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European Reference Materials

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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European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information
Reference materials sales
Retieseweg 111
B-2440 Geel, Belgium
E-mail: jrc-irmm-rm-sales@ec.europa.eu
Tel.: +32 (0)14 571 705
Fax: +32 (0)14 590 406

http://irmm.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

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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

M. Ulberth-Buchgraber, V. Morales, J. Charoud-Got, H. Emteborg, A. Held

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

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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]	0.65	0.04	% (m/m) [4]
Diglyceride content [2]	0.136	0.015	% (m/m) [4]
Triglyceride content [2]	<0.1 [6]	-	% (m/m) [4]
Total glycerol content [2]	0.187	0.009	% (m/m) [4]
Water content [3]	0.0205	0.0024	% (m/m) [4]

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]	9.8	0.5	h
Acid value [4]	0.184	0.015	mg KOH/g
Iodine value [5]	112	4	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.

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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)

FUNDACIÓN CETENA, Noain, ES
(measurements partially under the scope of ISO/IEC 17025 accreditation ENAC 69/LE1062)

INNOVHUB - Stazioni Sperimentali per l'Industria, Milan, IT
(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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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".

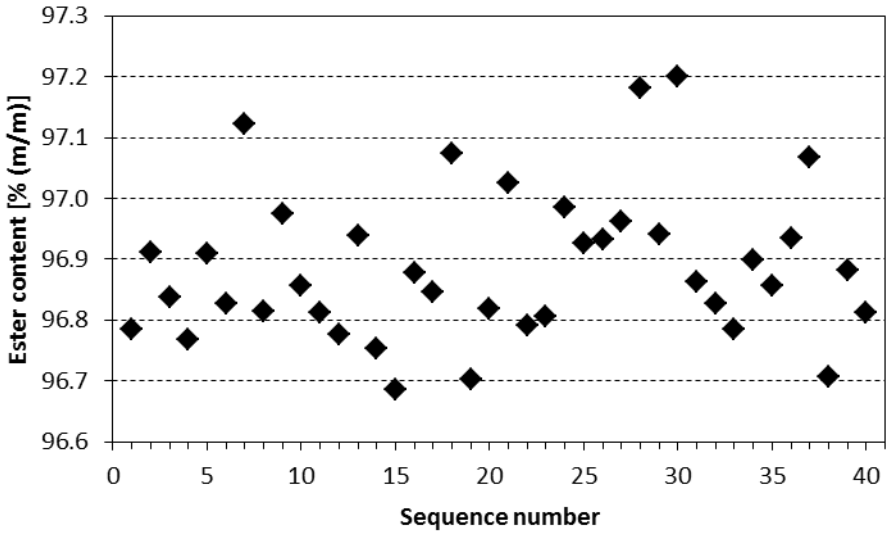

Figure A1: Individual measurement replicates for ester content, against sequence number.

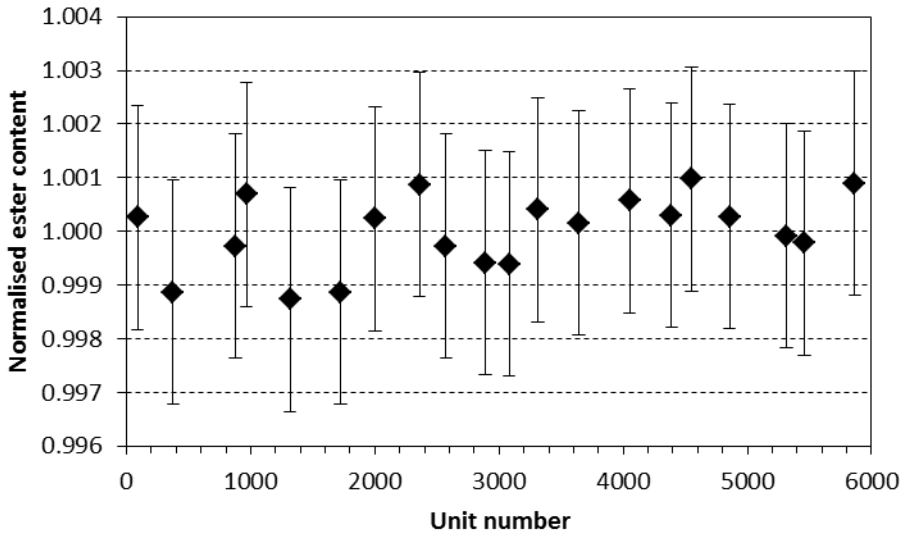

Figure A2: Normalised unit means for ester content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

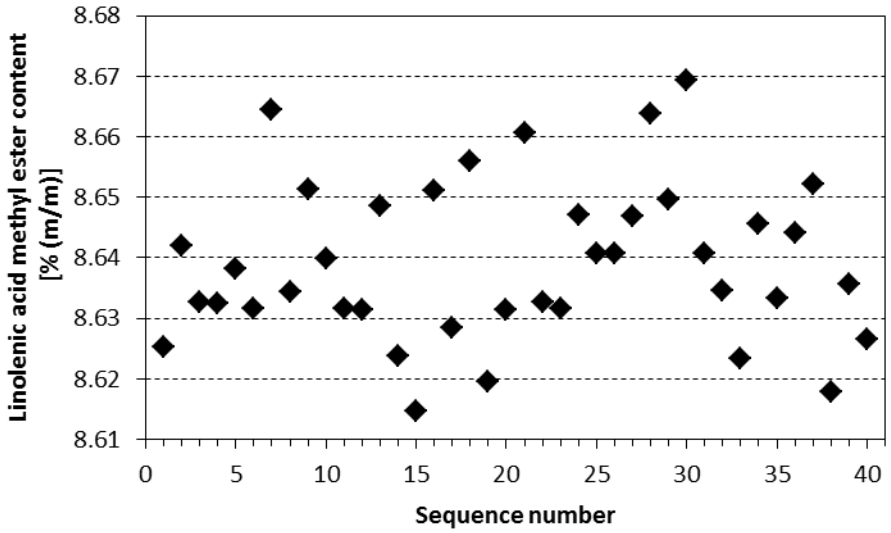

Figure A3: Individual measurement replicates for linolenic acid methyl ester content, against sequence number.

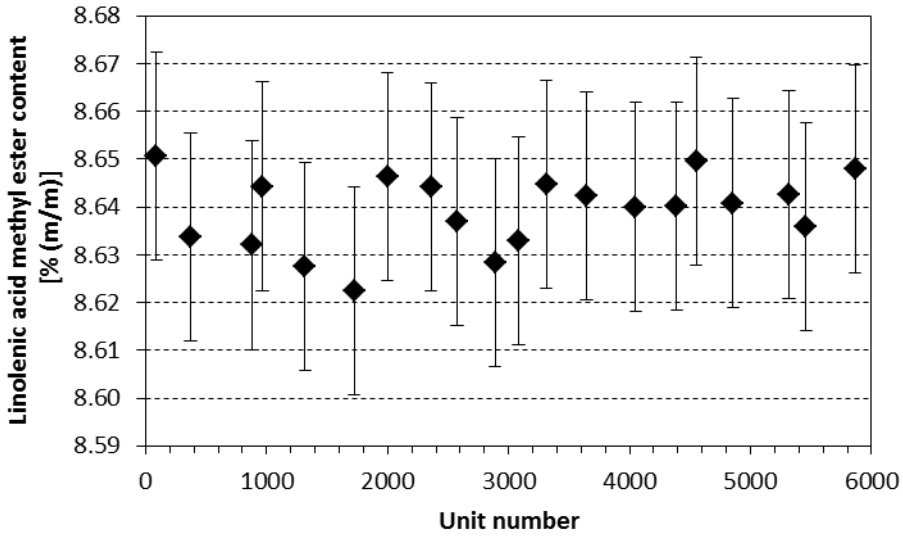

Figure A4: Unit means for linolenic acid methyl ester content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

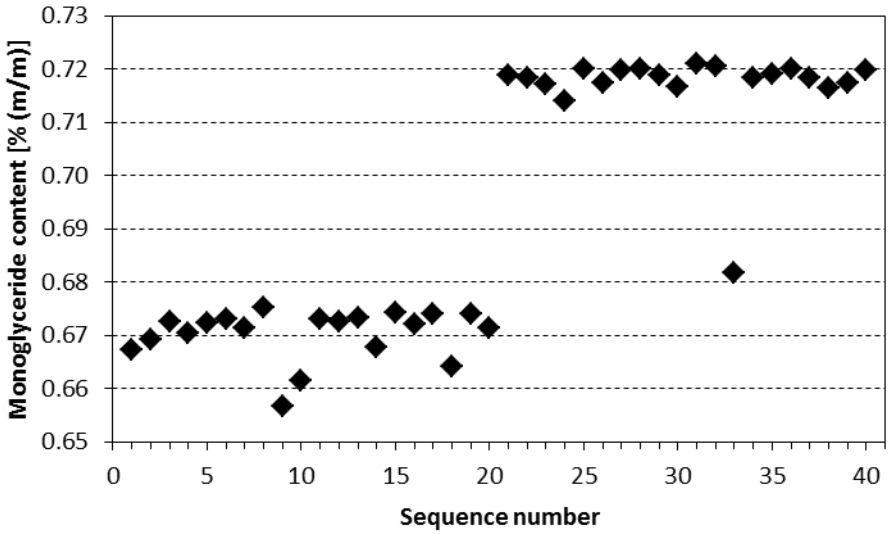

Figure A5: Individual measurement replicates for monoglyceride content, against sequence number.

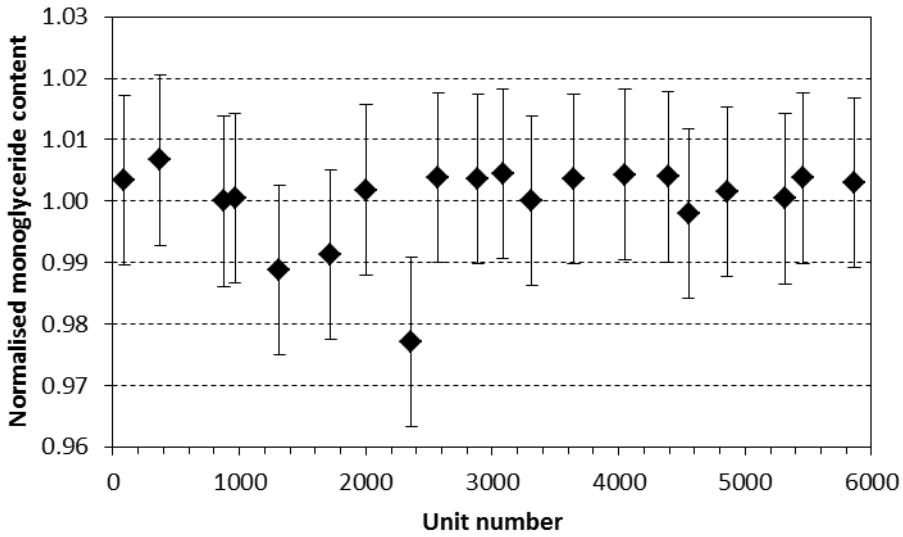

Figure A6: Normalised unit means for monoglyceride content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

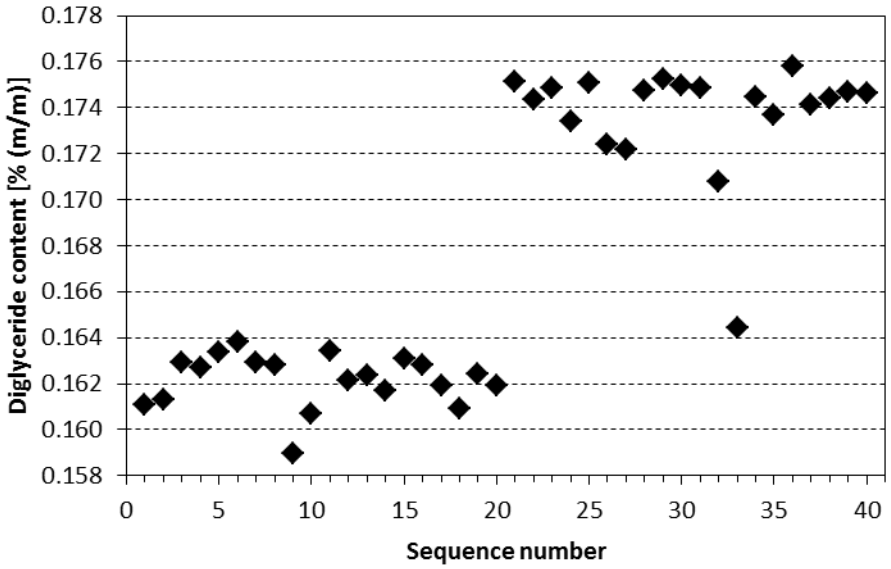

Figure A7: Individual measurement replicates for diglyceride content, against sequence number.

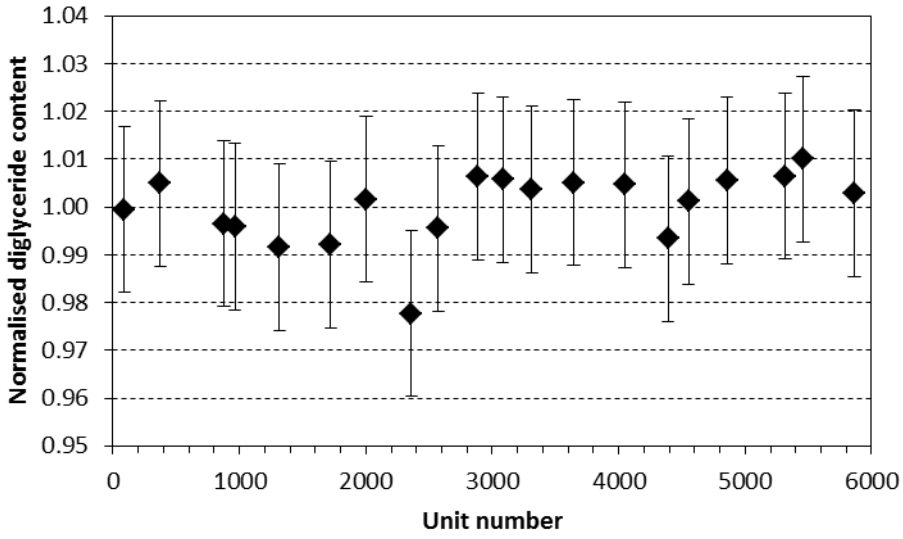

Figure A8: Normalised unit means for diglyceride content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

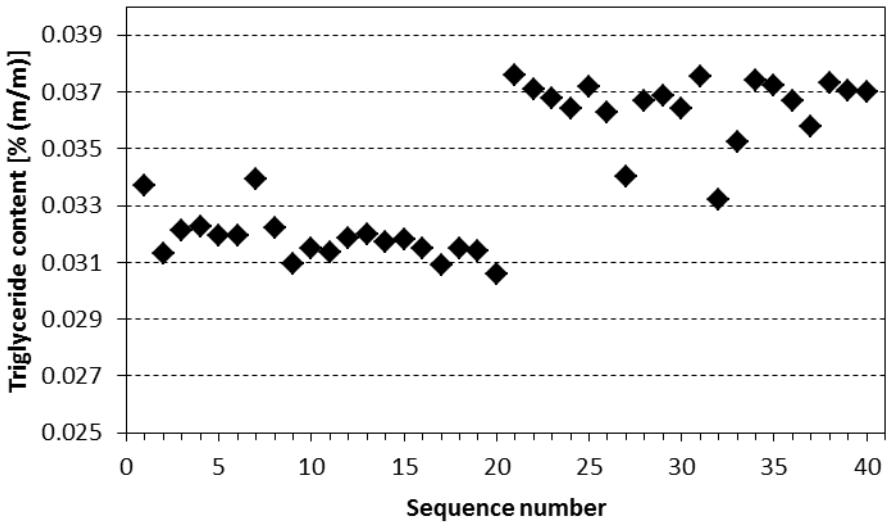

Figure A9: Individual measurement replicates for triglyceride content, against sequence number.

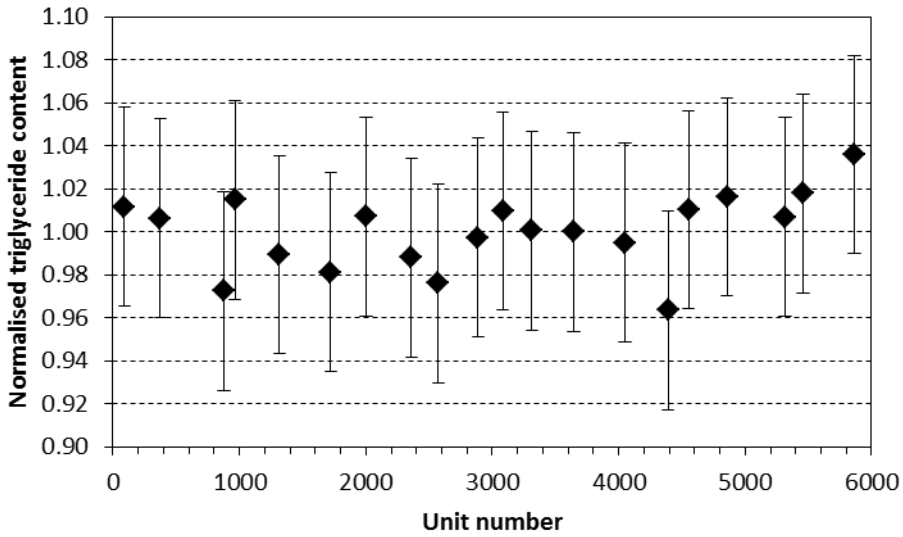

Figure A10: Normalised unit means for triglyceride content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

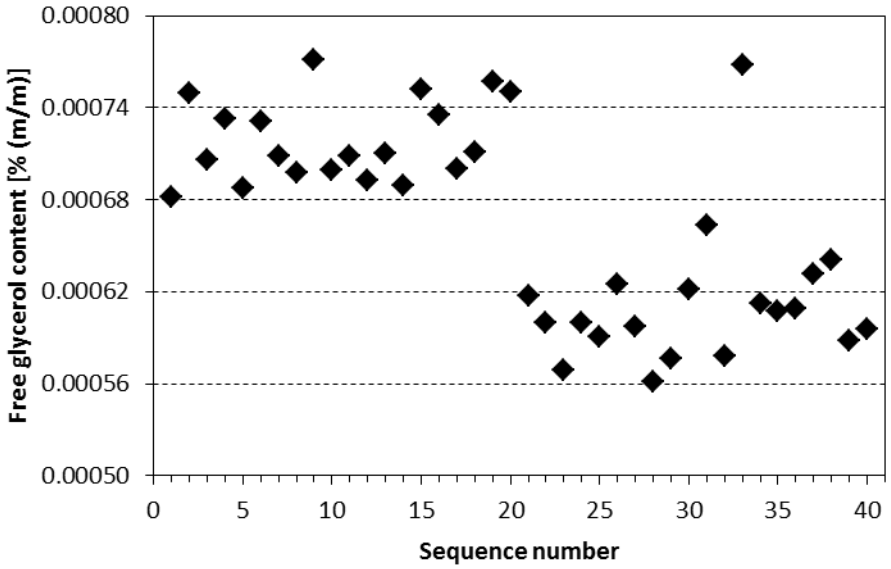

Figure A11: Individual measurement replicates for free glycerol content, against sequence number.

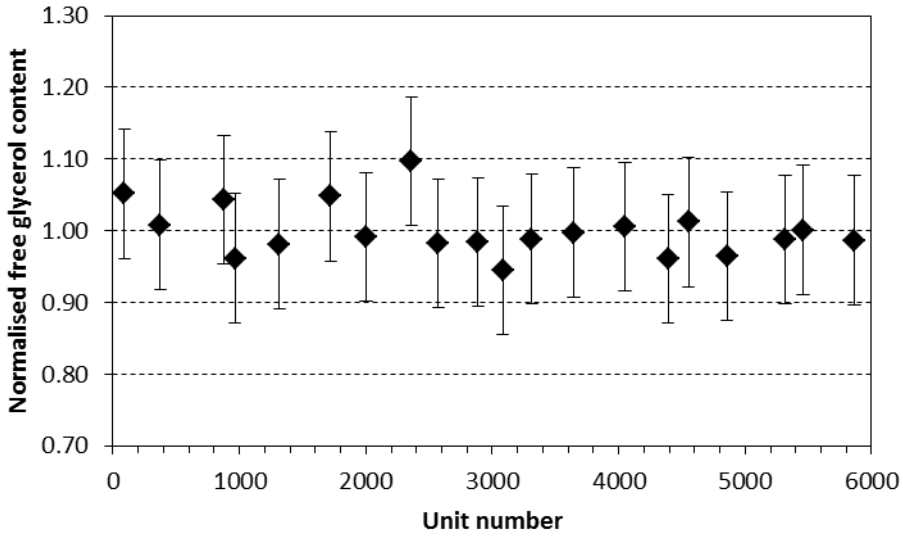

Figure A12: Normalised unit means for free glycerol content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

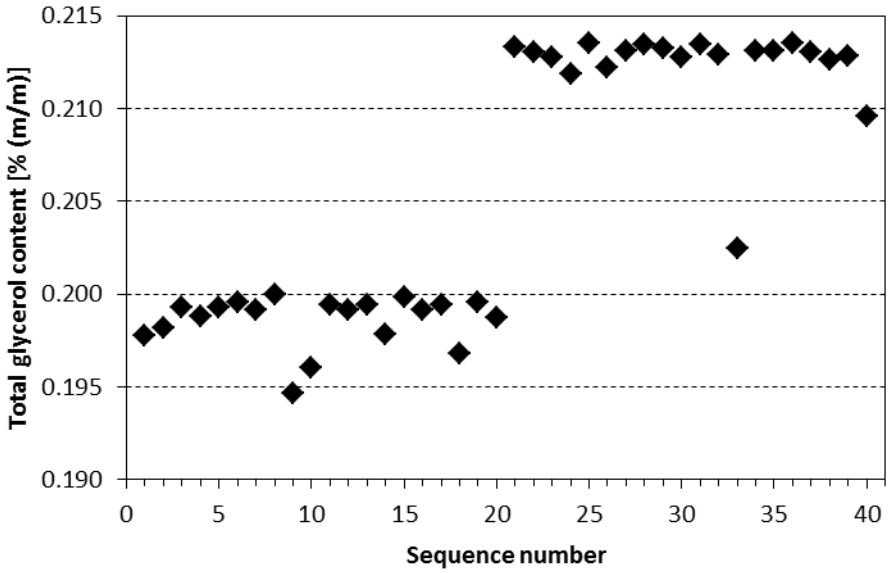

Figure A13: Individual measurement replicates for total glycerol content, against sequence number.

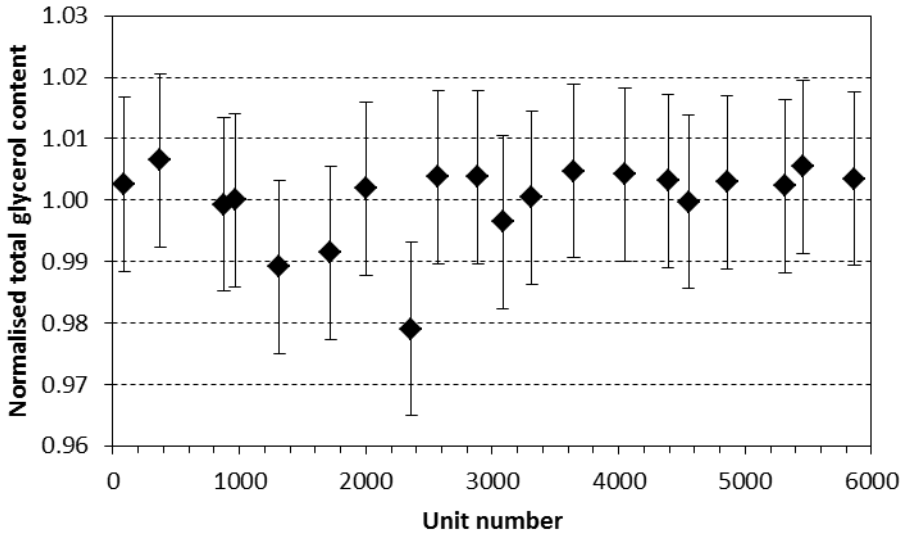

Figure A14: Normalised unit means for total glycerol content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

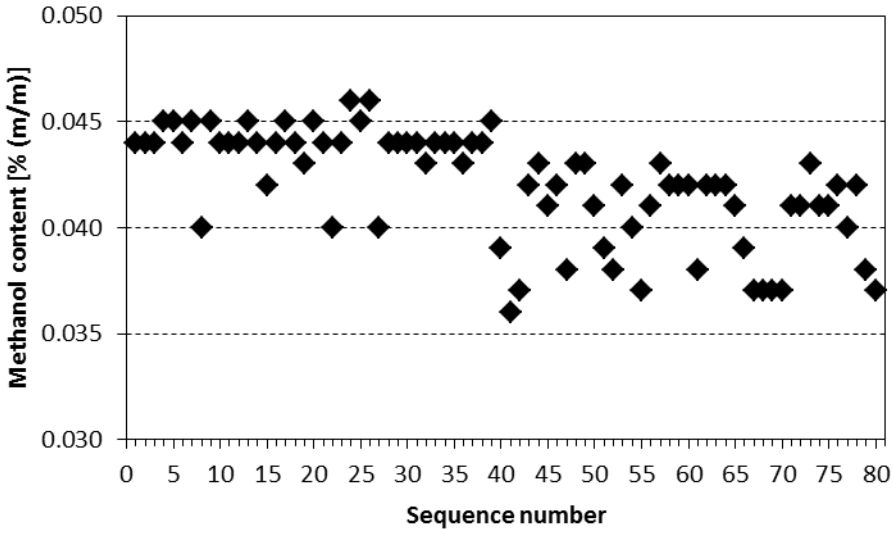

Figure A15: Individual measurement replicates for methanol content, against sequence number.

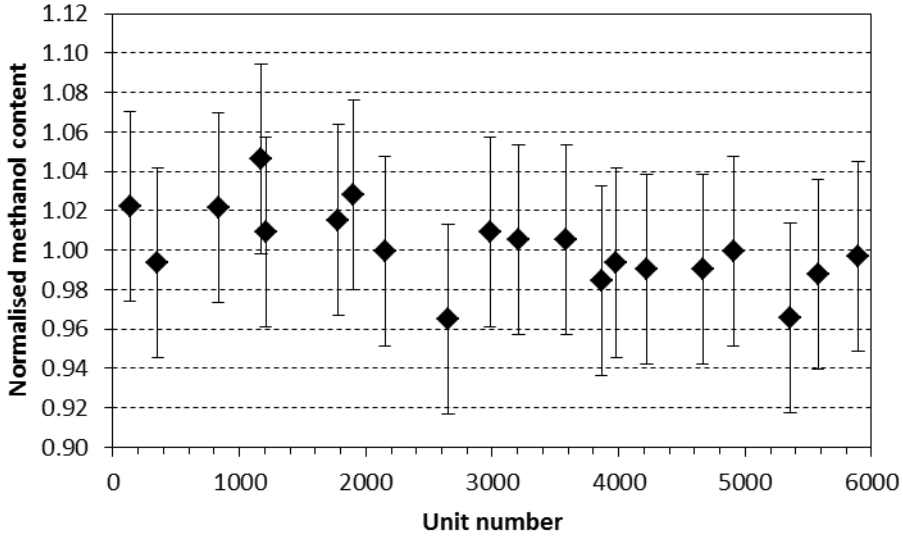

Figure A16: Normalised unit means for methanol content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

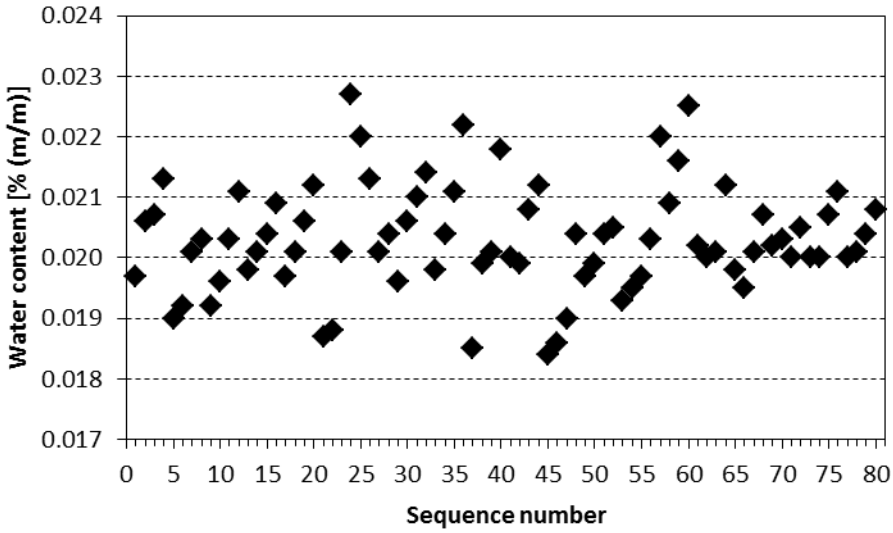

Figure A17: Individual measurement replicates for water content, against sequence number.

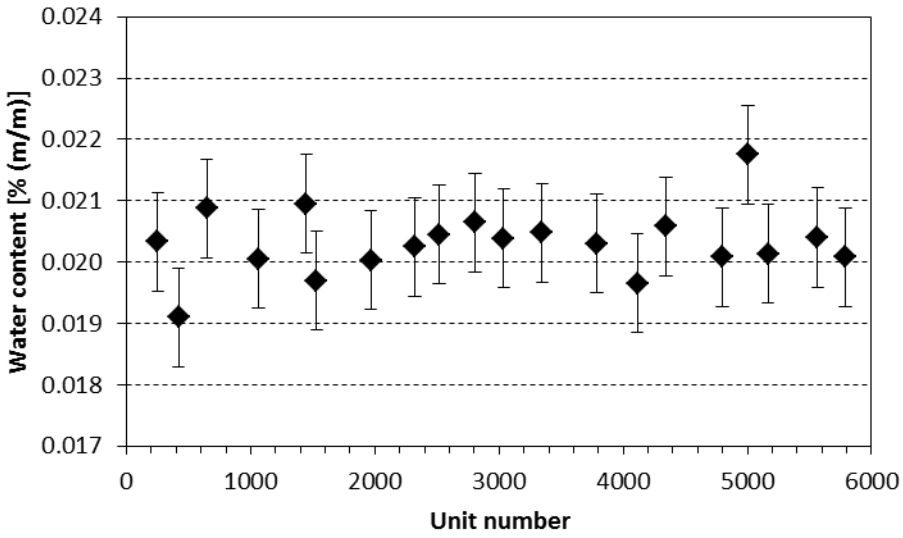

Figure A18: Unit means for water content, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

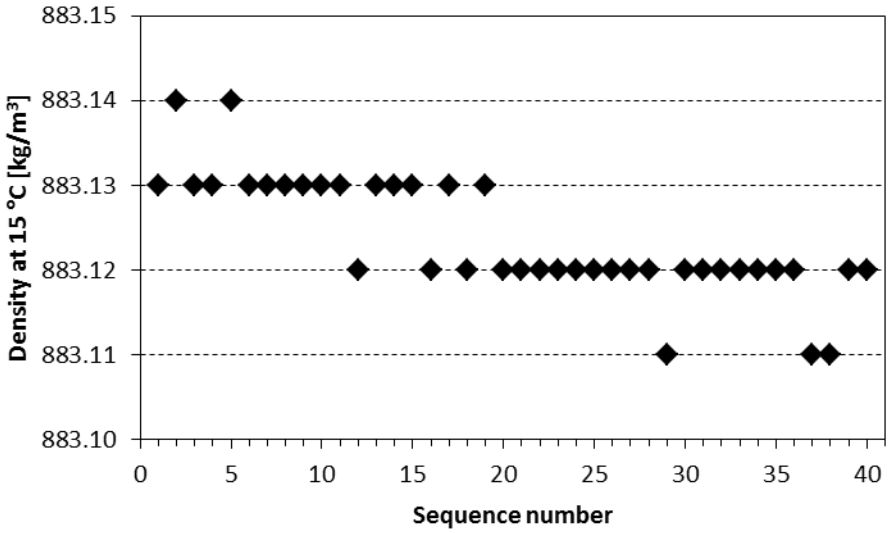

Figure A19: Individual measurement replicates for density at 15 °C, against sequence number.

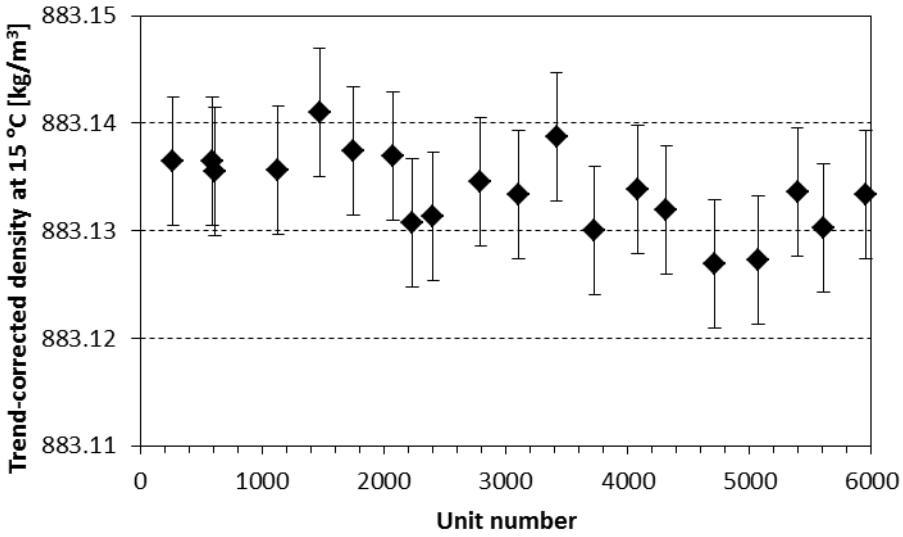

Figure A20: Analytical trend corrected unit means for density at 15 °C, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

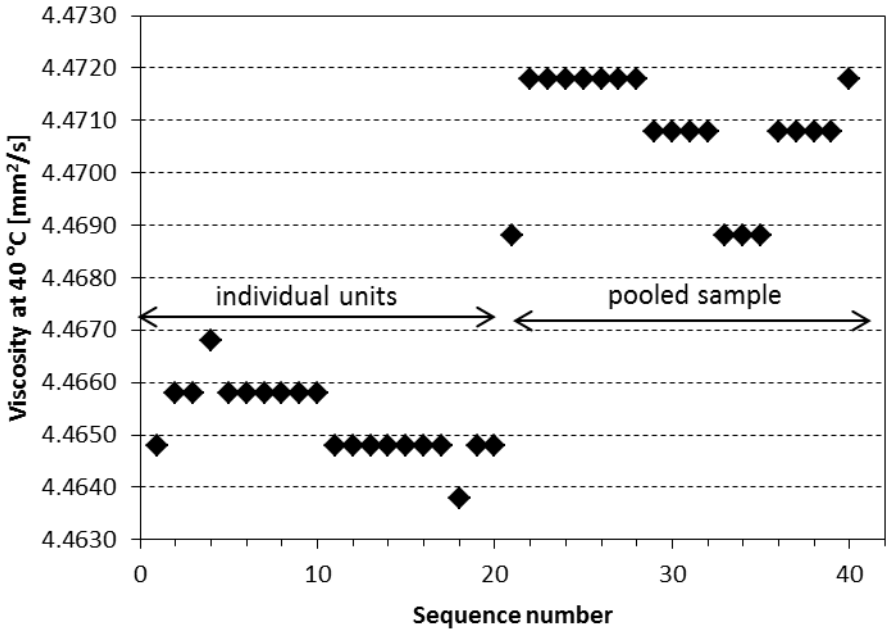

Figure A21: Individual measurement replicates for viscosity at 40 °C, against sequence number. (Sequence number: measurements on 20 individual units and 20 measurements from pooled sample)

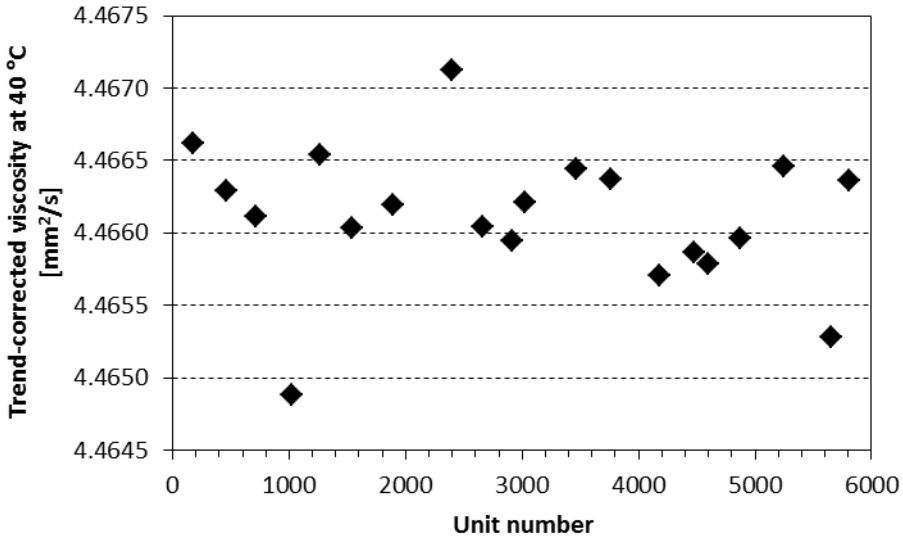

Figure A22: Analytical trend corrected unit means for viscosity at 40 °C, against unit number.

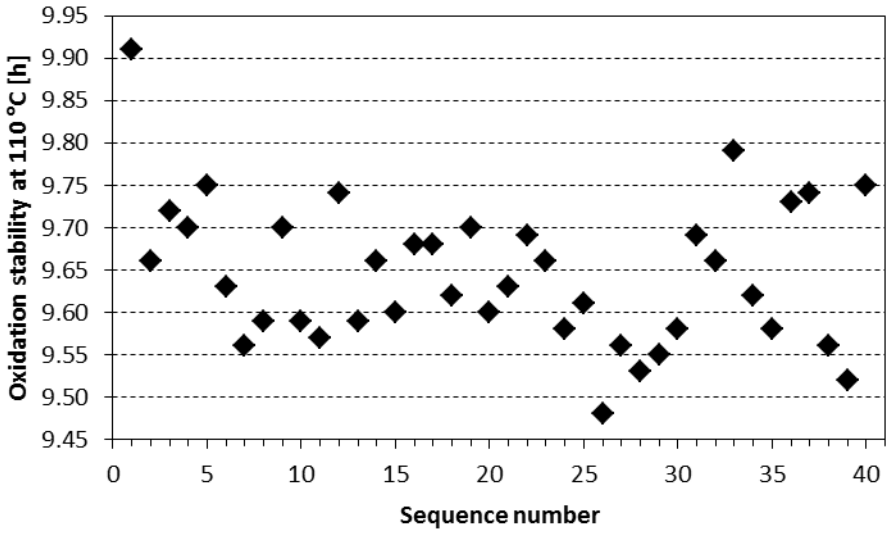

Figure A23: Individual measurement replicates for oxidation stability at 110 °C, against sequence number.

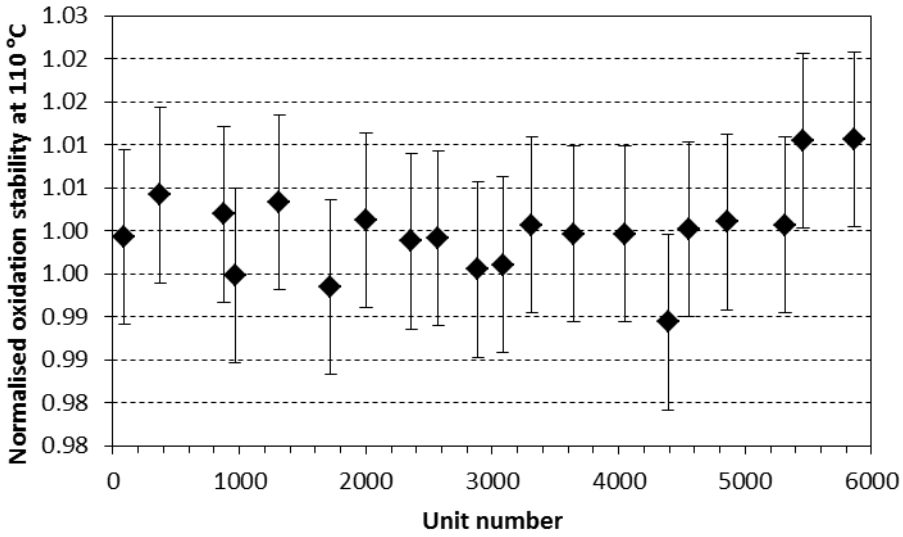

Figure A24: Normalised unit means for oxidation stability at 110 °C, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

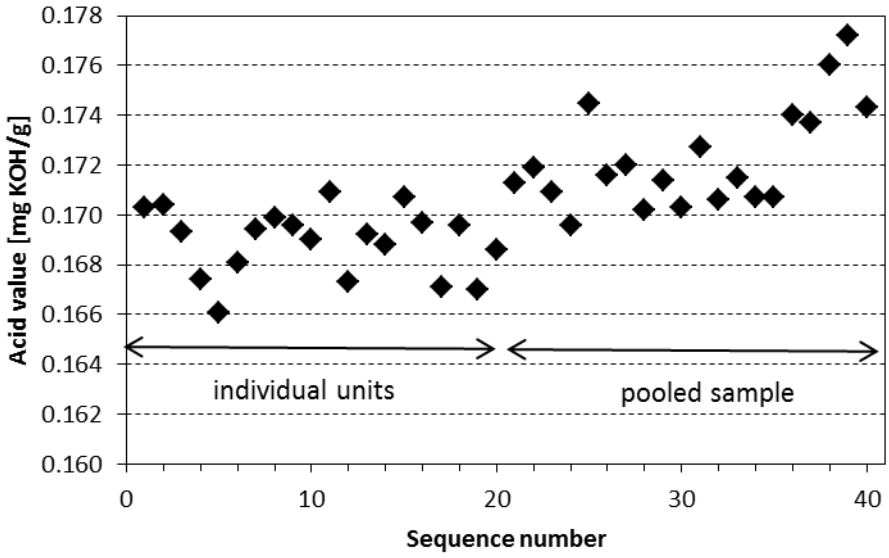

Figure A25: Individual measurement replicates for acid value, against sequence number (Sequence number: measurements on 20 individual units and 20 measurements from pooled sample).

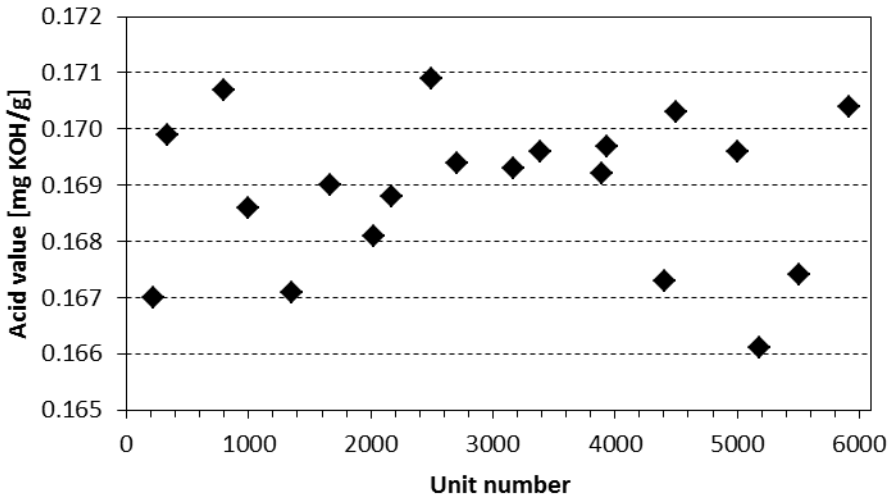

Figure A26: Unit means for acid value, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

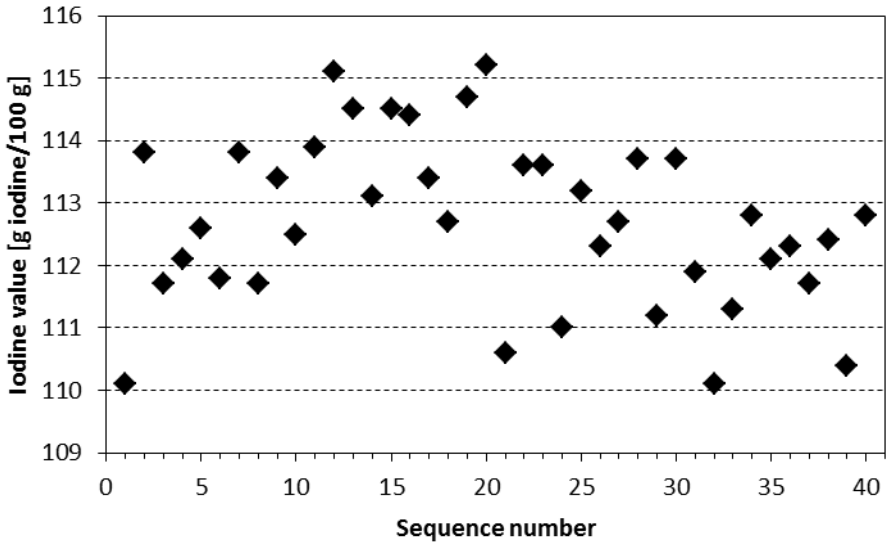

Figure A27: Individual measurement replicates for iodine value, against sequence number.

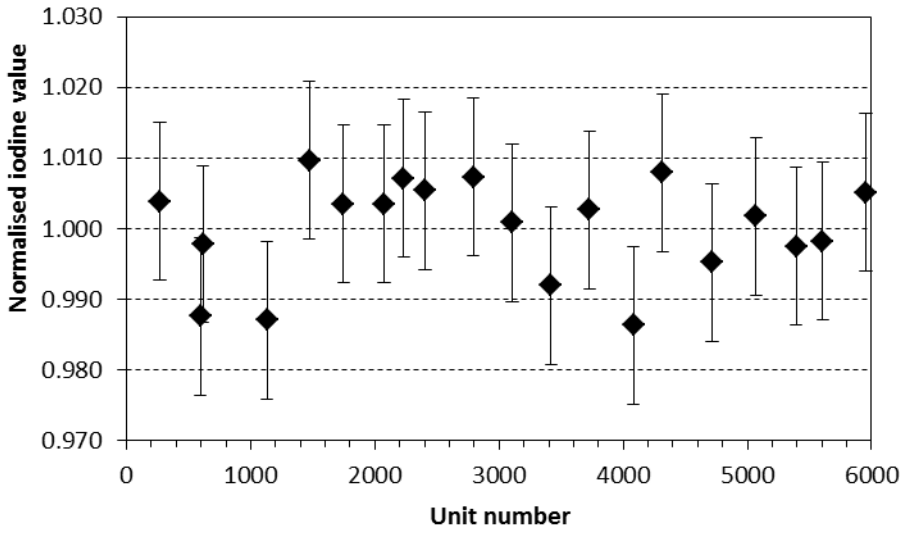

Figure A28: Normalised unit means for iodine value, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

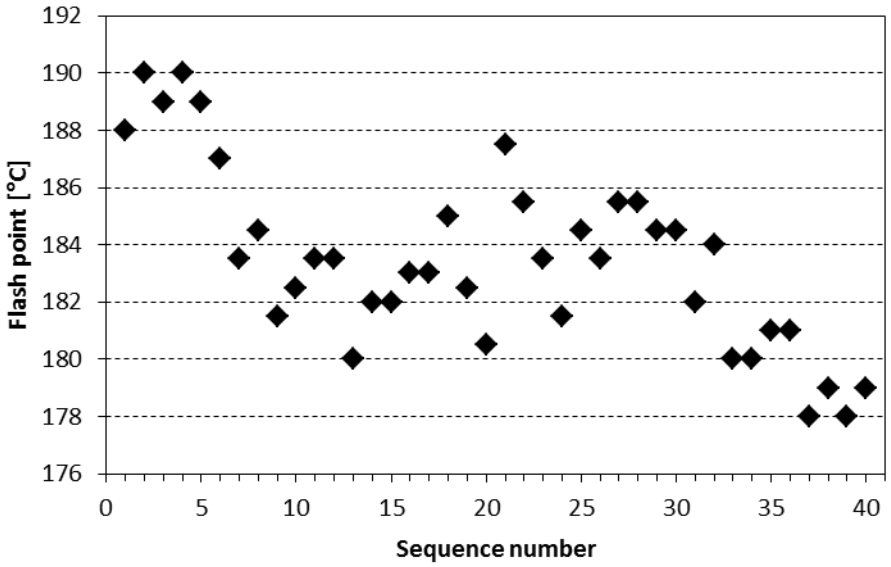

Figure A29: Individual measurement replicates for flash point, against sequence number.

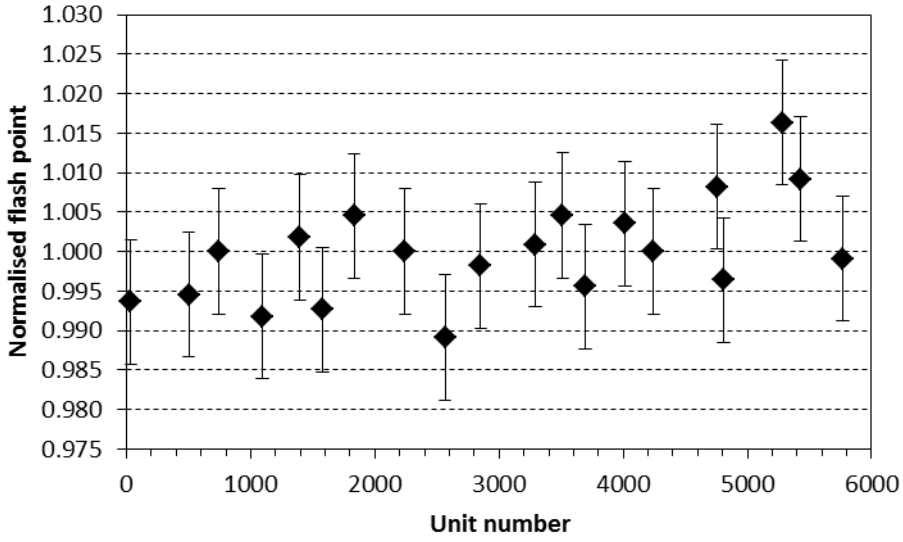

Figure A30: Normalised unit means for flash point, against unit number. Vertical bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

Annex B: Results of the short-term stability measurements

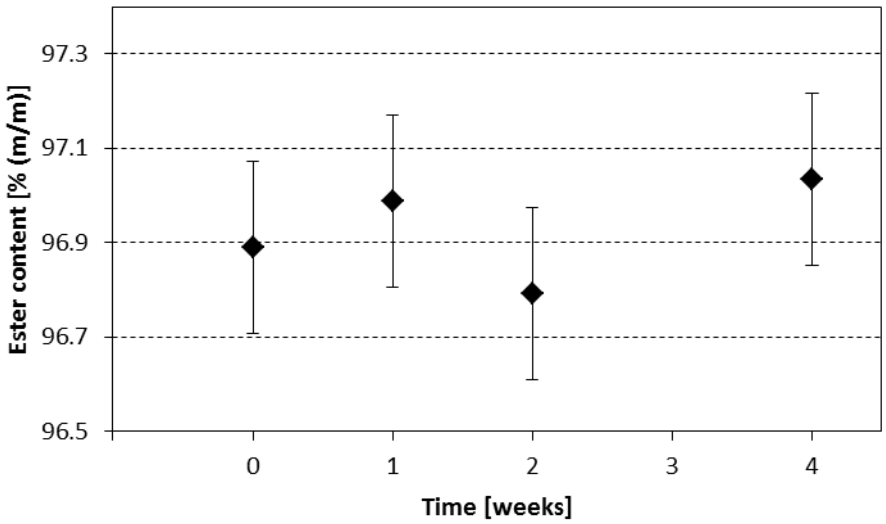

Figure B1: Ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

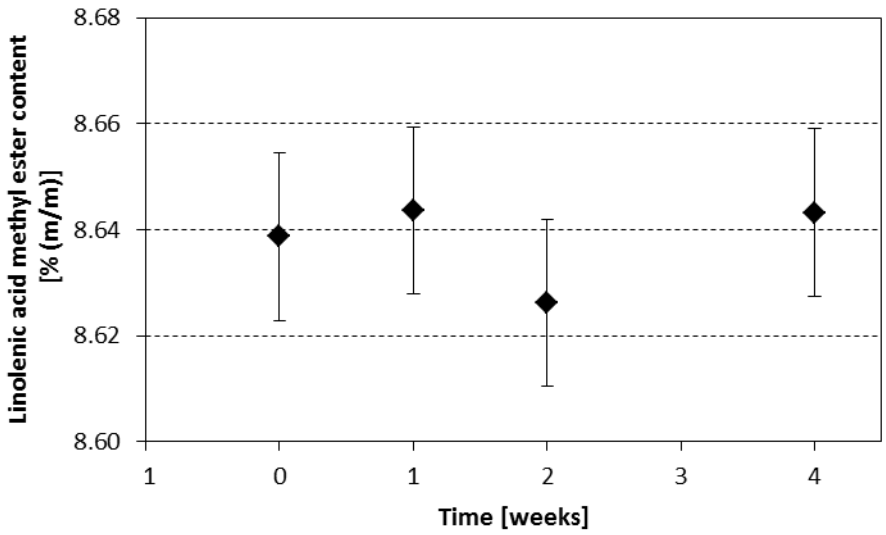

Figure B2: Linolenic acid methyl ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

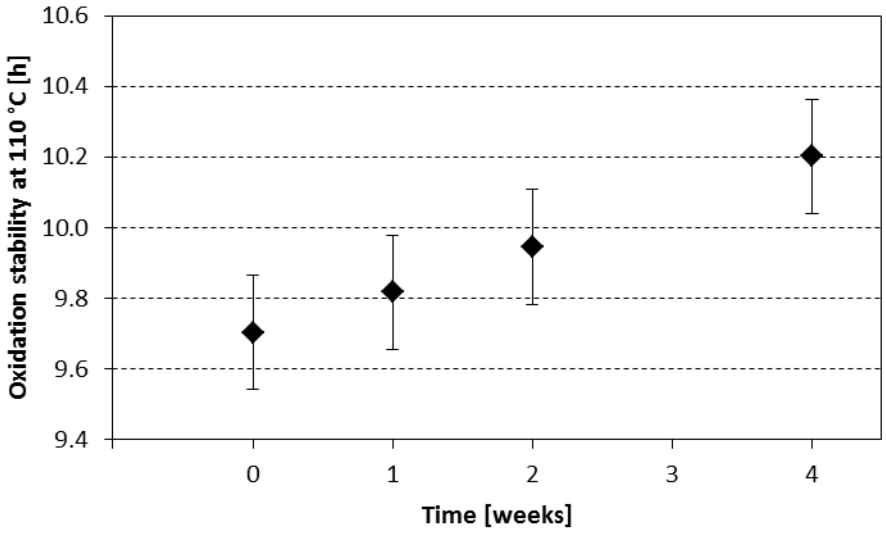

Figure B3: Oxidation stability means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

Annex C: Results of the long-term stability measurements

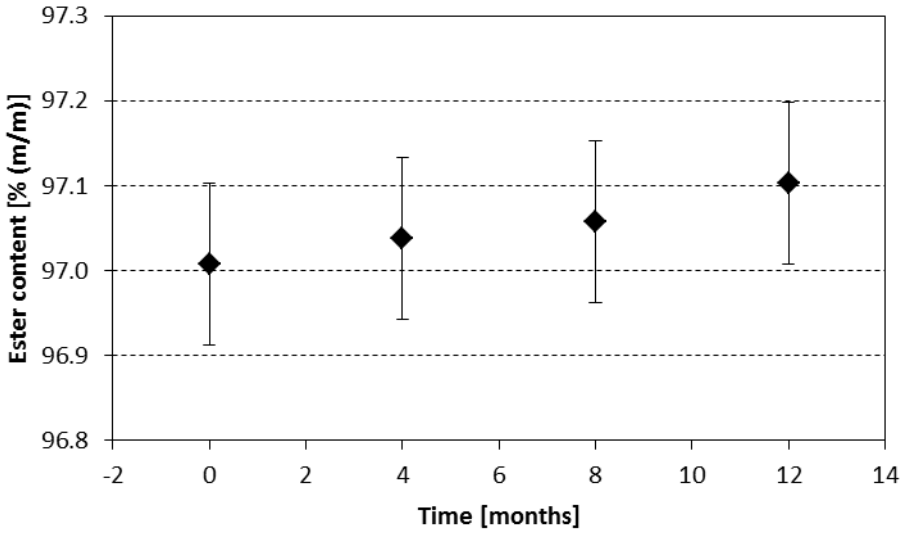

Figure C1: Ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

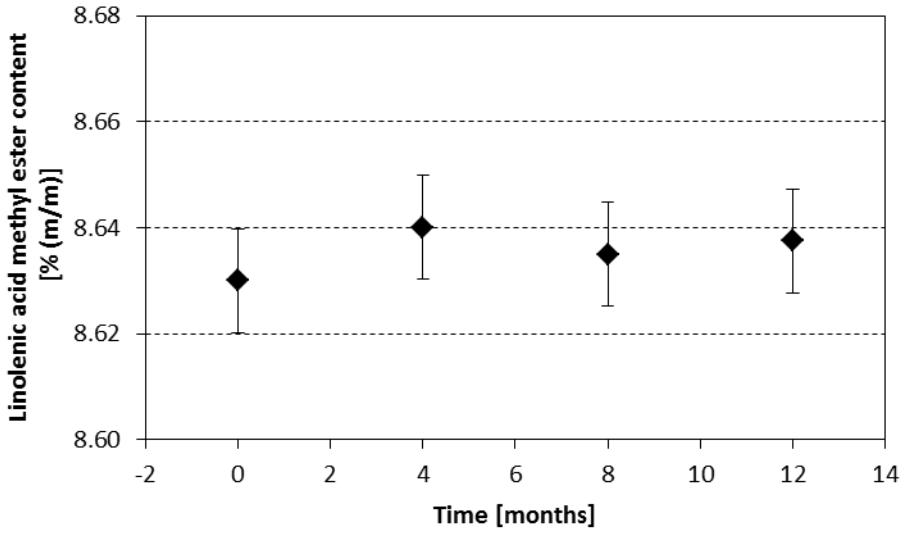

Figure C2: Linolenic acid methyl ester content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

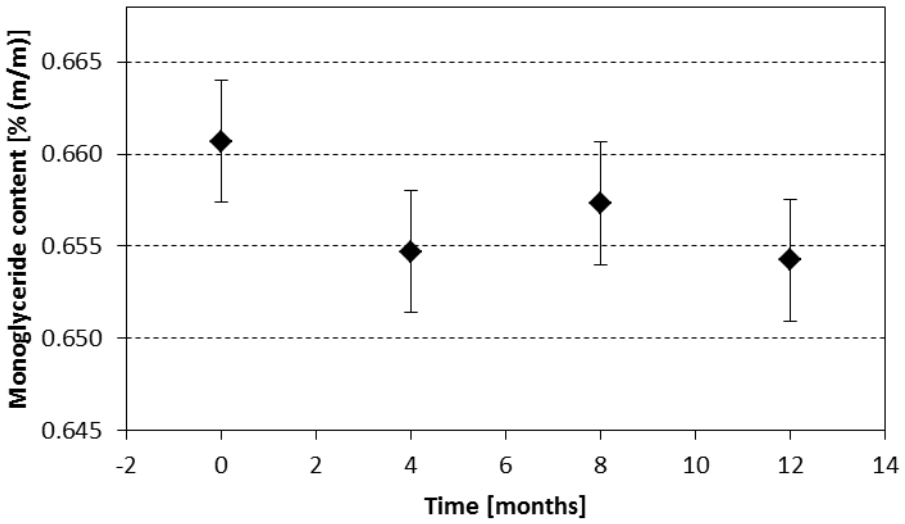

Figure C3: Monoglyceride content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

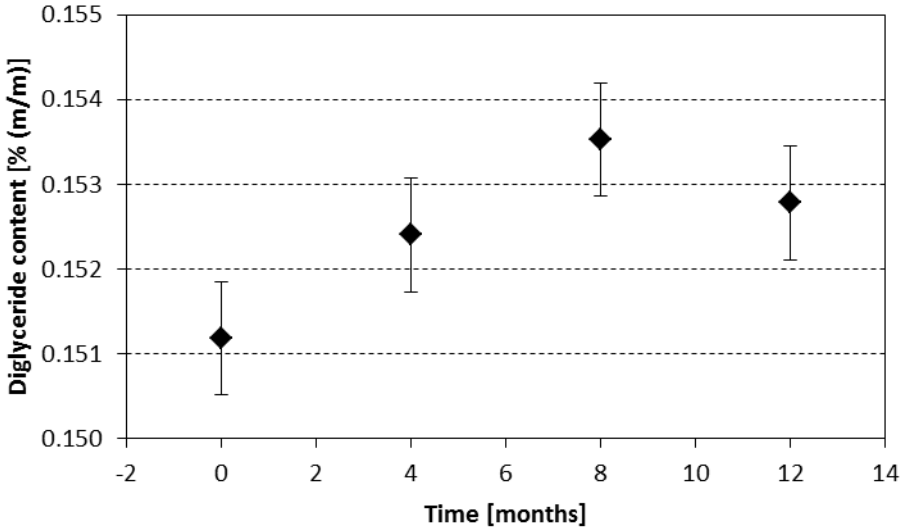

Figure C4: Diglyceride content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

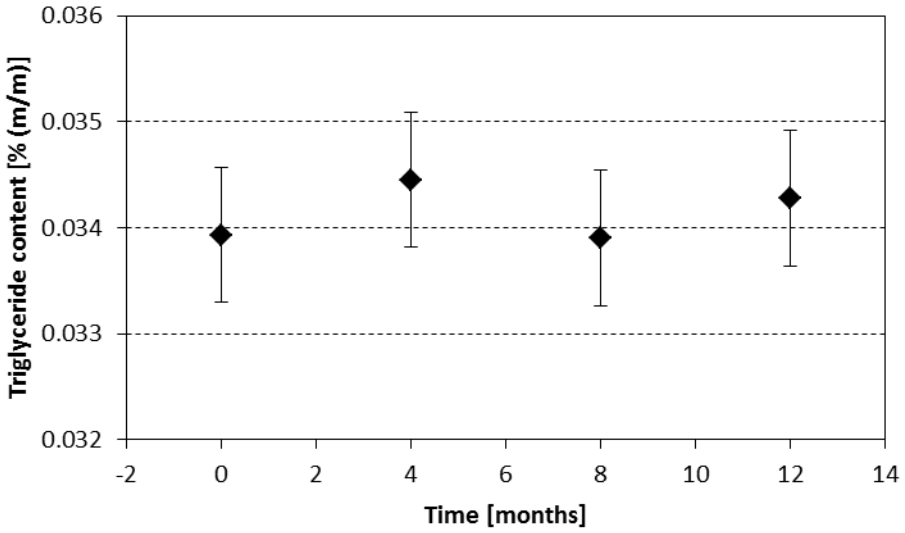

Figure C5: Triglyceride content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

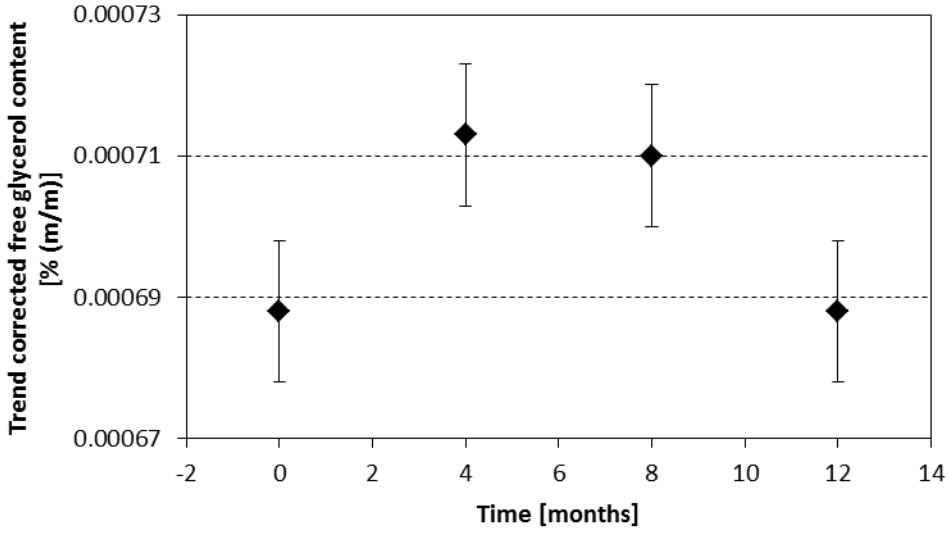

Figure C6: Analytical trend corrected free glycerol content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

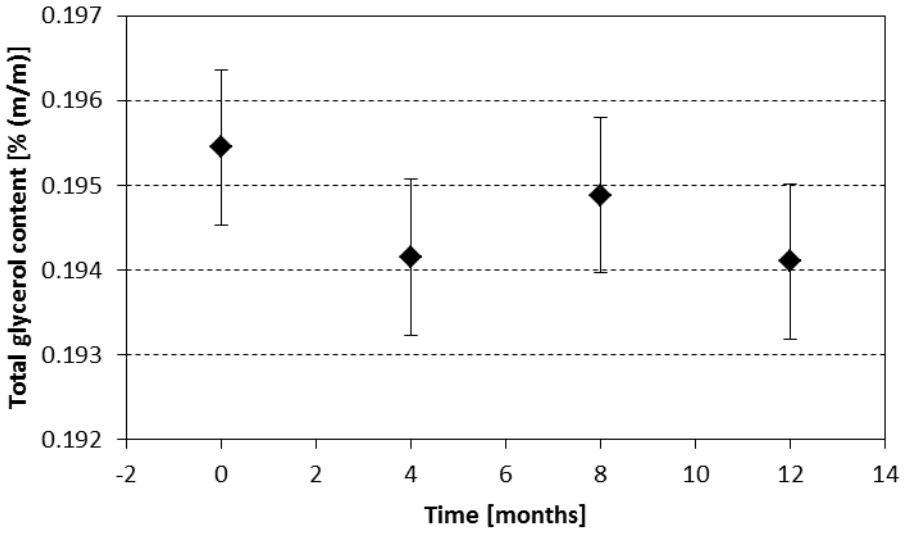

Figure C7: Total glycerol content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

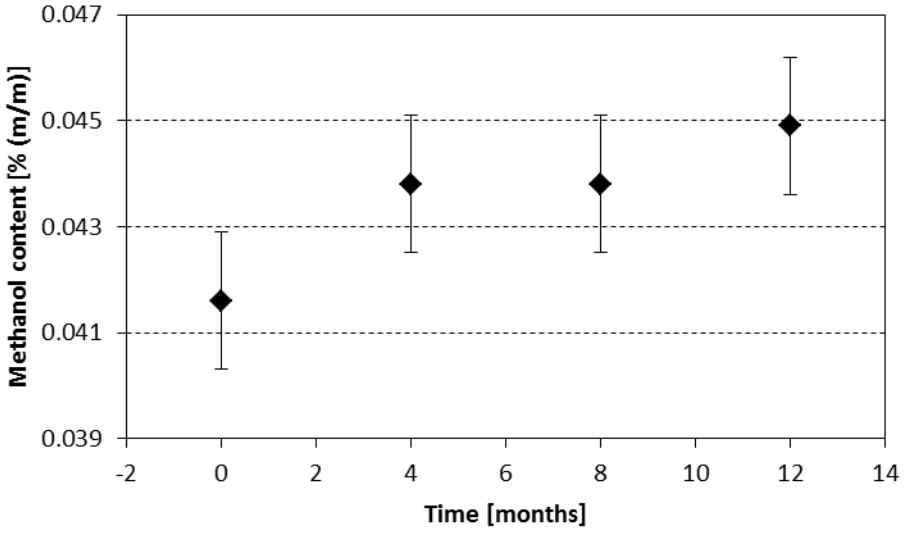

Figure C8: Methanol content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

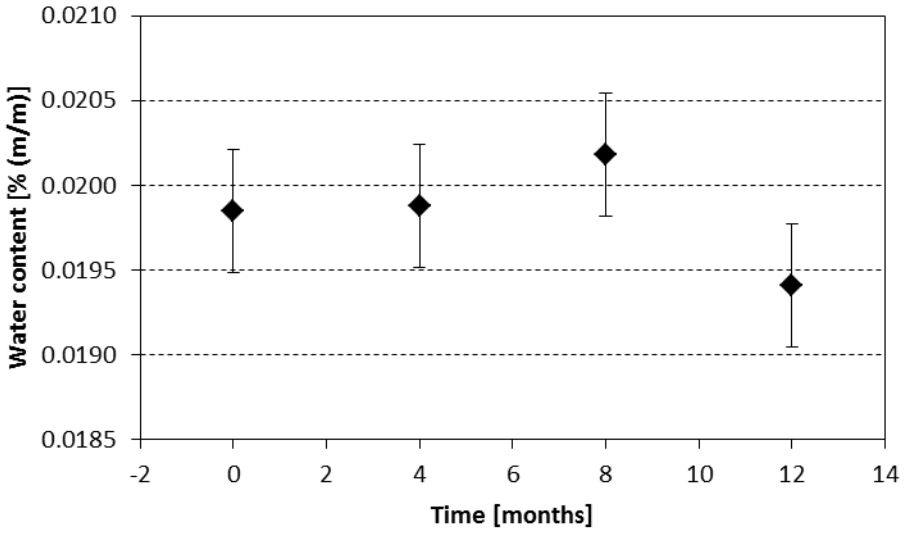

Figure C9: Water content means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

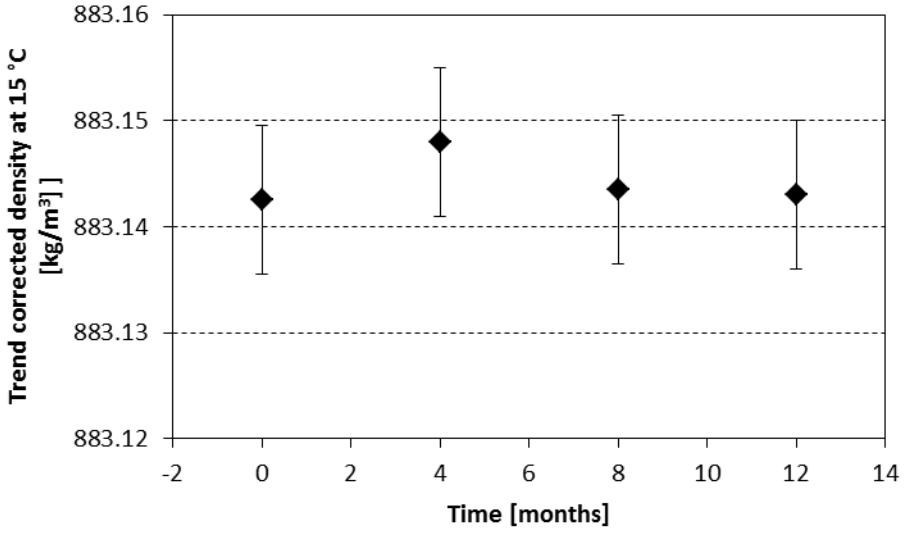

Figure C10: Analytical trend corrected density means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

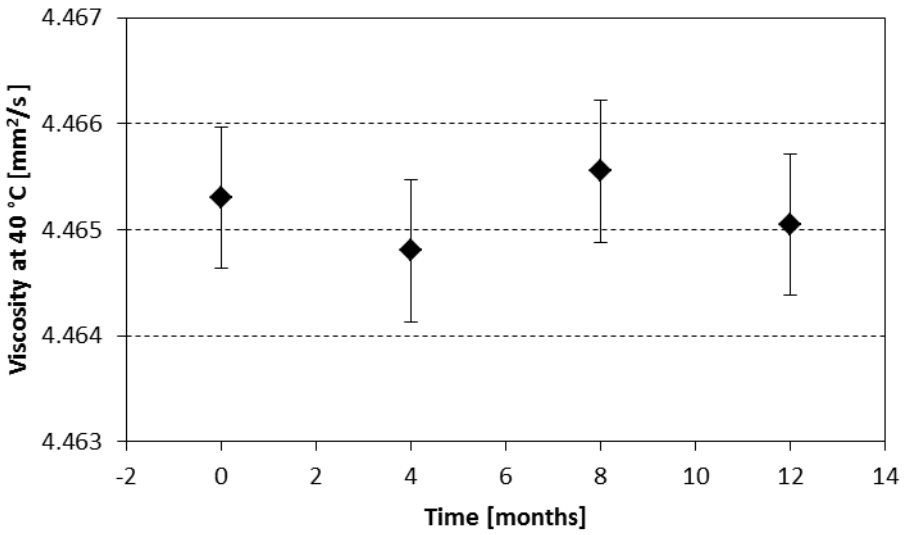

Figure C11: Viscosity means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

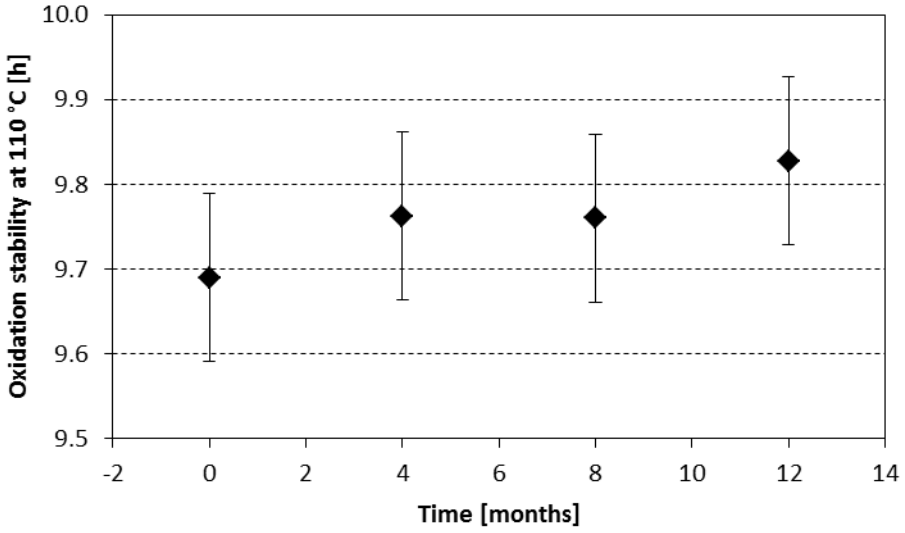

Figure C12: Oxidation stability means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

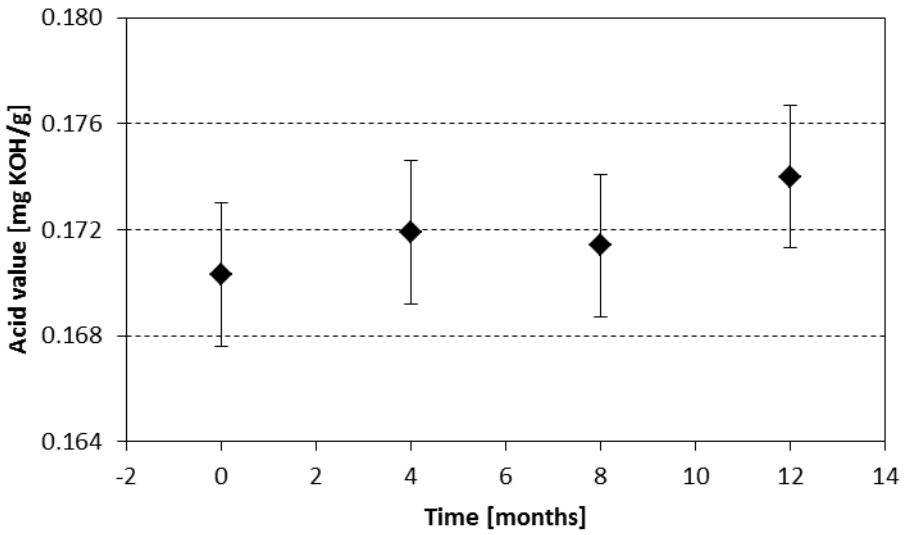

Figure C13: Acid value means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

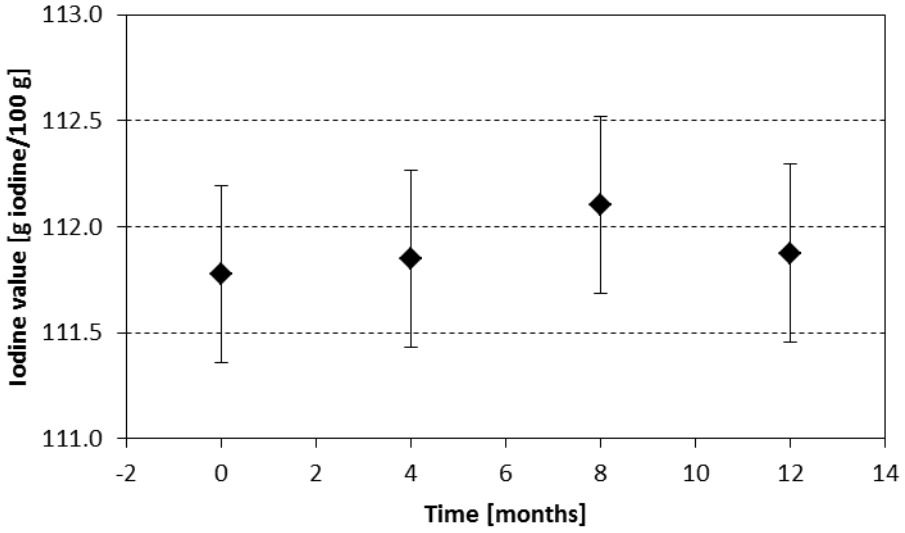

Figure C14: Iodine value means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

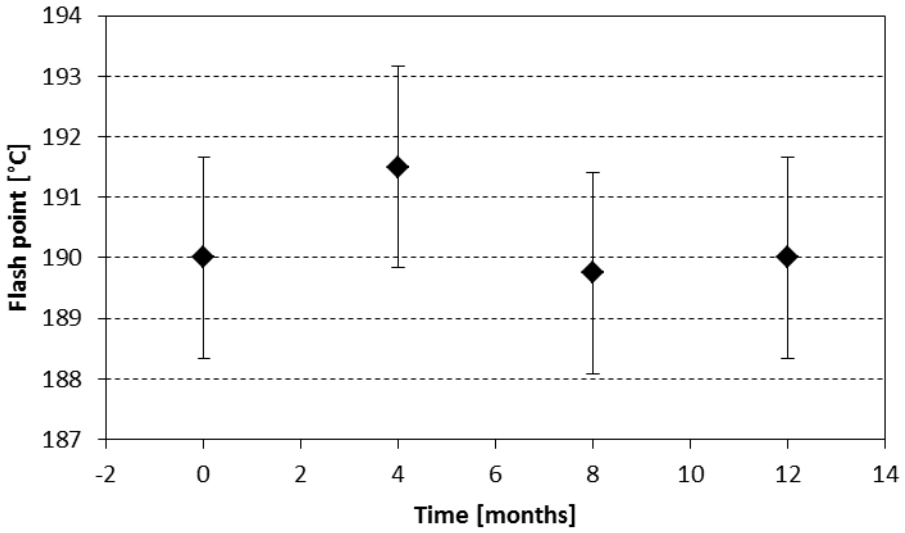

Figure C15: Flash point means measured at each time-point. Vertical bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

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*

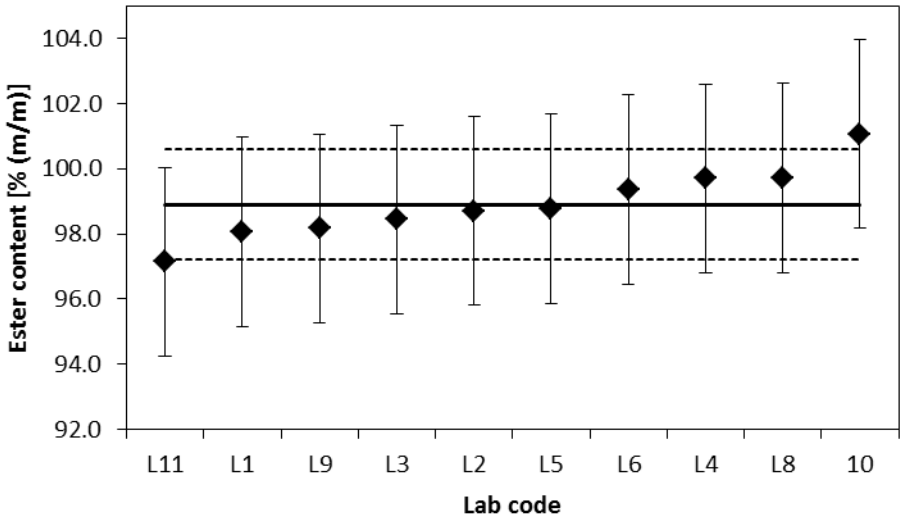

Figure E1: Results of the characterisation study for the mass fraction of ester content in biodiesel measured using EN 14103 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

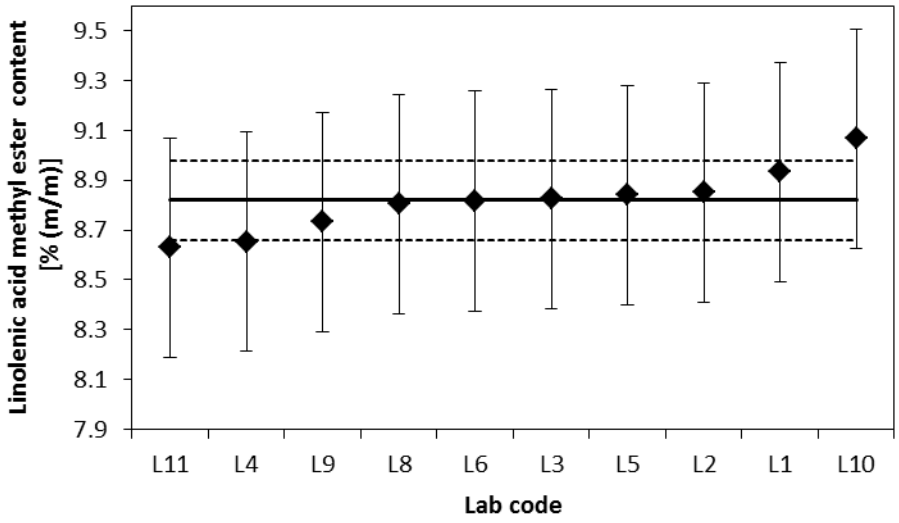

Figure E2: Results of the characterisation study for the mass fraction of linolenic acid methyl ester content in biodiesel measured using EN 14103 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

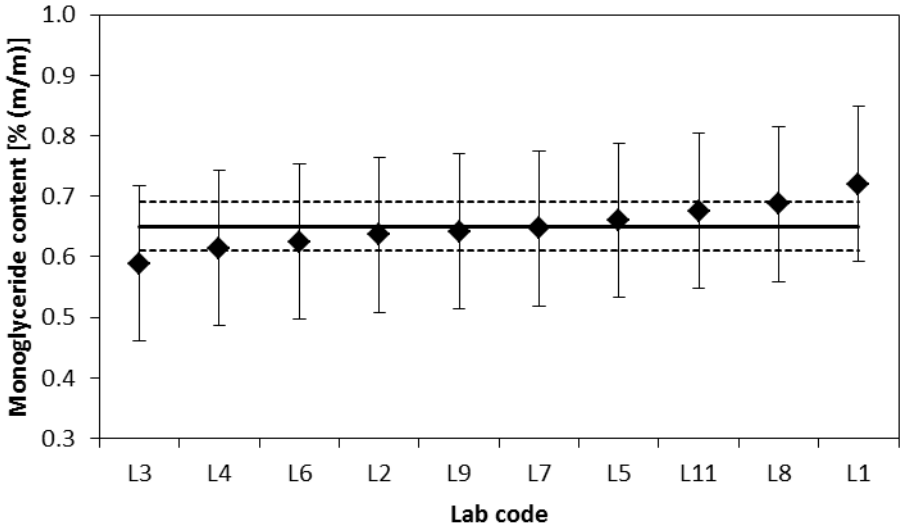

Figure E3: Results of the characterisation study for the mass fraction of monoglyceride content in biodiesel measured using EN 14105 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

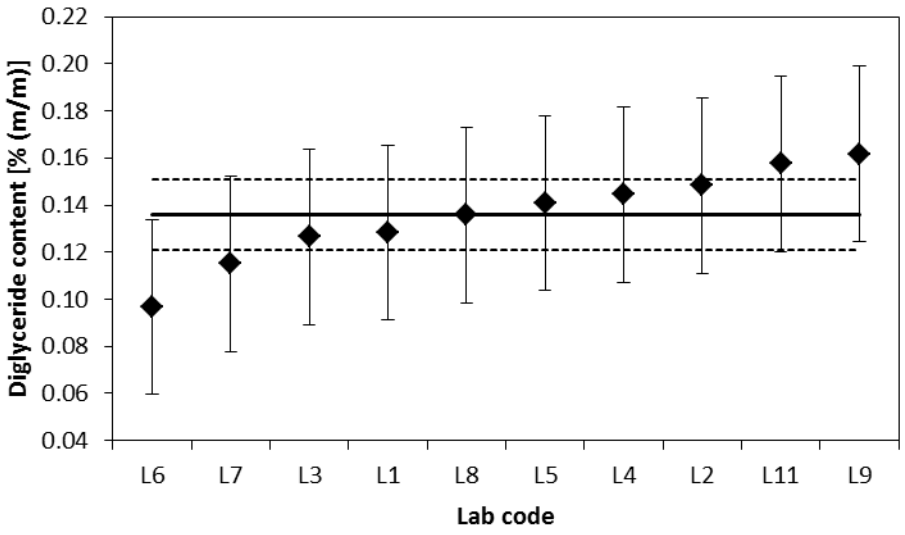

Figure E4: Results of the characterisation study for the mass fraction of diglyceride content in biodiesel measured using EN 14105 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

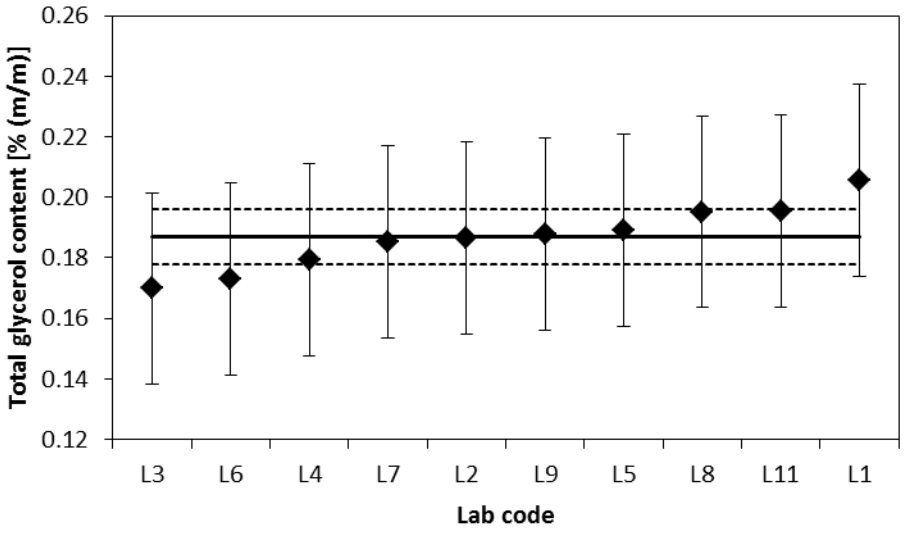

Figure E5: Results of the characterisation study for the mass fraction of total glycerol content in biodiesel measured using EN 14105 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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

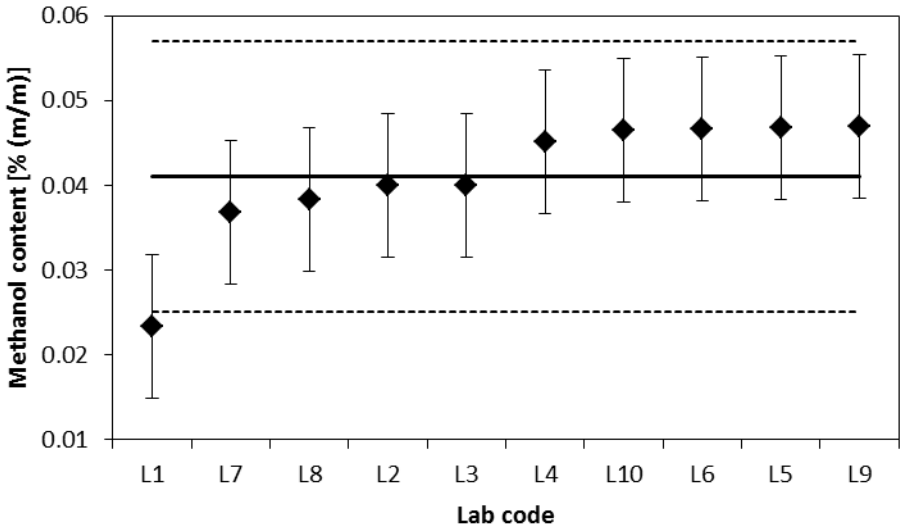

Figure E6: Results of the characterisation study for the mass fraction of methanol content in biodiesel measured using EN 14110 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

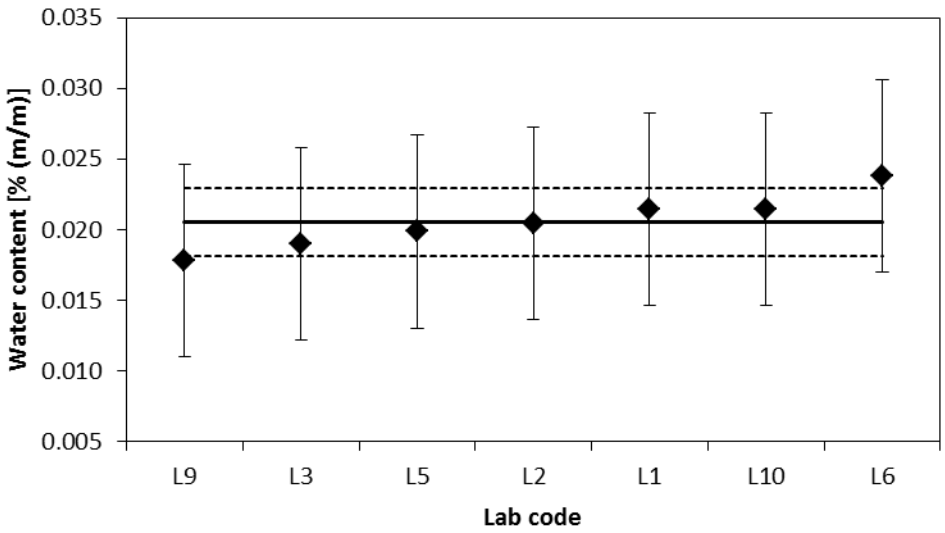

Figure E7: Results of the characterisation study for the mass fraction of water content in biodiesel measured using EN ISO 12937 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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

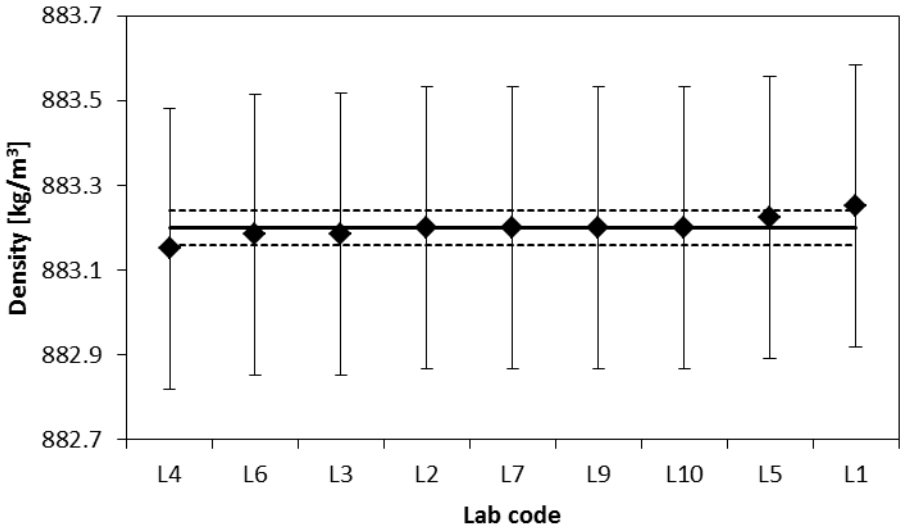

Figure E8: Results of the characterisation study for density in biodiesel measured using EN ISO 12185 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

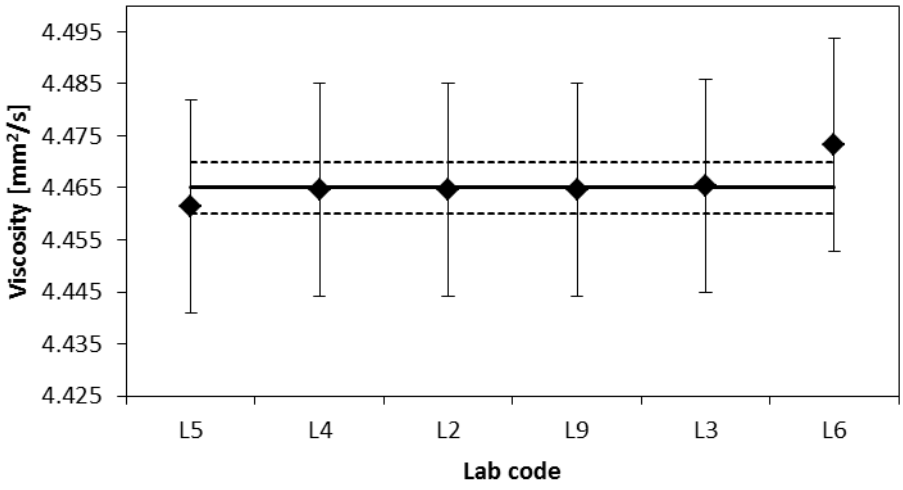

Figure E9: Results of the characterisation study for viscosity in biodiesel measured using EN ISO 3104 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

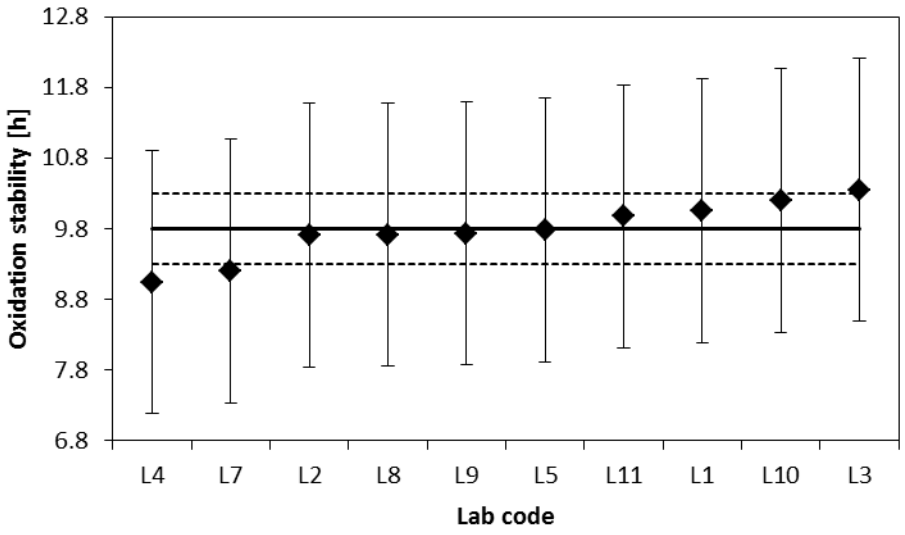

Figure E10: Results of the characterisation study for the oxidation stability of biodiesel measured using EN 14112 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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

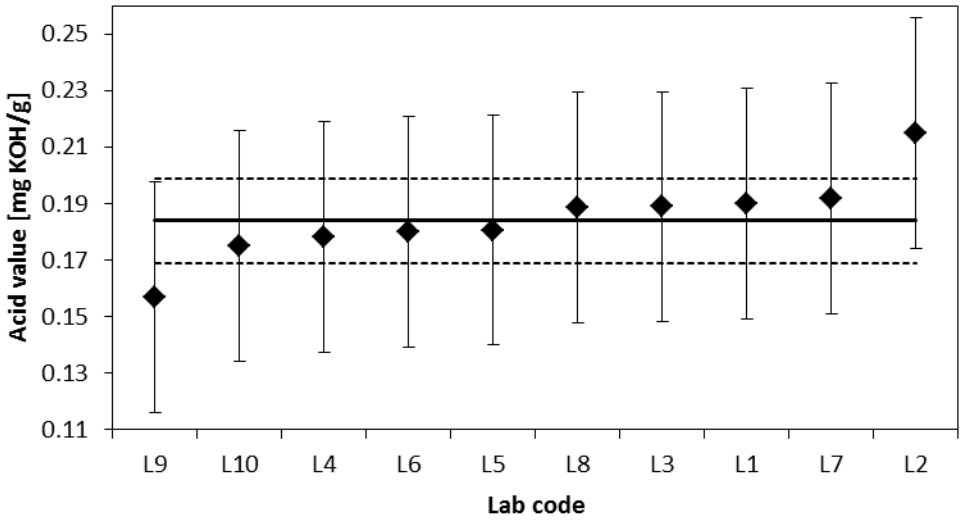

Figure E11: Results of the characterisation study for the acid value of biodiesel measured using EN 14104 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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

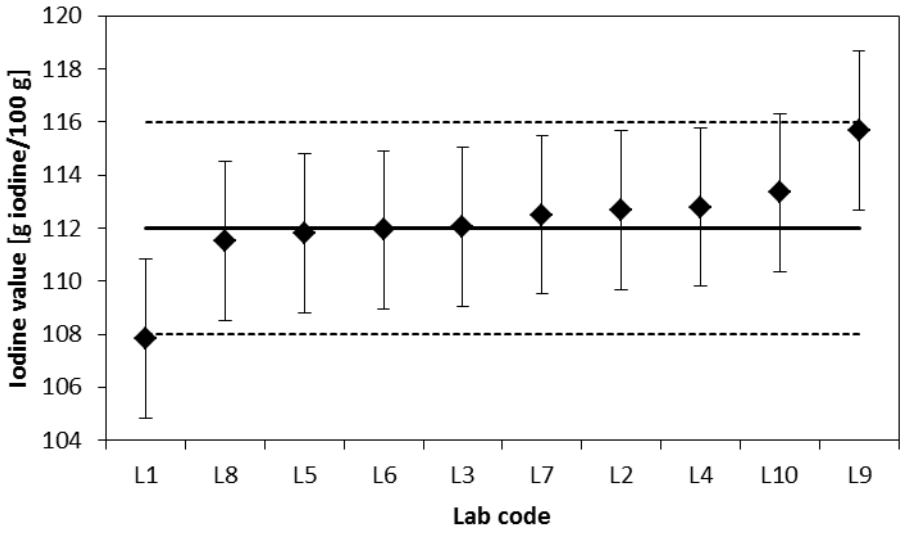

Figure E12: Results of the characterisation study for the iodine value of biodiesel measured using EN 14111 (continuous line: certified value; dashed line: expanded uncertainty with k=2; error bars: expanded measurement uncertainty as given in Table D2)

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*

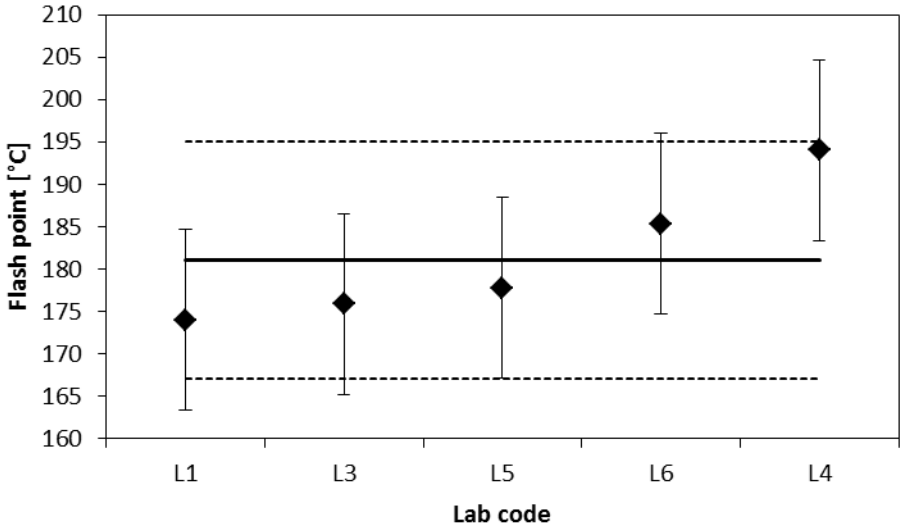

Figure E13: Results of the characterisation study for the flash point of biodiesel measured using EN ISO 3679 (continuous line: certified value; dashed line: expanded uncertainty with k=2.8; error bars: expanded measurement uncertainty as given in Table D2)

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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