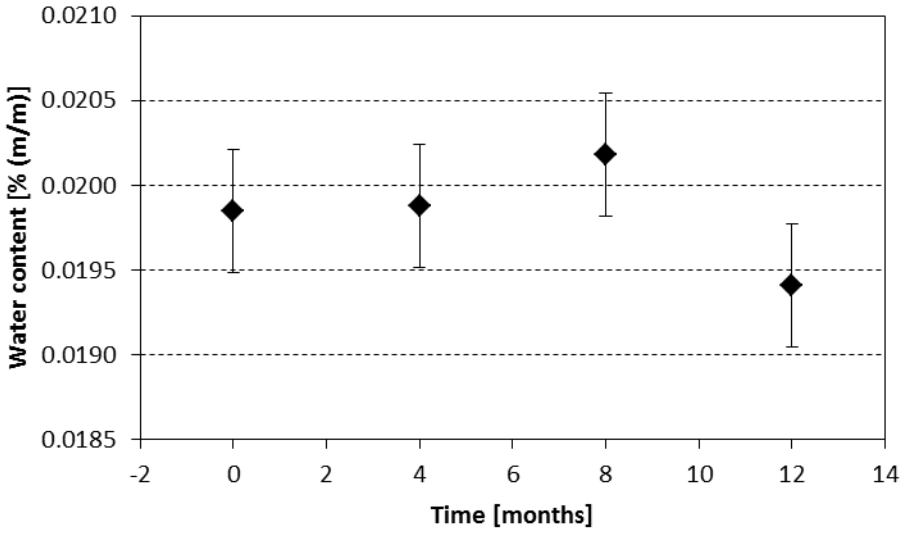

Figure C9: Water content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

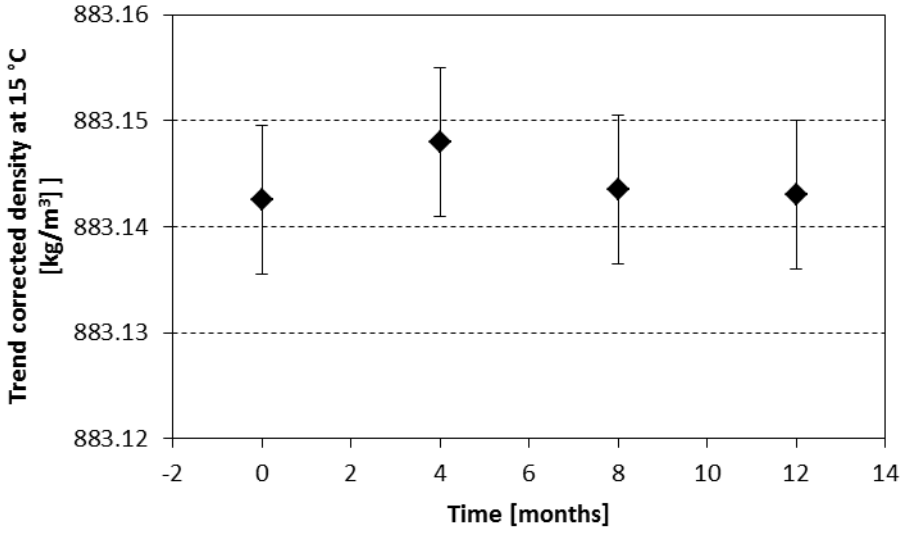

Figure C10: Analytical trend corrected density means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

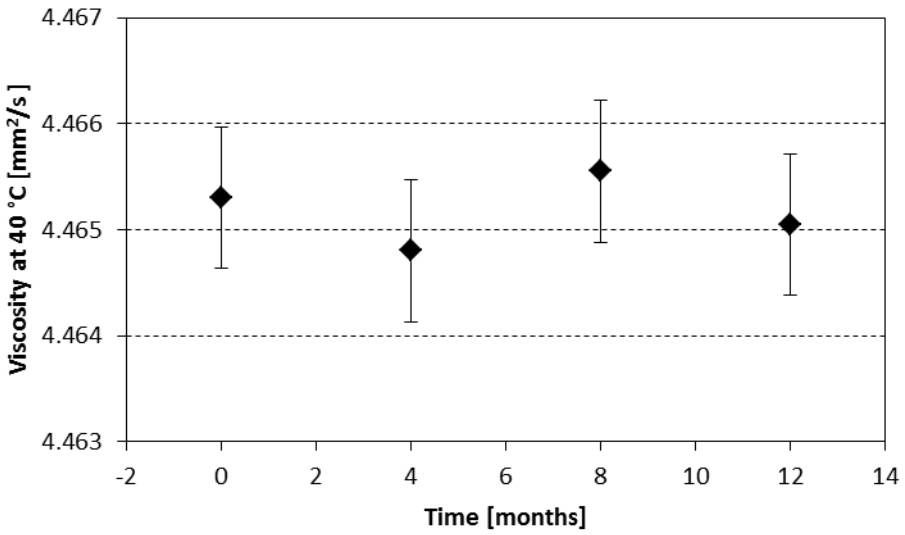

Figure C11: Viscosity means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

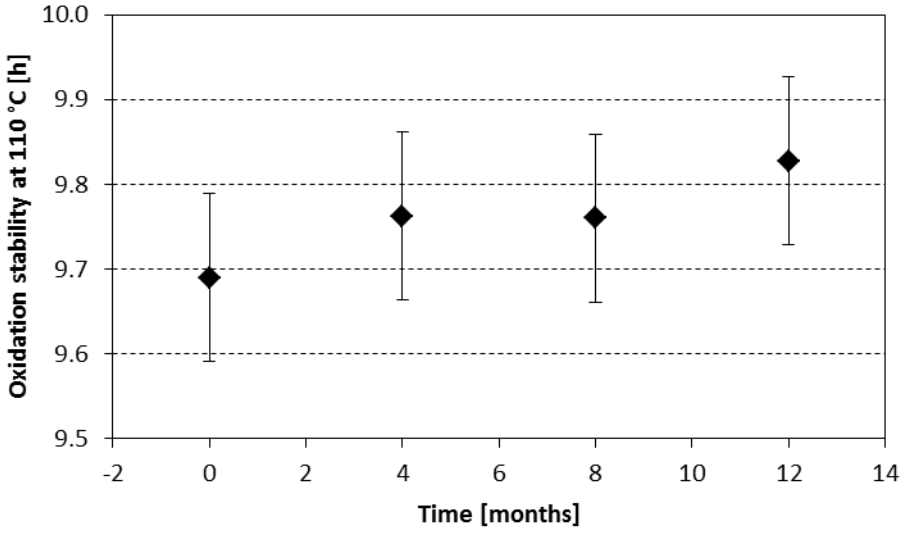

Figure C12: Oxidation stability means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

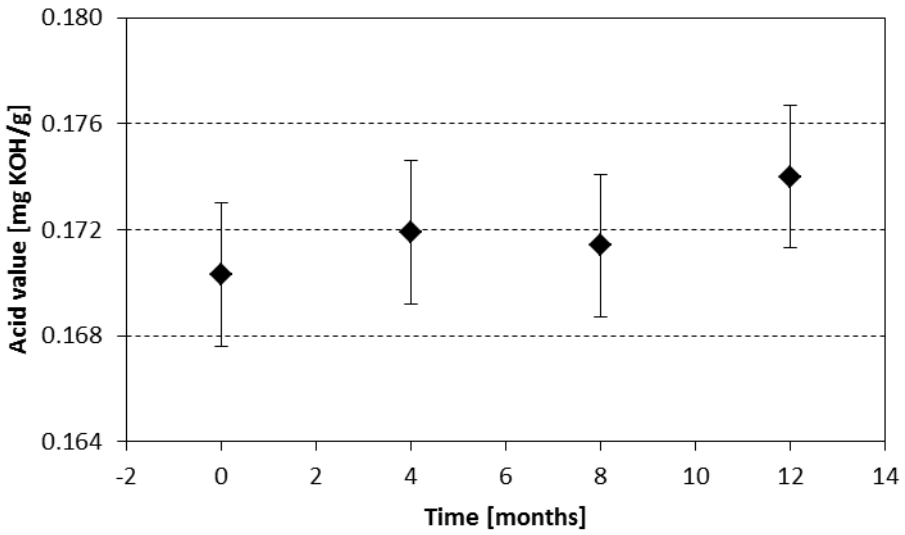

Figure C13: Acid value means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

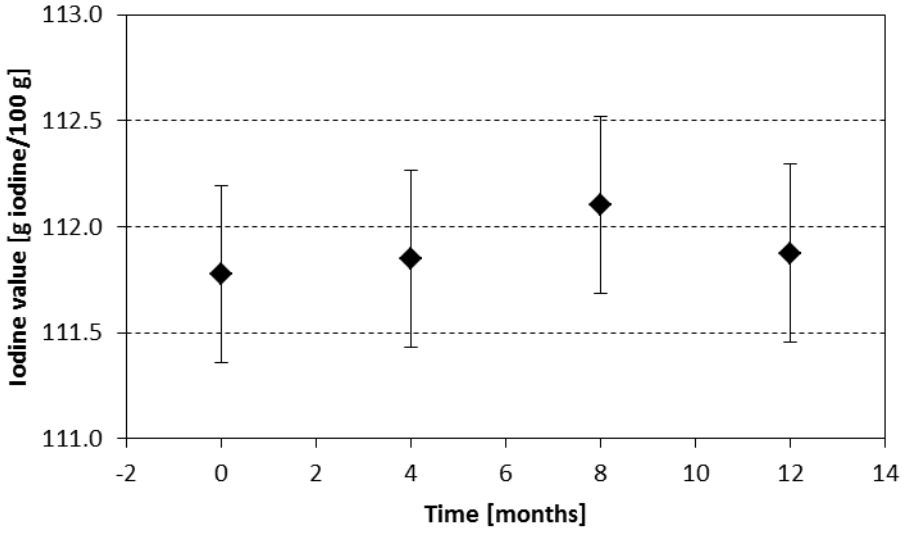

Figure C14: Iodine value means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

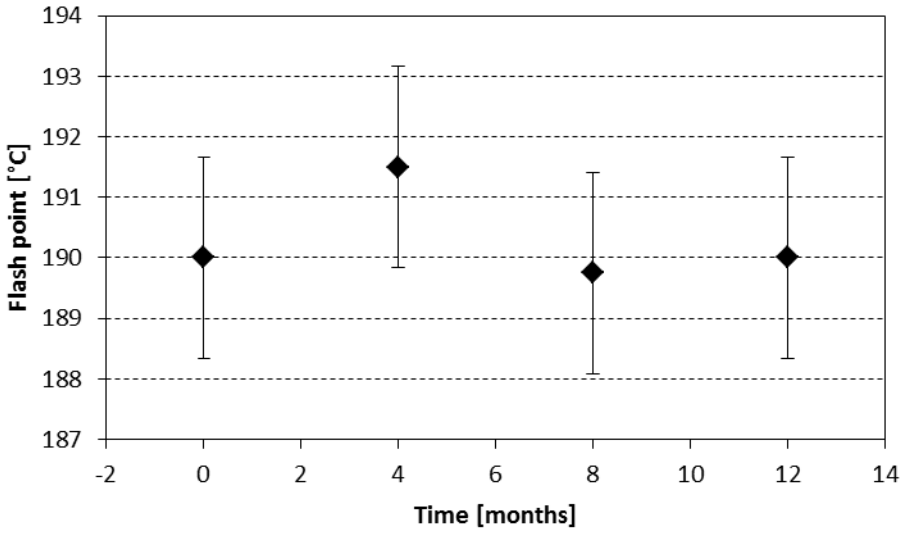

Figure C15: Flash point means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

Annex D: Summary of methods used in the characterisation study

Table D1. Overview on scope and principles of documentary standards

Standard Reference	EN 14103:2011	EN 14105:2011
Technical Body	CEN/TC 307 - Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis	CEN/TC 307 - Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis
Title	Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of **ester and linolenic acid methyl ester contents**	Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of **free and total glycerol and mono-, di-, triglyceride contents**
Scope	The purpose of this document is to describe a procedure for the determination of the ester content in fatty acid methyl esters (FAME) intended for incorporation into diesel oil. It also allows determining the linolenic acid methyl ester content. It allows verifying that the ester content of FAME is greater than 90 % (m/m) and that the linolenic acid content is between 1 % (m/m) and 15 % (m/m). This method is suitable for FAME which contains methyl esters between C6 and C24. NOTE For the purposes of this European Standard, the terms "% (m/m)" and "%(v/v)" are used to represent respectively the mass and volume fractions.	The purpose of this European Standard is to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters (FAME) intended for addition to mineral oils. The total glycerol content is then calculated from the obtained results. Under the conditions described, the quantification limits are 0.001 % (m/m) for free glycerol, 0.10 % (m/m) for all glycerides (mono-, di- and tri-). This method is suitable for FAME prepared from rapeseed, sunflower, soybean, palm, animal oils and fats and mixture of them. It is not suitable for FAME produced from or containing coconut and palm kernel oils derivatives because of overlapping of different glyceride peaks. NOTE For the purposes of this European Standard, the term "% (m/m)" is used to represent respectively the mass fraction.
Principle	Determination of the percentage of total methyl esters of fatty acids and the percentage of linolenic acid methyl ester present in the sample, by gas chromatography according to a procedure using internal calibration (nonadecanoic acid methyl ester).	Transformation of the glycerol and of the mono- and diglycerides into more volatile and stable silyl derivatives in presence of pyridine and of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). Analysis of the sample after silylation, by gas chromatography on a short capillary column with thin film thickness, with an on-column injector or equivalent device, and flame ionization detection. After a calibration procedure, the quantification of glycerol is carried out in presence of the internal standard 1,2,4-butanetriol. Mono-, di- and triglycerides are directly evaluated in presence of an internal standard for each glyceride category: - glyceryl monononadecanoate (Mono C19) for monoglycerides; - glyceryl dinonadecanoate (Di C38) for diglycerides; - glyceryl trinonadecanoate (Tri C57) for triglycerides.

Standard Reference	EN 14110:2003	EN ISO 12937:2000
Technical Body	CEN/TC 307 - Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis	CEN/TC 19 - Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin.
Title	Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of **methanol** content	Petroleum products - Determination of **water** - Coulometric Karl Fischer titration method (ISO 12937:2000)
Scope	This European Standard specifies a method for the determination of methanol content in fatty acid methyl esters (FAME) for use as diesel fuel and domestic heating fuel. The method is applicable for a concentration range from 0.01 % to 0,5 % (m/m) methanol. The method is not applicable to mixtures of FAME which contain other low boiling components.	This International Standard specifies a method for the direct determination of water in petroleum products boiling below 390 °C. It covers the mass fraction range 0.003 % (m/m) to 0.100 % (m/m). It is not applicable to products containing ketones or to residual fuel oils.
Principle	The sample is heated at 80 °C in a hermetically sealed vial to allow desorption of contained methanol into the gas phase. When equilibrium is reached, a defined part of the gas phase is injected into a gas chromatograph, where methanol is detected with a flame ionisation detector. Normally methanol is the only peak in the chromatogram. The amount of methanol is calculated by reference to an external calibration. Methanol can also be determined after addition of an internal standard to the sample before heating, followed by calculation with the use of an internal calibration factor. NOTE If only manual equipment is available then only internal standard calibration should be used.	A sample is visually inspected. If clear and bright, and free from both water droplets and particulate matter on swirling, a weighed portion is injected into the titration vessel of a coulometric Karl Fischer apparatus in which iodine for the Karl Fischer reaction is generated coulometrically at the anode. When all the water has been titrated, excess iodine is detected by an electrometric end-point detector and the titration is terminated. Based on the stoichiometry of the reaction, one mole of iodine reacts with one mole of water, thus the quantity of water is proportional to the total integrated current according to Faraday's Law. If the sample is not clear and bright, or water droplets or particulate matter are observed on swirling, a portion of a solution of sodium dioctylsulfosuccinate is added prior to homogenizing with a mixer. A weighed portion is then treated as described above.

Standard Reference	EN ISO 12185:1996	EN ISO 3104:1996
Technical Body	CEN/TC 19 - Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin.	CEN/TC 19 - Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin.
Title	Crude petroleum and petroleum products - Determination of **density** - Oscillating U-tube method (ISO 12185:1996)	Petroleum products - Transparent and opaque liquids - Determination of **kinematic viscosity and calculation of dynamic viscosity** (ISO 3104:1994)
Scope	Gives a method for the determination, using an oscillation U-tube densitometer, of the density of crude petroleum and related products within the range 600 kg/m^3 to 1 100 kg/m^3 which can be handled as single-phase liquids at the test temperature and pressure.	This International Standard specifies a procedure for the determination of the kinematic viscosity, V, of liquid petroleum products, both transparent and opaque, by measuring the time for a volume of liquid to flow under gravity through a calibrated glass capillary viscometer. The dynamic viscosity, q, can be obtained by multiplying the measured kinematic viscosity by the density, p, of the liquid. NOTE 1 The result obtained from this International Standard is dependent upon the behaviour of the sample and is intended for application to liquids for which primarily the shear stress and shear rates are proportional (Newtonian flow behaviour). If, however, the viscosity varies significantly with the rate of shear, different results may be obtained from viscometers of different capillary diameters. The procedure and precision values for residual fuel oils, which under some conditions exhibit non-Newtonian behaviour, have been included.
Principle	A small (typically less than 1 ml) portion of the test sample is introduced into a temperature-controlled sample cell. The oscillation frequency is noted, and the density of the test sample calculated using cell constants previously determined by measuring the oscillation frequencies when the cell is filled with calibration fluids of known density.	The time is measured for a fixed volume of liquid to flow under gravity through the capillary of a calibrated viscometer under a reproducible driving head and at a known and closely controlled temperature. The kinematic viscosity is the product of the measured flow time and the calibration constant of the viscometer under gravity. Kinematic viscosity, V: Resistance to flow of a fluid under gravity. NOTE 2 For gravity flow under a given hydrostatic head, the pressure head of a liquid is proportional to its density, p. For any particular viscometer, the time of flow of a fixed volume of fluid is directly proportional to its kinematic viscosity, V, where v = r/p, and where q is the dynamic viscosity coefficient.

Standard Reference	EN 14112:2003	EN 14104:2003
Technical Body	CEN/TC 307 - Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis	CEN/TC 307 - Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis
Title	Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of **oxidation stability** (accelerated oxidation test)	Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of **acid value**
Scope	This European Standard specifies a method for the determination of the oxidation stability of fatty acid methyl esters (FAME) at 110 °C.	This European Standard specifies one titrimetric method for the determination of acid value in light coloured Fatty Acid Methyl Esters, hereinafter referred as FAME. It allows the determination of acid value within a range of 0,10 mg KOH/g to 1,00 mg KOH/g.
Principle	A stream of purified air is passed through the sample which has been brought to a specified temperature. The vapours released during the oxidation process, together with the air, are passed into a flask containing water which has been demineralized or distilled and contains an electrode for measuring the conductivity. The electrode is connected to a measuring and recording device. It indicates the end of the induction period when the conductivity begins to increase rapidly. This accelerated increase is caused by the dissociation of volatile carboxylic acids produced during the oxidation process and absorbed in the water.	A test portion is dissolved in a mixed solvent and titrated with a diluted solution of potassium hydroxide, using phenolphthalein as an indicator in order to detect the titration end point. The acid value is the number of milligrams of potassium hydroxide required to neutralise the free fatty acids present in 1 g of FAME, when determined in accordance with the procedure specified in this European Standard

Standard Reference	EN 14111:2003	EN ISO 3679:2004
Technical Body	CEN/TC 307 - Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis	CEN/TC 19 - Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin.
Title	Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of **iodine value**	Determination of **flash point** - Rapid equilibrium closed cup method (ISO 3679:2004)
Scope	This European Standard specifies a titrimetric method for the determination of iodine value in Fatty Acid Methyl Esters, hereinafter referred as FAME. The iodine value is defined as the mass of halogen, expressed as iodine, absorbed by the test portion when determined in accordance with the procedure specified in this European Standard, divided by the mass of the test portion. Iodine value is reported as grams of iodine per 100 g of FAME.	ISO 3679:2004 specifies a method for the determination of the closed cup flash point of paints (including water-borne paints), varnishes, paint binders, adhesives, solvents, petroleum, and related products having closed cup flash points within the range of - 30 degrees Celsius to 300 degrees Celsius. When used in conjunction with the flash detector (A.1.6), ISO 3679:2004 is also suitable for the determination of the flash point of fatty acid methyl esters (FAME).
Principle	A test portion is dissolved in a mixed solvent and then Wijs reagent is added. After a specified time, potassium iodide and water are added to the sample and the liberated iodine is titrated using a sodium thiosulfate standardized solution.	A test portion of specified volume is introduced into the test cup, which is maintained at the temperature of the estimated flash point of the material under test. After a specified time, a test flame is applied and the presence or absence of a flash observed. Further tests, with fresh test portions at different temperatures, are carried out until the flash point is determined to the sensitivity specified. Flash point is defined as the lowest temperature of the test portion (as measured in the prescribed manner), corrected to a barometric pressure of 101,3 kPa, at which application of a test flame causes the vapour of the test portion to ignite momentarily and the flame to propagate across the surface of the liquid under the specified conditions of test

Table D2: Precision data as laid down in respective documentary standards and estimated expanded measurement uncertainties thereof

Parameter	Unit	r	R	U_{meas}
Ester content	[% (m/m)]	1.01	4.16	2.90
Linolenic acid methyl ester content	[% (m/m)]	0.0283 + 0.0175 · C [1)]	0.3872 + 0.0285 · C	0.44
Monoglyceride content	[% (m/m)]	0.0787 · C + 0.0059	0.1867 · C + 0.0654	0.128
Diglyceride content	[% (m/m)]	0.0989 · C + 0.0042	0.1885 · C + 0.0289	0.037
Total glycerol content	[% (m/m)]	0.1092 · C - 0.0034	0.1902 · C + 0.0115	0.032
Methanol content	[% (m/m)]	0.056 · C + 0.001	0.221 · C + 0.003	0.0085
Water content	[% (m/m)]	0.01874 · C^0.5	0.06877 · C^0.5	0.0068
Density	[kg/m^3]	0.2	0.5	0.33
Viscosity	[mm^2/s]	0.0011 · C	0.0065 · C	0.020
Oxidation stability	[h]	0.09 · C + 0.16	0.26 · C + 0.23	1.86
Acid value	[mg KOH/g]	0.02	0.06	0.041
Iodine value	[g iodine/100 g]	3	5	2.99
Flash point	[°C]	1.9	15	10.6

[1)] C=Determined amount for respective parameter

Annex E: Results of the characterisation measurements

Table E1: Mass fraction of ester content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	97.6	98.0	98.9	98.5	97.8	97.6	98.1	0.54
L2	98.71	99.47	98.69	98.10	98.17	99.1	98.7	0.53
L3	98.59	97.96	98.36	98.53	98.88	98.36	98.45	0.31
L4	99.45	99.80	99.31	99.68	99.99	99.95	99.70	0.27
L5	98.737	98.712	98.756	98.845	98.829	98.780	98.777	0.05
L6	98.6	99.0	99.8	99.8	99.4	99.6	99.4	0.48
L8	100.00	99.46	99.70	100.00	99.70	99.39	99.71	0.26
L9	98.1	97.6	98.5	97.6	98.7	98.5	98.2	0.49
L10	100.7	101	102	101	100.9	100.8	101.1	0.47
L11	97.23	97.22	97.06	97.06	97.17	97.14	97.15	0.07
Results not used for certification								
L7	*99.1*	*99.9*	*99.5*	*98.5*	*99.8*	*99.1*	*99.3*	*0.53*

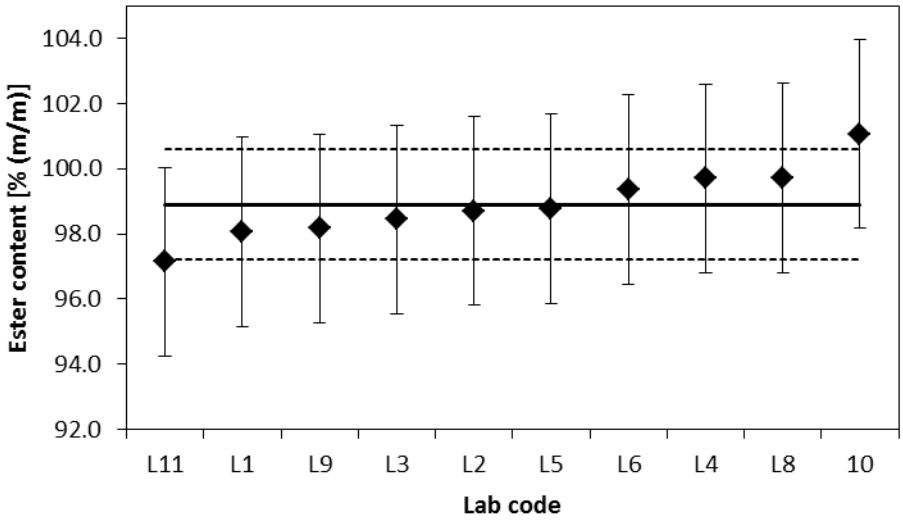

Figure E1: Results of the characterisation study for the mass fraction of ester content in biodiesel measured using EN 14103 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E2: Mass fraction of linolenic acid methyl ester content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	8.9	9.0	8.9	9.0	8.9	8.9	8.9	0.58
L2	8.91	8.75	8.86	8.76	8.90	8.92	8.85	0.86
L3	8.84	8.79	8.81	8.80	8.88	8.83	8.83	0.37
L4	8.56	8.72	8.75	8.78	8.59	8.52	8.65	1.27
L5	8.833	8.830	8.848	8.857	8.838	8.837	8.841	0.11
L6	8.7	8.8	8.9	8.9	8.8	8.8	8.8	0.85
L8	8.84	8.76	8.78	8.82	8.82	8.81	8.81	0.33
L9	8.7	8.7	8.7	8.7	8.8	8.8	8.7	0.59
L10	9.1	9.1	9.2	9.0	9.0	9.0	9.1	0.90
L11	8.64	8.65	8.62	8.61	8.63	8.63	8.63	0.16
Results not used for certification								
L7	*8.78*	*8.8*	*8.82*	*8.86*	*8.83*	*8.88*	*8.83*	*0.42*

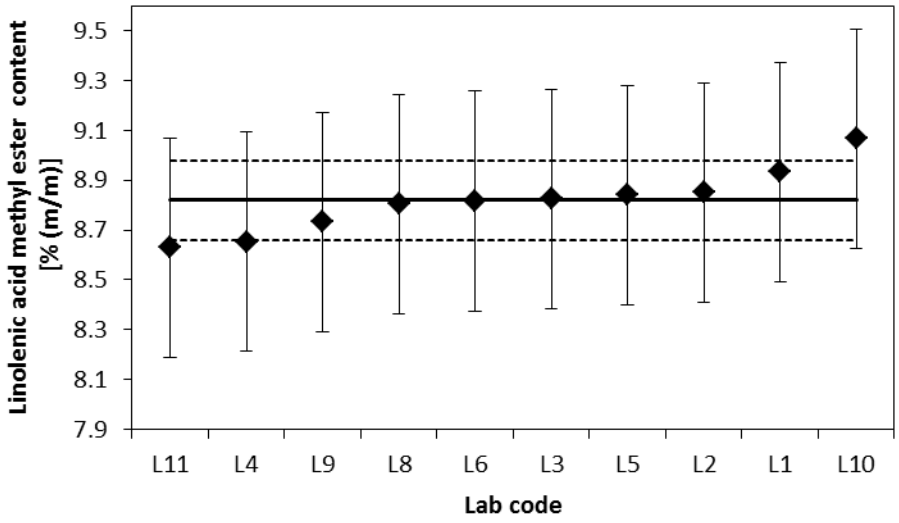

Figure E2: Results of the characterisation study for the mass fraction of linolenic acid methyl ester content in biodiesel measured using EN 14103 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E3: Mass fraction of monoglyceride content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	0.74	0.71	0.70	0.72	0.74	0.71	0.72	2.32
L2	0.589	0.629	0.641	0.654	0.663	0.642	0.636	4.08
L3	0.5846	0.6361	0.5585	0.6055	0.5505	0.5978	0.5888	5.37
L4	0.640	0.593	0.609	0.612	0.613	0.619	0.614	2.49
L5	0.6608	0.6705	0.6523	0.6497	0.6742	0.6545	0.6603	1.53
L6	0.63	0.64	0.60	0.61	0.66	0.61	0.63	3.61
L7	0.64	0.62	0.67	0.65	0.64	0.66	0.65	2.71
L8	0.681	0.679	0.682	0.682	0.708	0.695	0.688	1.66
L9	0.66	0.65	0.63	0.65	0.63	0.63	0.64	2.07
L11	0.69	0.69	0.70	0.70	0.64	0.64	0.68	4.15
Results not used for certification								
L10	*0.67*	*0.66*	*0.76*	*0.76*	*0.78*	*0.81*	*0.74*	*8.24*

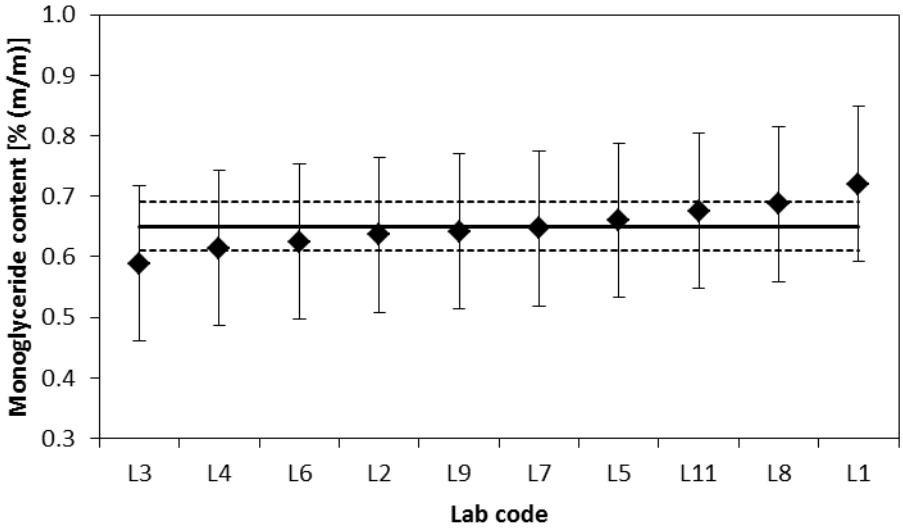

Figure E3: Results of the characterisation study for the mass fraction of monoglyceride content in biodiesel measured using EN 14105 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E4: Mass fraction of diglyceride content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	0.13	0.13	0.13	0.13	0.13	0.12	0.13	3.18
L2	0.141	0.151	0.148	0.149	0.152	0.149	0.148	2.62
L3	0.1311	0.1325	0.1273	0.1319	0.1107	0.1259	0.1266	6.49
L4	0.152	0.142	0.133	0.143	0.146	0.151	0.145	4.81
L5	0.1411	0.1414	0.1436	0.1426	0.1439	0.1329	0.1409	2.90
L6	0.10	0.10	<0.1	<0.1	0.10	0.10	0.10	
L7	0.11	0.11	0.12	0.12	0.11	0.12	0.12	4.76
L8	0.134	0.136	0.134	0.133	0.145	0.133	0.136	3.40
L9	0.17	0.16	0.16	0.16	0.16	0.16	0.16	2.53
L11	0.159	0.160	0.161	0.160	0.152	0.153	0.158	2.42
Results not used for certification								
L10	*0.14*	*0.14*	*0.14*	*0.14*	*0.17*	*0.19*	*0.15*	*14.09*

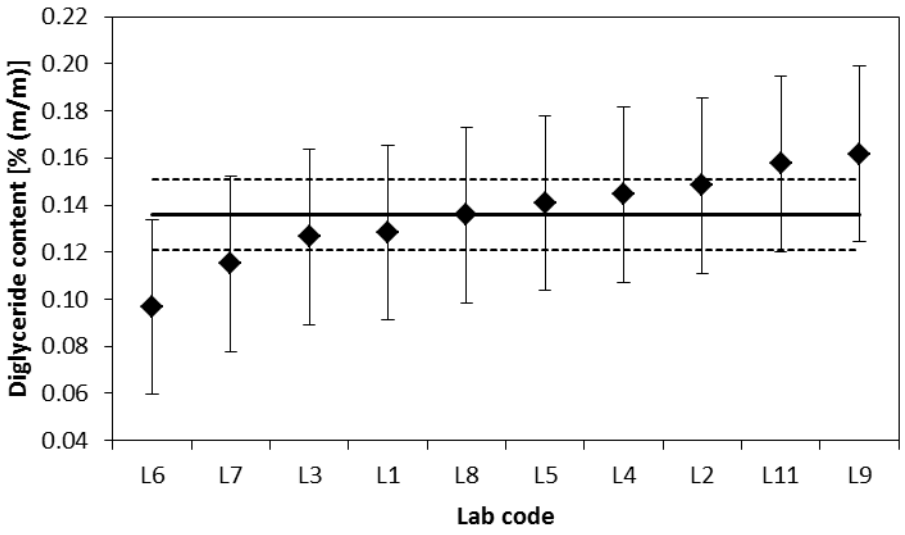

Figure E4: Results of the characterisation study for the mass fraction of diglyceride content in biodiesel measured using EN 14105 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E5: Mass fraction of triglyceride content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L8	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
L11	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
Results not used for certification								
L10	*<0.1*	*<0.1*	*<0.1*	*<0.1*	*<0.1*	*<0.1*	*<0.1*	

Table E6: Mass fraction of free glycerol content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	0.004	0.003	0.004	0.004	0.002	0.003	0.003	24.49
L2	0.003	0.003	0.002	0.003	0.002	0.002	0.003	21.91
L3	0.0011	0.0014	0.0011	0.0010	0.0014	0.0013	0.0012	14.16
L4	0.0014	0.0015	0.0013	0.0013	0.0016	0.0015	0.0014	8.45
L5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
L6	0.006	0.005	0.004	0.003	0.003	0.003	0.00400	31.62
L7	0.0041	0.0037	0.0032	0.0036	0.0042	0.0032	0.0037	11.66
L8	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
L9	<0.001	<0.001	0.00102	<0.001	0.00102	0.00103	<0.001	
L11	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Results not used for certification								
L10	*0.002*	*0.002*	*0.001*	*0.003*	*0.003*	*0.003*	*0.002*	*34.99*

Table E7: Mass fraction of total glycerol content in biodiesel recalculated excluding the free glycerol and/or triglyceride fractions that were below the LOQs using the formula from EN 14105:2011 (total glycerol = free glycerol + 0,255 monoglycerides + 0,146 diglycerides + 0,103 triglycerides)

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	0.21	0.20	0.20	0.21	0.21	0.20	0.21	2.11
L2	0.174	0.185	0.187	0.192	0.193	0.187	0.186	3.68
L3	0.169	0.183	0.162	0.175	0.158	0.172	0.170	5.28
L4	0.187	0.173	0.176	0.178	0.179	0.181	0.179	2.58
L5	0.1891	0.1916	0.1873	0.1865	0.1929	0.1863	0.1890	1.47
L6	0.181	0.183	0.157	0.159	0.186	0.173	0.1731	7.29
L7	0.18	0.18	0.19	0.19	0.18	0.19	0.19	2.62
L8	0.193	0.193	0.193	0.193	0.202	0.197	0.195	1.77
L9	0.193	0.189	0.185	0.189	0.185	0.185	0.188	1.76
L11	0.199	0.199	0.203	0.201	0.185	0.186	0.195	4.04
Results not used for certification								
L10	*0.19*	*0.19*	*0.22*	*0.22*	*0.23*	*0.24*	*0.21*	*8.60*

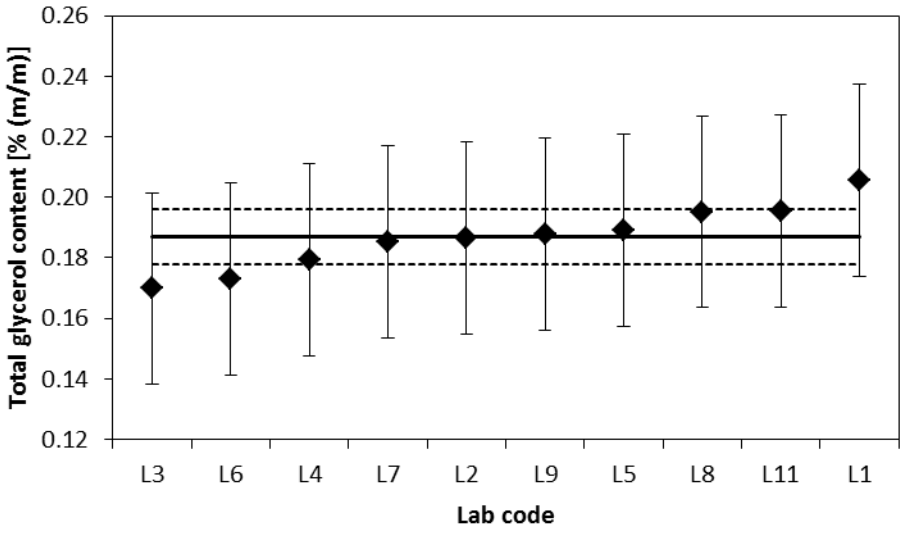

Figure E5: Results of the characterisation study for the mass fraction of total glycerol content in biodiesel measured using EN 14105 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E8: Mass fraction of methanol content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	0.03	0.03	0.02	0.02	0.02	0.02	0.02	22.13
L2	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.00
L3	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.00
L4	0.041	0.039	0.047	0.047	0.048	0.049	0.045	9.12
L5	0.04666	0.04617	0.04805	0.04759	0.04676	0.04567	0.04682	1.88
L6	0.05	0.04	0.04	0.05	0.05	0.05	0.05	11.07
L7	0.042	0.041	0.031	0.041	0.033	0.033	0.037	13.57
L8	0.038	0.038	0.039	0.039	0.038	0.038	0.038	1.35
L9	0.050	0.048	0.045	0.047	0.046	0.046	0.047	3.81
L10	0.0527	0.0495	0.0392	0.0403	0.0482	0.0492	0.0465	11.75

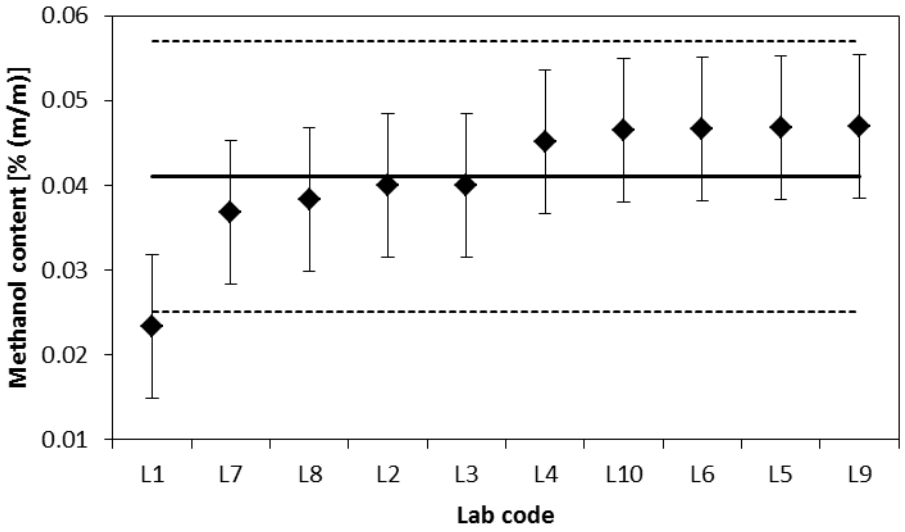

Figure E6: Results of the characterisation study for the mass fraction of methanol content in biodiesel measured using EN 14110 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E9: Mass fraction of water content in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [% (m/m)]	replicate 2 [% (m/m)]	replicate 3 [% (m/m)]	replicate 4 [% (m/m)]	replicate 5 [% (m/m)]	replicate 6 [% (m/m)]	mean [% (m/m)]	RSD [%]
L1	0.0224	0.0223	0.0211	0.0201	0.0210	0.0216	0.0214	4.06
L2	0.0202	0.0211	0.0207	0.0215	0.0195	0.0197	0.0205	3.86
L3	0.01857	0.01957	0.01876	0.01885	0.01915	0.01895	0.01898	1.84
L5	0.019594	0.019820	0.019549	0.020272	0.019425	0.020464	0.019854	2.13
L6	0.0230	0.0240	0.0240	0.0240	0.0230	0.0250	0.0238	3.16
L9	0.01784	0.01827	0.01751	0.01829	0.01737	0.01744	0.01779	2.33
L10	0.0221	0.0220	0.0206	0.0207	0.0216	0.0215	0.0214	2.97
Results not used for certification								
L4	*0.0221*	*0.0224*	*0.0185*	*0.0193*	*0.0202*	*0.0198*	*0.0204*	*7.63*
L7	*0.0317*	*0.0319*	*0.0305*	*0.0303*	*0.0303*	*0.0298*	*0.0308*	*2.76*

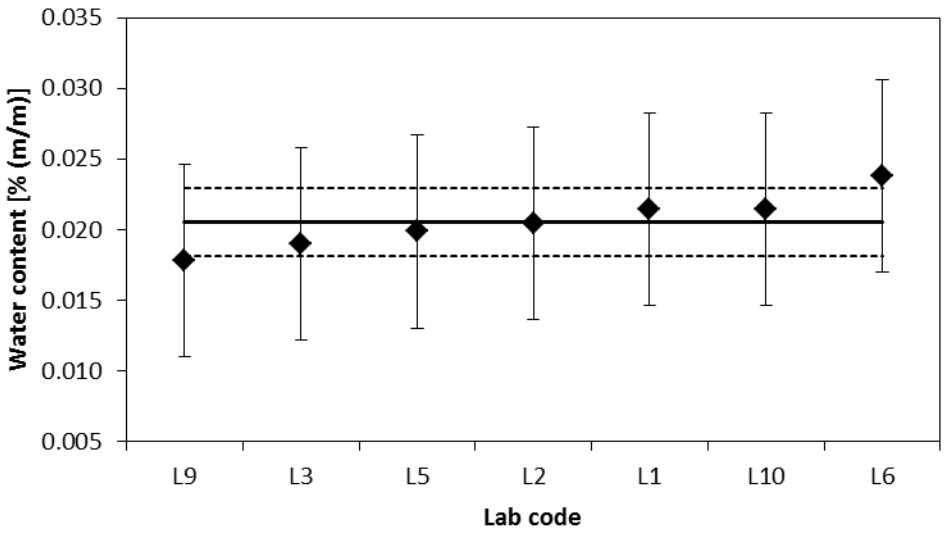

Figure E7: Results of the characterisation study for the mass fraction of water content in biodiesel measured using EN ISO 12937 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E10: Density in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [kg/m^3]	replicate 2 [kg/m^3]	replicate 3 [kg/m^3]	replicate 4 [kg/m^3]	replicate 5 [kg/m^3]	replicate 6 [kg/m^3]	mean [kg/m^3]	RSD [%]
L1	883.2	883.2	883.3	883.2	883.3	883.3	883.3	0.006
L2	883.2	883.2	883.2	883.2	883.2	883.2	883.2	0.000
L3	883.17	883.16	883.20	883.21	883.19	883.18	883.19	0.002
L4	883.15	883.18	883.14	883.14	883.14	883.15	883.15	0.002
L5	883.22	883.23	883.23	883.23	883.22	883.22	883.23	0.001
L6	883.2	883.2	883.2	883.1	883.2	883.2	883.2	0.005
L7	883.2	883.2	883.2	883.2	883.2	883.2	883.2	0.000
L9	883.2	883.2	883.2	883.2	883.2	883.2	883.2	0.000
L10	883.2	883.2	883.2	883.2	883.2	883.2	883.2	0.000

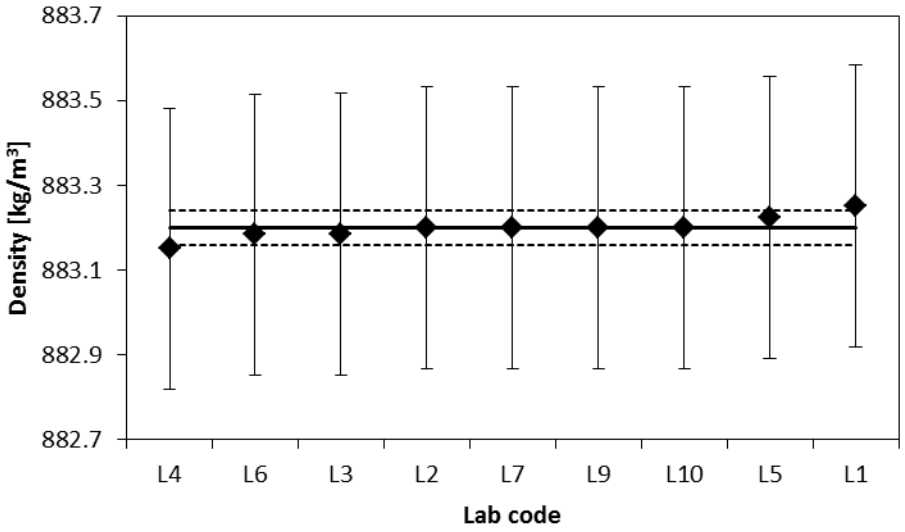

Figure E8: Results of the characterisation study for density in biodiesel measured using EN ISO 12185 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E11: Viscosity in biodiesel as reported by each individual lab

Laboratory code	replicate 1 [mm^2/s]	replicate 2 [mm^2/s]	replicate 3 [mm^2/s]	replicate 4 [mm^2/s]	replicate 5 [mm^2/s]	replicate 6 [mm^2/s]	mean [mm^2/s]	RSD [%]
L2	4.462	4.465	4.467	4.464	4.463	4.467	4.465	0.05
L3	4.4655	4.4650	4.4655	4.4658	4.4652	4.4655	4.4654	0.01
L4	4.4648	4.4638	4.4648	4.4658	4.4638	4.4648	4.4646	0.02
L5	4.4627	4.4616	4.4598	4.4611	4.4622	4.4607	4.4614	0.02
L6	4.474	4.474	4.473	4.475	4.470	4.473	4.473	0.04
L9	4.466	4.464	4.465	4.462	4.466	4.465	4.465	0.03
Results not used for certification								
L1	*4.448*	*4.453*	*4.454*	*4.455*	*4.460*	*4.451*	*4.454*	*0.09*
L7	*4.5130*	*4.5110*	*4.4950*	*4.5020*	*4.4990*	*4.5035*	*4.5039*	*0.15*
L10	*4.4660*	*4.4695*	*4.4774*	*4.4684*	*4.4686*	*4.4701*	*4.4700*	*0.09*

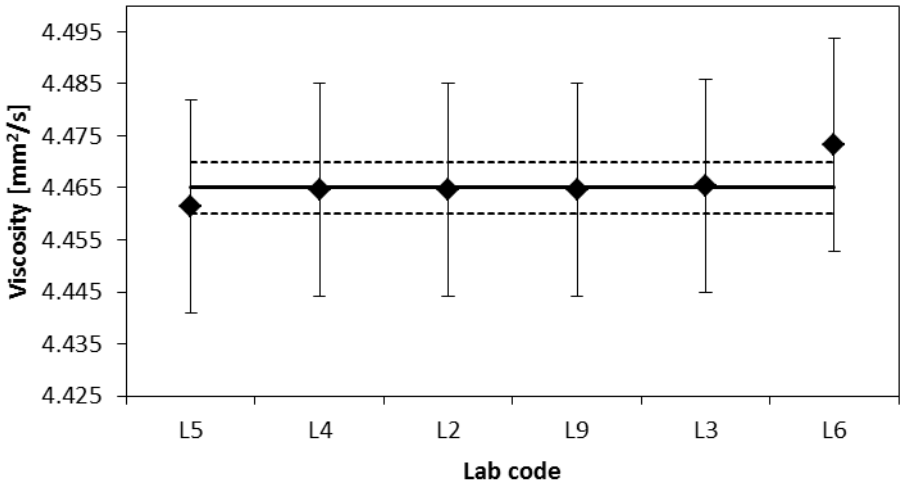

Figure E9: Results of the characterisation study for viscosity in biodiesel measured using EN ISO 3104 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E12: Oxidation stability of biodiesel as reported by each individual lab

Laboratory code	replicate 1 [h]	replicate 2 [h]	replicate 3 [h]	replicate 4 [h]	replicate 5 [h]	replicate 6 [h]	mean [h]	RSD [%]
L1	10.20	10.00	10.10	10.20	9.90	9.90	10.05	1.37
L2	9.77	9.60	9.69	9.68	9.76	9.74	9.71	0.66
L3	10.16	10.31	10.39	10.54	10.31	10.38	10.35	1.21
L4	9.11	9.05	9.09	8.97	9.04	8.98	9.04	0.63
L5	9.67	9.70	9.72	9.75	9.90	9.92	9.78	1.09
L7	9.10	9.00	9.10	9.30	9.30	9.40	9.20	1.68
L8	9.62	9.58	9.70	9.76	9.81	9.82	9.72	1.02
L9	9.82	9.75	9.62	9.57	9.85	9.79	9.73	1.16
L10	10.10	10.20	10.30	10.30	10.10	10.20	10.20	0.88
L11	9.99	9.91	10.16	9.95	10.01	9.83	9.98	1.11
Results not used for certification								
L6	*9.40*	*9.60*	*13.20*	*13.00*	*9.60*	*9.70*	*10.75*	*16.97*

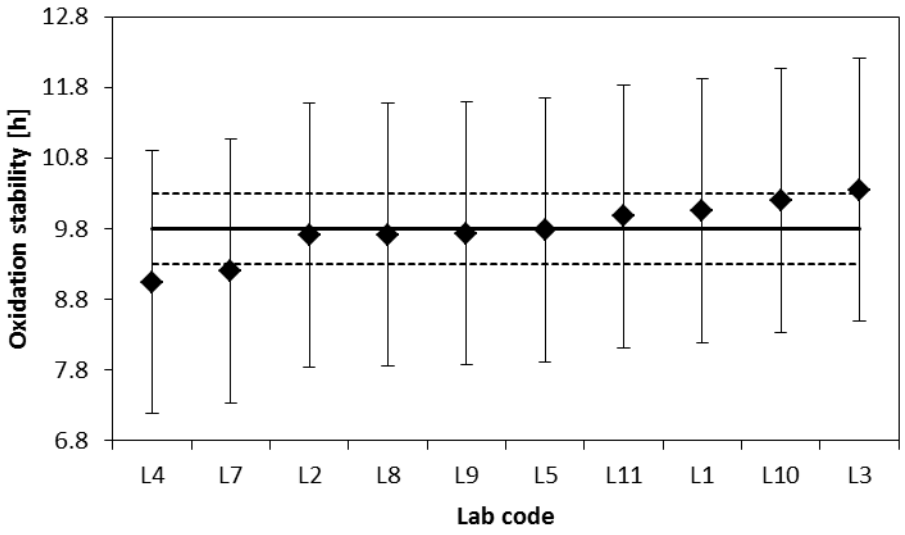

Figure E10: Results of the characterisation study for the oxidation stability of biodiesel measured using EN 14112 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E13: Acid value of biodiesel as reported by each individual lab

Laboratory code	replicate 1 [mg KOH/g]	replicate 2 [mg KOH/g]	replicate 3 [mg KOH/g]	replicate 4 [mg KOH/g]	replicate 5 [mg KOH/g]	replicate 6 [mg KOH/g]	mean [mg KOH/g]	RSD [%]
L1	0.19	0.19	0.18	0.18	0.20	0.20	0.19	4.71
L2	0.21	0.22	0.22	0.21	0.22	0.21	0.22	2.55
L3	0.19531	0.19576	0.18197	0.18194	0.18201	0.19586	0.18881	3.97
L4	0.1699	0.1708	0.1822	0.1809	0.1837	0.1804	0.1780	3.39
L5	0.1798	0.1798	0.1790	0.1829	0.1796	0.1827	0.1806	0.94
L6	0.18	0.18	0.18	0.17	0.19	0.18	0.18	3.51
L7	0.19	0.19	0.21	0.20	0.17	0.19	0.19	6.93
L8	0.200	0.183	0.183	0.184	0.195	0.186	0.189	3.84
L9	0.16	0.16	0.15	0.16	0.15	0.16	0.16	3.30
L10	0.17	0.17	0.16	0.19	0.17	0.19	0.18	7.00

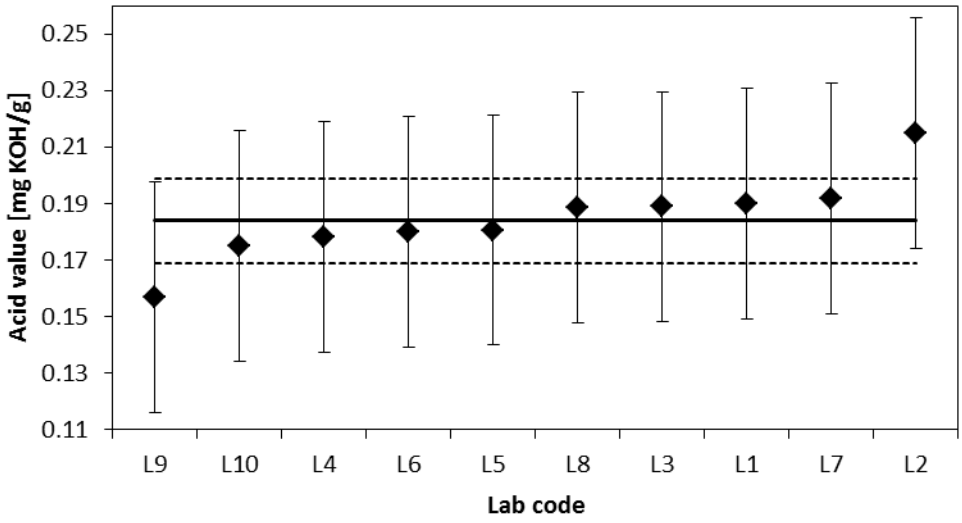

Figure E11: Results of the characterisation study for the acid value of biodiesel measured using EN 14104 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E14: Iodine value of biodiesel as reported by each individual lab

Laboratory code	replicate 1 [g iodine/100 g]	replicate 2 [g iodine/100 g]	replicate 3 [g iodine/100 g]	replicate 4 [g iodine/100 g]	replicate 5 [g iodine/100 g]	Replicate 6 [g iodine/100 g]	mean [g iodine/100 g]	RSD [%]
L1	108	108	107	108	107	109	108	0.70
L2	115	115	111	112	112	111	113	1.65
L3	113	111	111	110	113	114	112	1.39
L4	113.3	113.4	112.4	113	112.2	112.4	112.8	0.46
L5	111.3	111.9	111.6	111.5	112.1	112.4	111.8	0.37
L6	112.1	112.5	111.0	111.0	112.0	113.0	111.9	0.72
L7	113	111	113	113	112	113	113	0.74
L8	111.73	112.88	110.93	111.66	111.65	110.17	111.50	0.81
L9	115	116	115	117	116	115	116	0.71
L10	113	113	113	113	114	114	113	0.46

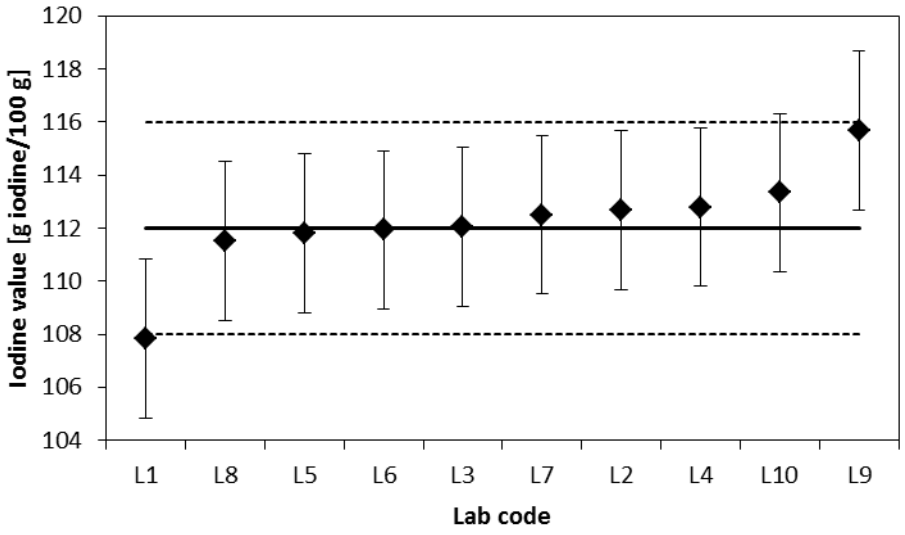

Figure E12: Results of the characterisation study for the iodine value of biodiesel measured using EN 14111 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

Table E15: Flash point of biodiesel as reported by each individual lab

Laboratory code	replicate 1 [°C]	replicate 2 [°C]	replicate 3 [°C]	replicate 4 [°C]	replicate 5 [°C]	Replicate 6 [°C]	mean [°C]	RSD [%]
L1	174	174	174	174	174	174	174	0.00
L3	173	174	177	177	177	177	176	1.04
L4	191.0	191.0	196.5	197.5	193.5	194.5	194.0	1.40
L5	176.8	176.8	180.4	180.4	175.9	176.2	177.8	1.17
L6	184	184	186	186	186	186	185	0.56
Results not used for certification								
L10	*177*	*177*	*178*	*178*	*176*	*176*	*177*	*0.45*

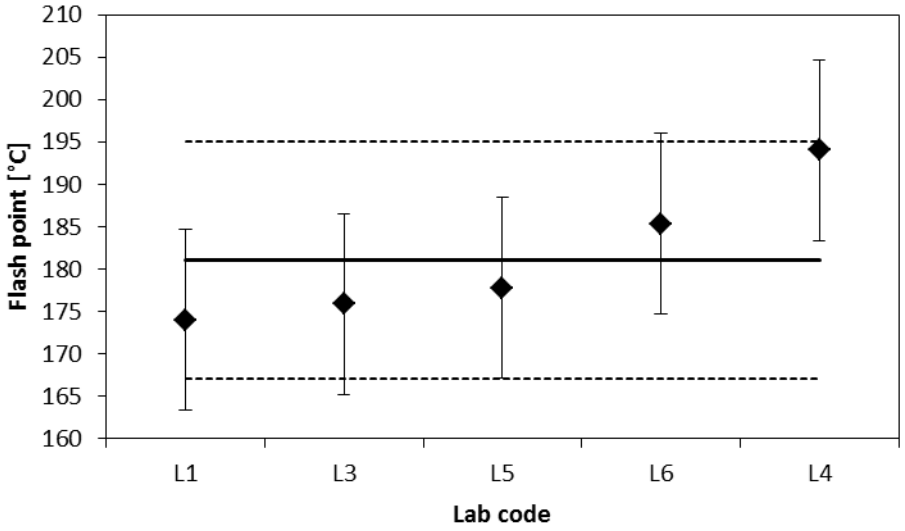

Figure E13: Results of the characterisation study for the flash point of biodiesel measured using EN ISO 3679 (continuous line: certified value; dashed line: expanded uncertainty with k=2.8; error bars: expanded measurement uncertainty as given in Table D2)

European Commission

EUR 26711 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: **The certification of the mass fraction of the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point of biodiesel: ERM® - EF001**
Author(s): M. Ulberth-Buchgraber, V. Morales, J. Charoud-Got, H. Emteborg, A. Held
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Abstract
This report describes the production of ERM®-EF001, a biodiesel material certified for the ester, linolenic acid methyl ester, monoglyceride, diglyceride, triglyceride, total glycerol and water content, density, viscosity, oxidation stability, acid value, iodine value and flash point. The material was produced following ISO Guide 34:2009.
A rapeseed oil fatty acid methyl ester with the addition of an antioxidant (butylhydroxytoluene) was selected as the base material. It was provided by a biodiesel producer located in Germany. The material was filled in amber glass ampoules. To keep the material homogenous throughout the filling it was gently bubbled with argon.
Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006. The minimum sample intake is defined by the required sample volume stipulated in the respective documentary standard.
The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, and instability and to characterisation.
The material is intended for the quality control or assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in glass bottles containing 27 mL of biodiesel closed under argon atmosphere.
The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

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