BIODIESEL
UME BIOFMET CRM 01

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ABBREVIATIONS

ANOVA	analysis of variance
α	significance level
BAM	Bundesanstalt für Materialforschung und -prüfung, Germany
BRML-INM	National Metrology Institute, Romania
CRM	certified reference material
DTI	Danish Technological Institute, Denmark
EMPIR	European Metrology Programme for Innovation and Research
FAME	Fatty Acid Methyl Ester
GUM	Central Office of Measures, Poland
HR ICP-MS	High resolution ICP-MS
ICP-MS	Inductively coupled plasma mass spectrometry
ID MS	Isotope Dilution Mass Spectrometry
ICP-OES	Inductively coupled plasma optical emission spectroscopy
IMBIH	Institute of Metrology, Bosnia and Herzegovina
IS	internal standard
ISO	International Organization for Standardization
LGC	LGC Paragon Scientific Ltd. Prenton, United Kingdom
$MS_{between}$	mean square between-bottle from ANOVA
MS_{within}	mean square within-bottle from ANOVA
MSTFA	N-Methyl-N-trimethylsilyl-trifluoroacetamide
n	number of replicates per unit
QC	quality control
PTB	Physikalisch Technische Bundesanstalt, Germany
RME	rapeseed methyl ester
RSD	relative standard deviation
s	standard deviation
s_{bb}	between-bottle standard deviation
SGT	single Grubbs' test
SI	International System of Units
SME	soy methyl ester
s_{wb}	within-bottle standard deviation
U_{AV}	expanded uncertainty of assigned value
u_{bb}	standard uncertainty related to possible between-bottle heterogeneity
u^*_{bb}	standard uncertainty of heterogeneity that can be hidden by method repeatability
U_{CRM}	expanded uncertainty of certified value
UME	TÜBİTAK National Metrology Institute, Türkiye
u_{char}	standard uncertainty related to characterization
u_{lts}	standard uncertainty related to long term stability

The subscript "rel" is added when a variable is expressed in relative terms (e.g. as percent)

ABSTRACT

Biomass is a key element in biofuels. It can be defined as a fuel produced through contemporary biological processes, and its increased use can support the EU's aims of reducing greenhouse gas emissions. Information on the nature and the quality of the biomass or biofuel is important in order to support the optimization of their combustion with respect to realizing higher efficiencies and lower emissions during energy production.

BIOFMET project aims to establish advanced traceable measurement standards for the determination of the calorific value and mass fractions of impurities.

This report describes the production of a biodiesel reference material: UME BIOFMET CRM 01, certified for calorific value, density, viscosity and mass fractions of Ca, K, Mg, Na, P and S elements. The material was produced in accordance with requirements of ISO 17034 standard.

The raw material for the CRM is Biodiesel (B100 composed of 80 % RME [rapeseed methyl ester] and 20 % SME [soy methyl ester]) which was produced in Romania. The material was spiked with Ca, K, Mg, Na and P standards in mineral oil.

Homogeneity and stability of the material were assessed in accordance with ISO 33405 standard. The material was characterized by an interlaboratory comparison among competent laboratories.

Uncertainties of the certified values were calculated in accordance with GUM "Guide to the Expression of Uncertainty in Measurement" and includes characterization, homogeneity, stability components.

The material is intended for method development and validation in determination of calorific value, density, viscosity and mass fractions of Ca, K, Mg, Na, P and S elements and for quality control purposes. The CRM is available in glass bottles containing approximately 500 mL of material.

INTRODUCTION

Energy has a crucial role in life which is needed for heating, lighting, cooking in households and for every transport activity. Fossil fuels (coal, gas, and oil) currently account for about 79 % of world energy consumption, nuclear energy for 7 %, and renewable energy sources for 14 % [1]. One of the renewable energy source is biomass and a definition adopted by EU legislation for biomass is "the biodegradable fraction of products, waste and residues from agriculture (including vegetal and animal substances), forestry and related industries". When biomass is burned or digested, the organic carbon is recycled in a global process known as the carbon cycle. In this process, the CO_2 that was absorbed as the plants grew is simply returned to the atmosphere when the biomass is burned. Therefore, if the growth and harvest cycle is maintained, there will be no net release of CO_2, therefore biomass is regarded as a carbon neutral energy source that does not emit CO_2 into the atmosphere when burned. Biomass can be used as feedstock for energy production either by direct combustion or through conversion to biofuels such as biodiesel, ethanol or biogas.

Biodiesel is produced by a chemical process called the transesterification of fatty acids produced from vegetable oils. Biodiesel is generated via a chemical process known as transesterification, which converts fatty acids derived from vegetable oils, animal fats, or recycled greases. In the EU, rapeseed oil (canola) is the predominant feedstock for biodiesel production, while soybean oil is commonly used in the USA; other feedstocks include sunflower oil and used cooking oils. All diesel engines can run on biodiesel or blends of biodiesel with normal diesel. Emissions of carbon dioxide are less for biodiesel

than they are for fossil fuel diesel. Emissions of hydrocarbons and soot are also lower for biodiesel than for fossil-fuel-derived diesel. In addition, biodiesel releases fewer solid particles and, because it contains less sulfur, does not create too much SO_2, which contributes to acid rain.

FAME (Fatty Acid Methyl Ester) is the generic chemical term for biodiesel derived from renewable sources. Relevant characteristics, requirements and test methods for FAME to define the product to be used as automotive diesel fuel and in heating applications are given in EN 14214:2012+A2:2019 standard [2].

Laboratories performing sampling and tests in this field need matrix CRMs enabling appropriate quality control. National metrology institutes and designated institutes with proven metrological capabilities for the production and certification of such materials are necessary for the provision of quality data. The EMPIR joint research project BIOFMET [3] developed capacity to produce CRMs for biofuel analysis by transferring the theoretical and practical know-how between the partners and combining their skills to focus on biofuel CRM production according to ISO 17034:2016 [4] and ISO 33405:2024 [5].

UME BIOFMET CRM 01, the production of which was carried out by a project consortium described in this report, is intended to be used as a quality assurance and quality control tool especially by the laboratories involved in the quality control of the biodiesel used for automotive diesel fuel and in heating applications.

The parameters aimed to be certified in UME BIOFMET CRM01 are the following: calorific value, density, viscosity and mass fractions of the elements Ca, K, Mg, Na, P and S. The target concentration levels for elements were decided to meet laboratories' needs. Findings of these studies were partly published in a peer reviewed journal [6].

PARTICIPANTS

Laboratory/organizations involved in the production and their contributions are presented in Table 1.

Table 1. Laboratory/organisations involved and their contributions

Activity	Laboratory / Organization
Project management and data evaluation	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, Türkiye
Preliminary measurements	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, Türkiye
Processing	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, Türkiye
Homogeneity and Stability studies	PTB, Physikalisch Technische Bundesanstalt, Braunschweig, Germany BRML-INM, National Metrology Institute, Bucharest, Romania IMBIH, Institute of Metrology of Bosnia and Herzegovina, Sarajevo, B&H TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, Türkiye
Characterization Study (in alphabetical order)	BAM, Bundesanstalt für Materialforschung und prüfung, Berlin, Germany BRML-INM, National Metrology Institute, Bucharest, Romania DTI, Danish Technological Institute, Aarhus, Denmark GUM, Central Office of Measures, Warszawa, Poland IMBIH, Institute of Metrology of Bosnia and Herzegovina, Sarajevo, B&H LGC, Paragon Scientific Ltd. Prenton, Wirral, United Kingdom PTB, Physikalisch Technische Bundesanstalt, Braunschweig, Germany TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, Türkiye

MATERIAL PROCESSING

The raw material for the CRM Biodiesel (B100 composed of 80 % RME (rapeseed methyl ester) and 20 % SME (soy methyl ester)) was produced in Romania. Collected samples (total of 350 Liters in 70 x 5-liter metal canisters) were transported from Romania to TÜBİTAK UME for further processing.

For preliminary elemental content measurements, subsamples from 3 different canisters were taken and analyzed by TÜBİTAK UME. Results of this measurement are summarized in Table 2.

Table 2. Mass fraction levels of elements in biodiesel material

Parameter	Preliminary Measurement Result (mg/kg)	Target Range Level (mg/kg)
Ca	0.036	0.5 - 2.5
K	0.15	0.5 - 2.5
Mg	0.0023	0.2 - 1.0
Na	0.33	1.0 - 5.0
P	0.56	1.0 - 5.0
S	6.47	6.5 - 10

Results showed that the candidate raw material has low level of the target elements except sulfur, thus it was decided to spike Ca, K, Mg, Na and P elements to this material to reach target levels.

Material in canisters were filtered (0.7 µm, glass fiber) and combined in homogenization tank (320 L, HDPE). Spike mixture (prepared from Ca, K, Mg, Na and P element standards in mineral oil, Conostan) was added and the tank was filled with filtered biodiesel. Homogenization is performed by drawing and filling the content to the tank.

Filling and capping were done manually. A total of 553 units, each ~500 mL was filled and capped to amber colored glass bottles. All bottles were labelled following the filling order using automated labelling machine (Farmatek, Türkiye).

After this step, the bottles were stored at 4 °C in the dark environment. All stages of processing are summarized as a flow diagram in Annex 1. Details of the processing is also documented as a video: https://www.youtube.com/watch?v=DDnfvmhP20Y

HOMOGENEITY

Homogeneity study between the units is performed to show that the assigned values are valid for all units within the stated uncertainty. Homogeneity study between the units is performed with a number of samples representing the whole batch. In this project, 10 units were selected by using random stratified sampling for each of the participant laboratories. Homogeneity tests were carried out by measuring 2 or 3 sub-samples under repeatability conditions. The samples to be analysed were introduced to the instruments by random order to find out any trend arising from analytical and/or filling sequences. For Ca, Mg and P, data supplied for homogeneity samples by BRML was evaluated as

technically invalid due to high variance on some of the individual units. Alternatively, short-term stability sample data was used to evaluate the homogeneity of these parameters.

Grubbs test (one sided) was applied to all data for the presence of outlier at 99 % confidence level and outliers were detected for two parameters. Two of the outliers detected for one parallel measurement result out of three measurements of one unit for Tri-Glycerides and density parameters are excluded from the calculations. Data was visually checked whether all individual data follow a unimodal distribution using histograms and normal probability plots. It was found that the distribution was normal and unimodal. 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 presented in Table 3.

Table 3. Statistical Evaluation of Homogeneity Results for Biodiesel

Parameter (Lab)	Is there a Trend?		Number of Outliers		Distribution
	Analytical sequence	Filling sequence	All data	Unit averages	All data
Calorific Value (PTB)	No	No	-	-	Normal/unimodal
Mono-Glycerides (BRML)	No	No	-	-	Normal/unimodal
Di-Glycerides (BRML)	No	No	-	-	Not Normal/unimodal
Tri-Glycerides (BRML)	No	No	2	-	Not Normal/unimodal
Free Glycerol (BRML)	No	No	-	-	Normal/unimodal
Total Glycerol (BRML)	No	No	-	-	Not Normal/unimodal
Methyl Linoleate (IMBIH)	No	No	-	-	Normal/unimodal
Methyl Palmitoleate (IMBIH)	No	No	-	-	Normal/unimodal
Methyl Palmitate (IMBIH)	No	No	-	-	Normal/unimodal
Methyl 11-Octadecenoate (IMBIH)	No	No	-	-	Not Normal/unimodal
Methyl Stearate (IMBIH)	No	No	-	-	Normal/unimodal
Methyl cis-11-Eicosenoate (IMBIH)	No	No	-	-	Normal/unimodal
Calcium (BRML)	No	No	-	-	Normal/unimodal
Magnesium (BRML)	No	No	-	-	Not Normal/unimodal
Phosphorus (BRML)	No	No	-	-	Not Normal/unimodal
Potassium (BRML)	No	No	-	-	Normal/unimodal
Sodium (BRML)	Yes	No	-	-	Normal/unimodal
Sulfur (BRML)	No	No	-	-	Normal/unimodal
Viscosity (PTB)	No	No	-	-	Normal/unimodal
Density (PTB)	No	No	1	1	Normal/unimodal
Methanol (UME)	Yes	No	-	-	Normal/unimodal
Water (UME)	No	No	-	-	Normal/unimodal

Regression analyses were used to evaluate potential trends in each analytical run at 95 % and 99 % confidence levels. It is observed that there was a significant analytical trend at 95 % confidence level for the measurements of Na and Methanol. As the analytical sequence and the unit numbers were not

correlated, mathematical correction of the dataset for the significant analytical trend of the measurements was performed using the Equation (1) where trends were significant:

$$C_{Corrected} = C_{Measured} - b \cdot i \quad (1)$$

where;

b : slope of the linear regression,

i : position of the result in the analytical sequence.

The ANOVA allowed the calculation of the within- (s_{wb}) and between-unit homogeneity (s_{bb}), estimated as standard deviations, according to the equations (2) and (3):

$$s_{wb} = \sqrt{MS_{within}} \quad (2)$$

MS_{within} : Mean squares within-unit

s_{wb} is equivalent to the *s* of the method, provided that subsamples are representative for the whole unit.

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \quad (3)$$

$MS_{between}$: Mean squares between-unit,

n : Number of replicates per unit.

When $MS_{between}$ is smaller than MS_{within}, s_{bb} cannot be calculated. Instead, u^*_{bb}, the heterogeneity that can be hidden by the method repeatability [7], is calculated according to the equation (4):

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{v_{MSwithin}}} \quad (4)$$

$v_{MSwithin}$: Degrees of freedom of MS_{within}

The occurrence of $MS_{between} < MS_{within}$ can be seen, if material heterogeneity is smaller than that can be detected by the analytical methodology used.

For density parameter an outlying bottle mean was observed, and in this case alternative data evaluation was applied and between unit homogeneity was modeled as a rectangular distribution and equation (5) was applied for rectangular standard uncertainty (u_{rect}) of homogeneity.

$$u_{rect} = \frac{|Outlier\ value - Average\ value|}{\sqrt{3}} \quad (5)$$

For the parameters for which ANOVA was applied, the larger value of s_{bb} or u^*_{bb} is taken as uncertainty contribution for homogeneity, u_{bb} (Table 4).

Table 4. Results of the homogeneity study

Parameter	$s_{wb,rel}$, %	$s_{bb,rel}$, %	$u^*_{bb,rel}$, %	$u_{rec,rel}$, %	$u_{bb,rel}$,%
Calorific Value	0.042	0.023	0.02	-	0.023
Mono-Glycerides	0.18	0.28	0.06	-	0.28
Di-Glycerides	0.49	0.45	0.16	-	0.45
Tri-Glycerides	0.90	1.18	0.30	-	1.18
Free Glycerol	0.50	0.84	0.16	-	0.84
Total Glycerol	0.26	0.22	0.08	-	0.22
Methyl Linoleate	1.7	0.78	0.54	-	0.78
Methyl Palmitoleate	9.1	1.5	2.96	-	2.96
Methyl Palmitate	5.1	$MS_{between}<MS_{within}$	1.66	-	1.66
Methyl 11-Octadecenoate	0.84	0.82	0.17	-	0.82
Methyl Stearate	4.6	$MS_{between}<MS_{within}$	1.49	-	1.49
Methyl cis-11-Eicosenoate	5.1	3.25	1.69	-	3.25
Calcium	12	$MS_{between}<MS_{within}$	3.8	-	3.8
Magnesium	8.6	3.5	2.9	-	3.5
Phosphorus	13	$MS_{between}<MS_{within}$	4.2	-	4.2
Potassium	11	6.5	3.7	-	6.5
Sodium	25	2.9	8.2	-	8.2
Sulfur	3.4	$MS_{between}<MS_{within}$	1.1	-	1.1
Viscosity	0.024	0.031	0.011	-	0.031
Density	0.00097	0.00072	0.00032	0.0013	0.0013
Methanol	4.2	2.2	1.4	-	2.2
Water	1.2	3.2	0.57	-	3.2

The plotted data used for the evaluation of homogeneity can be found in Annex 2.

STABILITY

The stability of the units which are exposed to different environmental conditions that may occur during shipment and shelf life is tested and evaluated at defined storage conditions by reference material producers.

Stability studies were performed with isochronous design. For the short term stability (STS) test +45 °C temperature and five time points (0, 1, 2, 3 and 4 weeks) were tested. 10 units were selected for each laboratory by using a stratified sampling scheme covering whole batch. 32 samples were subjected to the test temperature for the specified time intervals. For the long-term stability test (LTS), 10 units for each laboratory were tested at +22 °C for 0, 2, 4, 6 and 8 months' time points.

Units were moved to +4 °C (reference temperature) after completion of the test time. All units were analyzed at the same time. Samples were analyzed under the repeatability conditions to determine the values for the parameters of interest.

Short Term Stability Results:

The results obtained from isochronous measurements were first grouped according to the time period and then evaluated for each time point.

The data for each parameter was first examined by single Grubbs test for both 95 % and 99 % confidence intervals to find out outliers. Number of detected outliers are given in the Table 5. Two outlying (one out of three parallel results of two different units) results were removed from the dataset for methyl palmitate reported by IMBIH.

Values calculated for each time point were plotted against the time. The relationship between variables were analyzed in order to determine if any significant change exists with the testing time (regression analysis). It was found that the slopes were significant for free glycerol reported by BRML, viscosity reported by PTB, and methanol reported by TÜBİTAK UME. The trend graphs of short term stability are shown in Annex 3. The relative short term stability uncertainty, $u_{sts,rel}$ for each parameter is calculated using Equation (6) for the required transfer time as described in [8], and results are given in Table 5:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \qquad (6)$$

where,

RSD : relative standard deviation of the points on the regression line as described in B.3.2 [5],
t_i : time point for each replicate expressed in weeks,
$\bar{t}$: mean of all time points expressed in weeks,
t : maximum time suggested for transfer (2 weeks).

Table 5. Short Term Stability (STS) test results

Parameter (Lab)	45 °C $u_{sts,rel}$ for 2 weeks (%)	Number of outliers in 95 % confidence interval [1]	Number of outliers in 99 % confidence interval [1]	Any significant trend in 95 % confidence interval?	Any significant trend in 99 % confidence interval?
Calorific Value (PTB)	0.023	-	-	No	No
Mono-Glycerides (BRML)	0.23	-	-	No	No
Di-Glycerides (BRML)	0.36	-	-	No	No
Tri-Glycerides (BRML)	1.1	-	-	No	No
Free Glycerol (BRML)	0.63 [2]	-	-	Yes	Yes
Total Glycerol (BRML)	0.16	-	-	No	No
Methyl Linoleate (IMBIH)	0.46	-	-	No	No
Methyl Palmitoleate (IMBIH)	1.5	-	-	No	No
Methyl Palmitate (IMBIH)	0.69	2	2	No	No
Methyl 11-Octadecenoate (IMBIH)	0.41	-	-	No	No
Methyl Stearate (IMBIH)	1.4	-	-	No	No
Methyl cis-11-Eicosenoate (IMBIH)	1.6	-	-	No	No
Calcium (BRML)	2.8	-	-	No	No
Magnesium (BRML)	3.1	-	-	No	No
Phosphorus (BRML)	3.3	-	-	No	No
Potassium (BRML)	2.8	-	-	No	No
Sodium (BRML)	4.8	-	-	No	No
Sulfur (BRML)	1.9	-	-	No	No
Viscosity (PTB)	0.13 [2]	1	-	Yes	No
Density (PTB)	0.0029	-	-	No	No
Methanol (UME)	2.5 [2]	-	-	Yes	Yes
Water (UME)	2.5	-	-	No	No

[1] Single Grubbs Test

[2] u_{sts} is calculated by taking into account the degradation (by combining the calculated uncertainty with u_{rec}= slope of reg. line/$\sqrt{3}$.

The material is found to be stable at 45 °C for up to 2 weeks. Thus, the samples can be safely dispatched under conditions where the temperatures do not exceed 45 °C for up to 2 weeks, i.e. at ambient temperature without applying any cooling elements.

Long Term Stability Results:

Shelf life of the CRM has been determined through long term stability measurements. For the measurements, for each partner two units for each of the months of 0, 2, 4, 6 and 8 have been stored at +22 °C and transferred to reference temperature (+4 °C) after each period of time to be measured isochronously afterwards. Eight units, designated as reference units, of the 'month 0' was stored at +4 °C. Detected outlying results were removed since they were observed only in one parallel measurement result out of three measurements of a unit.

The relative long term stability uncertainty, $u_{lts,rel}$ for each parameter is calculated using equation (7) for the required shelf life as [8]:

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \quad (7)$$

where

RSD *:* the relative standard deviation of the points on the regression line as described in B.3.2 [5],

t_i *:* the time point for each replicate expressed in months,

$\bar{t}$: the average of all time points expressed in months,

t : the proposed shelf life at 18 °C (12 months).

The uncertainty contribution u_{lts} was calculated for 12 months (t) at 22 °C. The graphs for long term stability are given in Annex 4.

Table 6. Long Term Stability (LTS) test results

Parameter (Lab)	22 °C $u_{lts,rel}$ for 12 months (%)	Number of outliers in 95 % confidence interval [1]	Number of outliers in 99 % confidence interval [1]	Any significant trend in 95 % confidence interval?	Any significant trend in 99 % confidence interval?
Calorific Value (PTB)	0.076	1	-	No	No
Mono-Glycerides (BRML)	0.79	-	-	No	No
Di-Glycerides (BRML)	1.3	-	-	No	No
Tri-Glycerides (BRML)	1.4	-	-	No	No
Free Glycerol (BRML)	1.6	-	-	No	No
Total Glycerol (BRML)	0.61	-	-	No	No
Methyl Linoleate (IMBIH)	1.1	1	-	No	No
Methyl Palmitoleate (IMBIH)	6.4	1	1	No	No
Methyl Palmitate (IMBIH)	3.5	-	-	No	No
Methyl 11-Octadecenoate (IMBIH)	1.2	1	-	No	No
Methyl Stearate (IMBIH)	2.8	-	-	No	No
Methyl *cis*-11-Eicosenoate (IMBIH)	4.0	2	-	No	No
Calcium (BRML)	5.3	-	-	No	No
Magnesium (BRML)	5.7	-	-	No	No
Phosphorus (BRML)	9.4	-	-	No	No
Potassium (BRML)	11.3	-	-	No	No
Sodium (BRML)	12.4	-	-	No	No
Sulfur (BRML)	6.3	-	-	No	No
Viscosity (PTB)	0.39 [2]	-	-	Yes	Yes
Density (PTB)	0.019 [2]	-	-	Yes	Yes
Methanol (UME)	8.4 [2]	-	-	Yes	Yes
Water (BRML)	11.7 [2]	-	-	Yes	Yes

[1] Single Grubbs Test

[2] u_{lts} is calculated by taking into account the degradation (by combining the calculated uncertainty with u_{rec}= slope of reg. line/ $\sqrt{3}$)

CHARACTERIZATION

According to ISO 17034, the characterization and the value assignment can be carried out in different ways. The approach chosen in this project is; characterization of a non-operationally and operationally defined measurands using two or more methods of demonstrable accuracy in two or more competent laboratories. The participating laboratories were partners and collaborators of the BIOFMET project consortium [3]. The detailed information about the laboratories is given in the participants section. The participating laboratories used validated methods for the characterization.

Each laboratory received two units of samples which were selected from the whole set of samples to represent the whole produced batch. The samples were selected randomly from the set of samples by the random stratified sampling technique. Each laboratory was asked to report at least three independent measurement results for each unit, together with their associated measurement uncertainty values and the approach used for the estimation of measurement uncertainty. In the reports, the details of the reference materials used in the calibration were also requested in order to assure the traceability of the reported results.

Measurement uncertainties were calculated according to the “Guide to the Expression of Uncertainty in Measurements (GUM)” and “EURACHEM/CITAC Guide Quantifying Uncertainty in Analytical Measurement” documents or estimated in accordance with ISO 17034:2016 and ISO 33405:2024. Equations (8, 9, 10) were used to calculate characterization standard uncertainty (u_{char}) stated by M. S. Lenson et al [9] for the cases where two method/laboratory results were available. In cases where more than two method/laboratory results were available, characterization standard uncertainty (u_{char}) is calculated using Equation (11) by taking into account the uncertainties and the standard deviation of the means reported by the participating laboratories. Value assignment of the material is performed by arithmetic averaging two or more method results.

$$u(B) = \frac{|x_{Method\ 1} - x_{Method\ 2}|}{2\sqrt{3}} \tag{8}$$

$$u(X) = \sqrt{(\tfrac{1}{2})^2 u^2(\text{Method 1}) + (\tfrac{1}{2})^2 u^2(\text{Method 2})} \tag{9}$$

$$u_{char} = \sqrt{u^2(X) + u^2(B)} \tag{10}$$

here,

$u(B)$: the standard uncertainty based on the difference on the difference of results of two methods,

$u(X)$: the standard uncertainty obtained by combining uncertainties of two methods,

u_{char} : the standard uncertainty of characterization by two methods.

$$u_{\text{char}} = \sqrt{\overline{u}^{\,2}_{\text{labs}} + \left(\frac{SD}{\sqrt{n}}\right)^2} \tag{11}$$

where;

u_{char} : Standard uncertainty arising from characterization,

$\overline{u}_{labs}$: Arithmetic mean of standard uncertainties reported by the participating laboratories,

SD : Standard deviation of accepted means of participating laboratories,

n : Number of laboratories with accepted results.

A list of laboratories with their abbreviations and their corresponding methodologies used for the measurements are summarized in Table 7. More details about the measurement methods are given in Annex 5. Characterization plots are given in Annex 6.

Table 7. Techniques used by participating laboratories

Parameter	BAM	BRML	DTI	IMBIH	GUM	LGC	PTB	UME
Calorific Value	-	ISOP-CAL	ISOP-CAL	-	-	-	ISOP-CAL	ISOP-CAL
Mono-Glycerides	-	GC-FID	-	-	-	-	-	-
Di-Glycerides	-	GC-FID	-	-	-	-	-	-
Tri-Glycerides	-	GC-FID	-	-	-	-	-	-
Free Glycerol	-	GC-FID	-	-	-	-	-	-
Total Glycerol	-	GC-FID	-	-	-	-	-	-
Methyl Linoleate	-	-	-	GC-MS	-	-	-	-
Methyl Palmitoleate	-	-	-	GC-MS	-	-	-	-
Methyl Palmitate	-	-	-	GC-MS	-	-	-	-
Methyl 11-Octadecenoate	-	-	-	GC-MS	-	-	-	-
Methyl Stearate	-	-	-	GC-MS	-	-	-	-
Methyl cis-11-Eicosenoate	-	-	-	GC-MS	-	-	-	-
Calcium	-	ICP-MS	-	-	-	-	-	ICP-OES
Magnesium	-	-	-	-	ICP-MS	-	-	HR ICP-MS ICP-OES
Phosphorus	-	-	-	-	-	-	-	HR ICP-MS ICP-OES
Potassium	-	-	-	-	ICP-MS	-	-	ICP-OES
Sodium	-	ICP-MS	-	-	ICP-MS	-	-	HR ICP-MS ICP-OES
Sulfur	ID ICP-MS	ICP-MS	-	-	-	-	-	ID ICP-MS HR ICP-MS ICP-OES
Viscosity	-	-	-	-	-	SVM	SVM	-
Density	-	-	-	-	-	DM	DM	-
Methanol	-	-	-	-	-	-	-	GC-FID
Water	-	COU-KFT	-	-	-	-	-	COU- o-KFT

COU-KFT : Coulometric Karl Fischer Titrimetry
COU-o-KFT : Coulometric Karl Fischer Titrimetry with Oven
DM : Density Meter
GC-FID : Gas Chromatography Flame İonization Detector
GC-MS : Gas Chromatography Mass Spectrometry
ICP-MS : Inductively Coupled Plasma Mass Spectrometry
ICP-OES : Inductively Coupled Plasma Optical Emission Spectrometry
ID ICP-MS : Isotope Dilution ICP-MS
ISOP-CAL : Isoperibol Calorimetry
SVM : Stabinger Viscometer

PROPERTY VALUE AND UNCERTAINTY ASSIGNMENT

Assigned values and uncertainties of the CRM were evaluated by applying approach in the characterization and uncertainty data that contribute to the homogeneity and stability assessments.

Data obtained in the characterization study were checked for normal distribution and outliers. Distributions were found to be normal, and no outlier was detected.

Unweighted mean value of characterization results is assigned as the property value of the reference materials.

Equation (12) is used to calculate the combined expanded uncertainty of the CRM:

$$\mathrm{U_{CRM}} = k\sqrt{\mathrm{u_{char}^2} + \mathrm{u_{bb}^2} + \mathrm{u_{lts}^2} + \mathrm{u_{sts}^2}} \qquad (12)$$

Uncertainty value on CRM certificate includes uncertainty contribution from characterization (u_{char}), homogeneity (u_{bb}), long term stability (u_{lts}) and short-term stability (u_{sts}). Expansion of uncertainty value of CRM was done with a coverage factor (k = 2) representing 95 % confidence level. Certified values, uncertainties and relative percent contribution of each component on uncertainty is given in Table 7.

Table 7. Certified values and uncertainties

Parameter (unit)	Certified value [1]	U_{CRM} (k=2)[1,2]	$U_{CRM,rel}$ (%, k=2)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
Gross Calorific Value [$q_{V,gr}$] [3] (J/g)	39901	97	0.25	0.089	0.023	0.023	0.076
Density at 15 °C [4] (g/cm^3)	0.88353	0.00035	0.040	0.0025	0.0013	0.0029	0.019
Kinematic Viscosity at 40 °C [5] (mm^2/s)	4.419	0.039	0.89	0.18	0.031	0.13	0.39
Ca [6] (mg/kg)	1.03	0.31	30	13	3.8	2.8	5.3
K [6] (mg/kg)	1.01	0.34	34	11	6.5	2.8	11
Mg [7] (mg/kg)	0.48	0.11	23	8.7	3.5	3.1	5.7
Na [8] (mg/kg)	1.70	0.57	33	5.8	8.2	4.8	12
P [9] (mg/kg)	2.13	0.51	24	2.8	4.2	3.2	10
S [10] (mg/kg)	8.7	1.4	16	4.0	1.1	1.9	6.3

[1] The certified values and the uncertainties are traceable to the International System of Units (SI).

[2] The expanded uncertainty of the certified value includes characterization, homogeneity, stability components and is stated as the standard uncertainty of measurement multiplied by the coverage factor k = 2, which for a normal distribution corresponds to a coverage probability of approximately 95 %. The standard uncertainty of measurement has been determined in accordance with GUM “Guide to the Expression of Uncertainty in Measurement”.

[3] Calculated from the arithmetic mean of the accepted results of gross calorific value at constant volume submitted by four laboratories applying DIN 51900-2 method.

[4] Calculated from the arithmetic mean of the accepted results submitted by two laboratories applying ASTM D4052 and EN ISO 12185 methods.

[5] Calculated from the arithmetic mean of the accepted results submitted by two laboratories applying ASTM D7042 and EN ISO 3104 methods.

[6] Calculated from the arithmetic mean of the accepted results submitted by two laboratories applying ICP-MS and ICP-OES methods.

[7] Calculated from the arithmetic mean of the accepted results submitted by two laboratories applying ICP-MS, HR ICP-MS and ICP-OES methods.

[8] Calculated from the arithmetic mean of the accepted results submitted by three laboratories applying ICP-MS, HR ICP-MS and ICP-OES methods.

[9] Calculated from the arithmetic mean of the accepted results submitted by one laboratory applying HR ICP-MS and ICP-OES methods.

[10] Calculated from the arithmetic mean of the accepted results submitted by three laboratories applying ICP-MS, HR ICP-MS, ID ICP-MS and ICP-OES methods.

INFORMATIVE VALUES

Parameters for which a consensus value cannot be assigned due to lack of multiple laboratory/method measurement results are given as informative value. Results for these parameters are given in Table 8 and Table 9.

Table 8. Informative values and uncertainties for biodiesel

Parameter (unit)	Assigned value	U_{AV} (k=2)	$U_{AV,rel}$ (%, k=2)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
Net Calorific Value [$q_{V,net}$] [1] (J/g)	37360	91	0.25	0.089	0.023	0.023	0.076
Mono-Glycerides[2] (g/100g)	0.566	0.019	3.3	1.4	0.28	0.23	0.79
Di-Glycerides[2] (g/100g)	0.1752	0.0080	4.6	1.8	0.45	0.36	1.2
Tri-Glycerides[2] (g/100g)	0.1432	0.0089	6.2	2.2	1.2	1.1	1.4
Free Glycerol[2] (g/100g)	0.0193	0.0027	14	6.5	0.84	0.63	1.6
Total Glycerol[2] (g/100g)	0.204	0.019	9.0	4.5	0.22	0.16	0.61
Methyl Linoleate[3] (g/100g)	28.7	2.2	7.7	3.6	0.78	0.46	1.1
Methyl Palmitoleate[3] (g/100g)	0.248	0.069	28	12	3.0	1.5	6.4
Methyl Palmitate[3] (g/100g)	8.67	0.93	11	3.6	1.7	0.69	3.5
Methyl 11-Octadecenoate[3] (g/100g)	58.4	3.1	5.3	2.2	0.82	0.41	1.2
Methyl Stearate[3] (g/100g)	2.40	0.31	13	5.4	1.5	1.4	2.8
Methyl cis-11-Eicosenoate[3] (g/100g)	1.09	0.21	19	7.8	3.3	1.6	4.0
Methanol[4] (g/100g)	0.176	0.035	20	3.6	2.2	2.5	8.4
Water[5] (mg/kg)	339	89	26	4.4	3.2	1.8	12

[1] Calculated from the certified gross calorific value at constant volume [qV,gr] by using the following equation: qV,net,= [qV,gr – (206 x hydrogen content of biofuel, in percentage by mass + 23.05 x water, in percentage by mass)] as defined in DIN 51900-1.
[2] Calculated from the arithmetic mean of the accepted results submitted by one laboratory applying EN 14105 method.
[3] Calculated from the arithmetic mean of the accepted results submitted by one laboratory applying GC-MS method aiming quantification of relative amount based on total area of the methyl esters.
[4] Calculated from the arithmetic mean of the accepted results submitted by one laboratory applying GC-FID method.
[5] Calculated from the arithmetic mean of the accepted results submitted by two laboratories applying EN ISO 12937 method with direct and oven sample introduction systems.

Table 9. Informative values for Carbon and Hydrogen element content

Element	Measurement Result [1] (g/100g)
C	77.29 ± 0.23
H	12.33 ± 0.16

* Values written with "±" sign represents standard deviation
[1] Arithmetic mean of the accepted analysis results (n = 12) by TÜBİTAK UME.

COMMUTABILITY

Commutability is defined as the mathematical relationship of the equation between the reference material and the results produced by the different measurement methods that can be used to measure

the routine samples it represents [10]. UME BIOFMET CRM 01 was produced from a regular biodiesel (80 % RME, 20 % SME) produced in Romania by spiking with a mixture of Ca, K, Mg, Na and P standards in mineral oil. The analytical behavior is expected to be the same as for a routine sample of biodiesel of similar content. It should be noted that the extractability of the five spiked elements (Ca, K, Mg, Na and P) from this CRM can be different to the extractability from an unspiked biodiesel sample tested by the user's laboratory due to the possibility that these elements might exist in different chemical forms.

TRACEABILITY

The metrological traceability of the CRM was ensured by using SI traceable calibration standards and using reference methods i.e., ID ICP-MS by the participating laboratories. The laboratories were asked to provide detailed information about the calibration standards and reference methods used in the measurements. Details about the measurement methods, calibration standards and quality control materials used by the participating laboratories are given in Annex 5.

INSTRUCTIONS FOR USE

Shipping conditions

This material can be safely dispatched under conditions where the temperature does not exceed 45 °C for up to two weeks, i.e. at ambient temperature without applying any cooling elements.

Storage conditions

The material should be stored at (22 ± 4) °C in a dark and clean environment. Bottle should be shaken before opening the cap. All precautions should be taken to prevent contamination and evaporation during the use of the material. In order to prevent contamination, it is recommended that the bottle should be opened in a clean environment and pipette should not be inserted into the bottle. After use, the bottle should be tightly recapped immediately. TÜBİTAK UME cannot be held responsible for changes that might happen to the material at the customer’s premises due to noncompliance with the instructions for use, and the storage conditions given.

Safety precautions

The material is manufactured for laboratory use only. General laboratory precautions should be followed during storage and use of the material. It is recommended to use and dispose of the material according to the existing safety rules.

Minimum sample intake

The minimum sample intake is defined by the required sample volume stipulated in the respective standard methods.

Use of Certified Value

For assessing the method performance, the measured values of the CRM are compared with the certified values [11]. The procedure can be described briefly as:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_{meas}) with the standard uncertainty of the certified value (u_{CRM}) using Equation (13):

$$u_{\Delta}=\sqrt{u_{meas}^2+u_{CRM}^2} \quad (13)$$

- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty (u_{Δ}) using a coverage factor of two (k = 2), corresponding to a confidence level of approximately 95 %.

If $\Delta m \leq U\Delta$, then it is assumed that there is no significant difference between the measurement result and the certified value at a confidence level of approximately 95 %.

An online application: CRM Result Evaluation-CRM RE to evaluate your measurement results and automatically create quality control charts is available through the link: https://rm.ume.tubitak.gov.tr/en/crm_re/

ACKNOWLEDGEMENTS

The work of this study is part of the 19 ENG09 BIOFMET project, which was funded within the framework of the EMPIR. The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States. Intern and scholar students; Berke Can, Elif Nur Kırbaş, Beyzanur Çobanoğlu, Rana Yaldız, Hikmet Küçük, Ayşenur Düzgün, Feyzanur Şentürk, Muhammed Faruk Kıran and Selina Kurunç are acknowledged for their contribution to the project. TÜBİTAK BIDEB 2247-C Intern Researcher Scholarship Program (STAR) is acknowledged for financial support of scholar students.

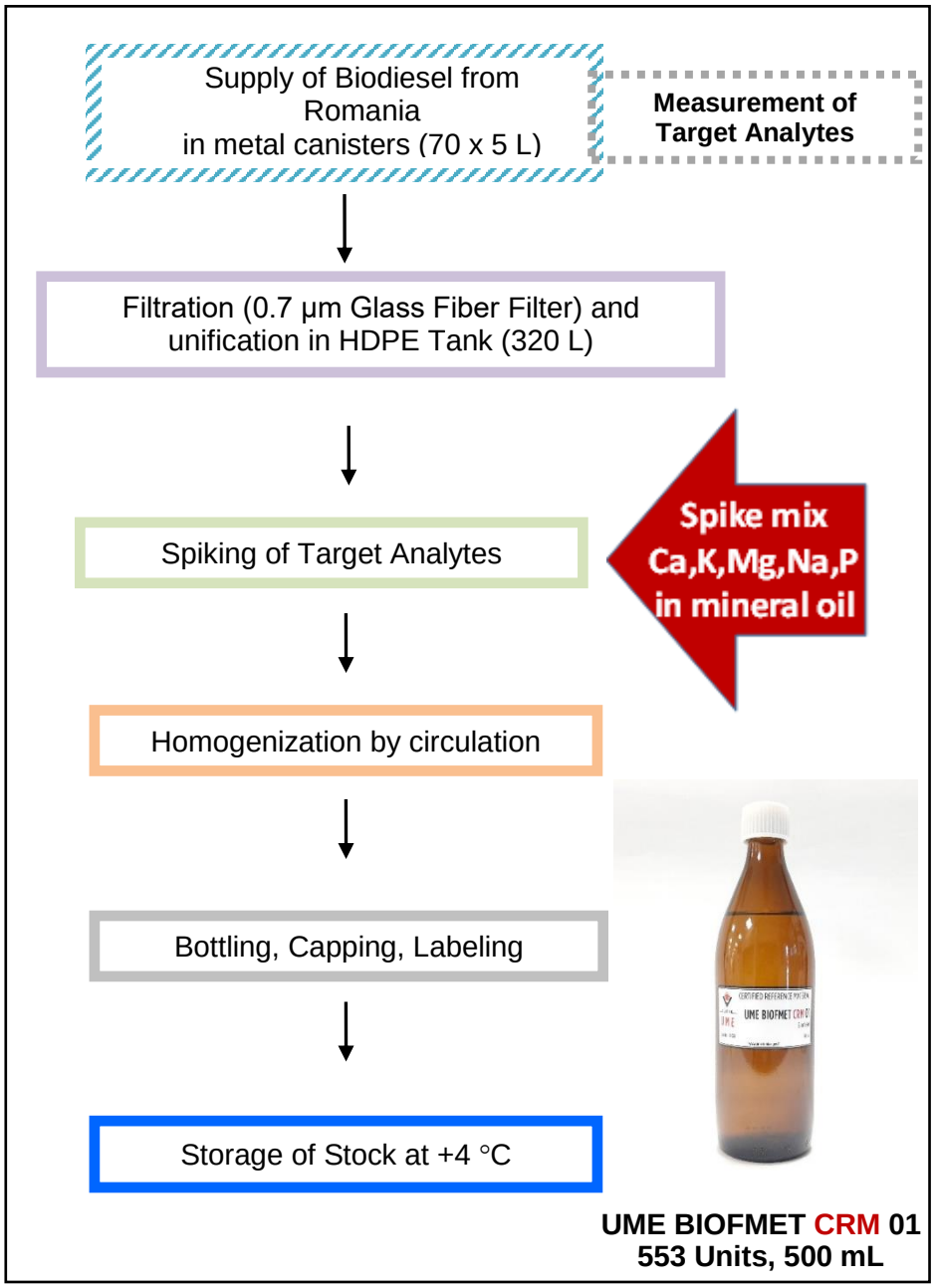
REFERENCES

[1] Mirjana Radovanović, Chapter 7 - Strategic priorities of sustainable energy development, Sustainable Energy Management (Second Edition), Planning, Implementation, Control and Security, 181-277 (2023)
[2] Liquid petroleum products — Fatty acid methyl esters (FAME) for use in diesel engines and heating applications — Requirements and test methods, EN 14214:2012+A2:2019
[3] BIOFMET project homepage; http://biofmet.eu/
[4] ISO 17034:2016. General requirements for the competence of reference materials producers
[5] ISO 33405:2024. Reference materials — Approaches for characterization and assessment of homogeneity and stability
[6] Isleyen, A., Özcan, K., Tunc, M. et al. Development of three biofuel CRMs for the quality parameters in biodiesel and wood pellet via a joint research project, Anal Bioanal Chem (2024) https://doi.org/10.1007/s00216-024-05694-y
[7] T. P. J. Linsinger, J. Pauwels, A. M. H. Van der Veen, H. Schimmel, A. Lamberty, Homogeneity and stability of reference materials, Accred. Qual. Assur. 6:20-25 (2001)
[8] A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem. 360:359-361 (1998)
[9] Lenson,M.S. An Approach to Combining Results From Multiple Methods Motivated by the ISO GUM, *Journal of Research of the National Institute of Standards and Technology*, *105*, 57 (2000)
[10] H. Vesper, H. Emons, M. Gnezda, C. P. Jain, W. G. Miller, R. Rej, G. Schumann, J. Tate, L. Thienpont, J. E. Vaks, Characterization and Qualification of Commutable Reference Materials for Laboratory Medicine; Approved Guideline, CLSI document C53-A, Clinical and Laboratory Standards Institute, Wayne, PA, USA (2010)
[11] For more information about comparison of a measurement result with the certified value please see ERM Application Note 1 https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes

REVISION HISTORY

Date	Remarks
27.12.2024	First issue.

ANNEX 1. Flow Diagram for the Preparation of the Biodiesel CRM



Details of the processing is documented as a video:
https://www.youtube.com/watch?v=DDnfvmhP20Y

ANNEX 2. Graphs for Homogeneity Studies

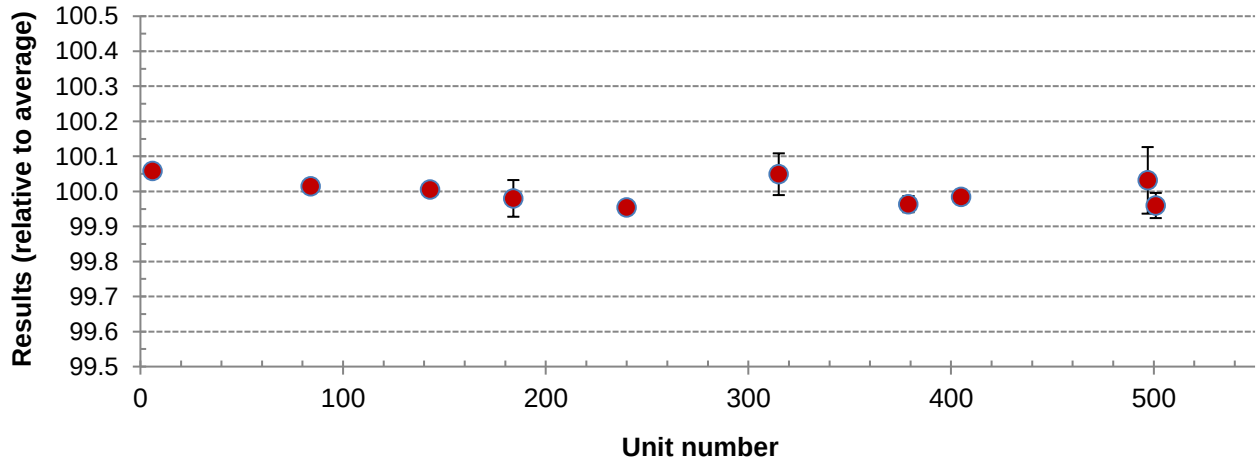


Figure A2.1. Between units homogeneity plot for Calorific Value by PTB

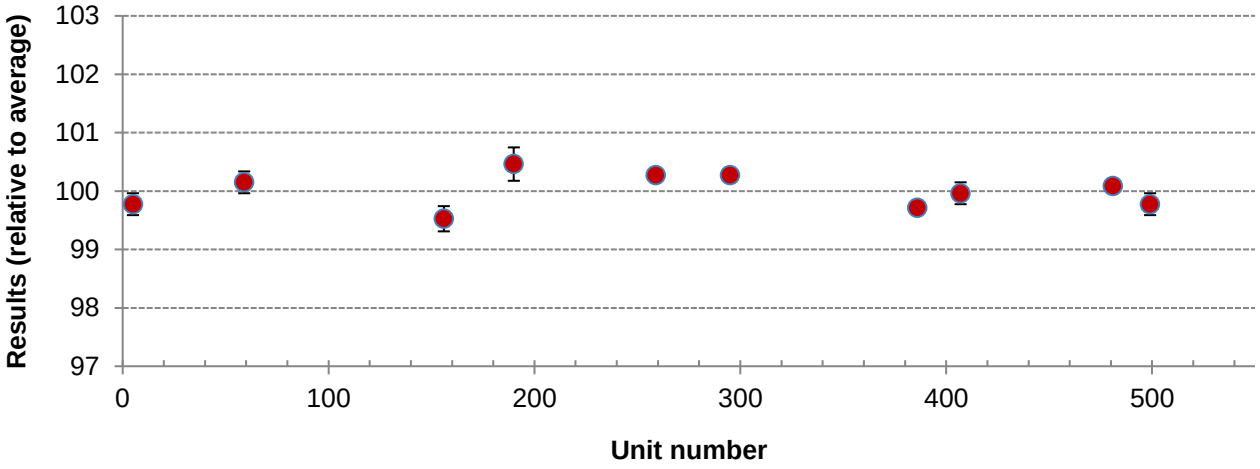


Figure A2.2. Between units homogeneity plot for Mono-Glyceride by BRML

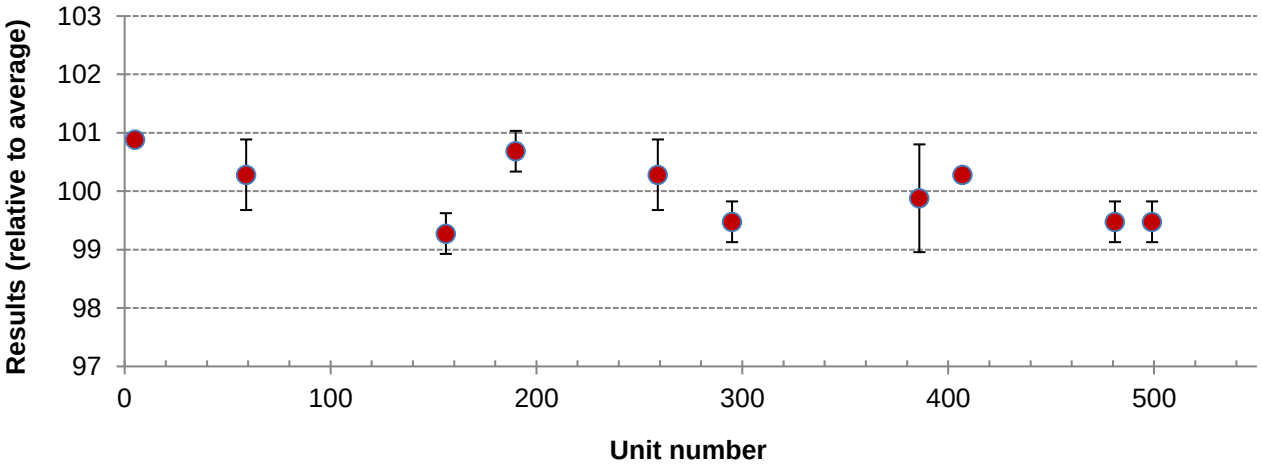


Figure A2.3. Between units homogeneity plot for Di-Glyceride by BRML

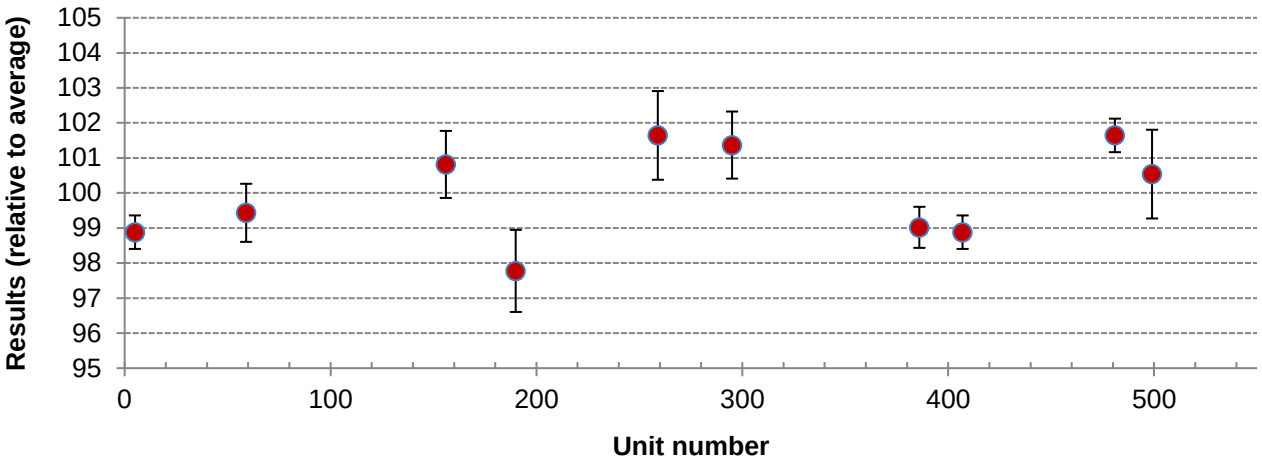


Figure A2.4. Between units homogeneity plot for Tri-Glyceride by BRML

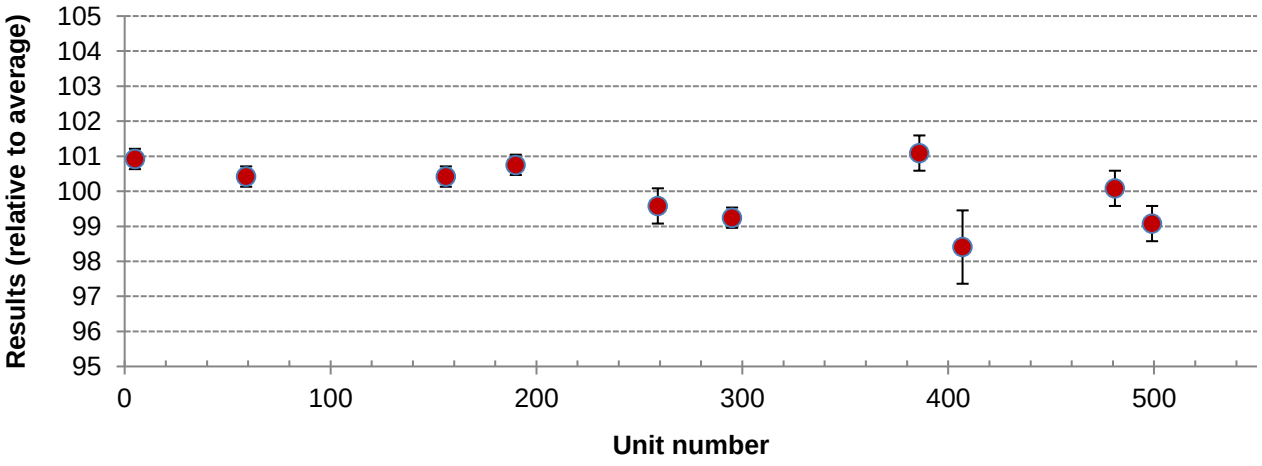


Figure A2.5. Between units homogeneity plot for Free Glycerol by BRML

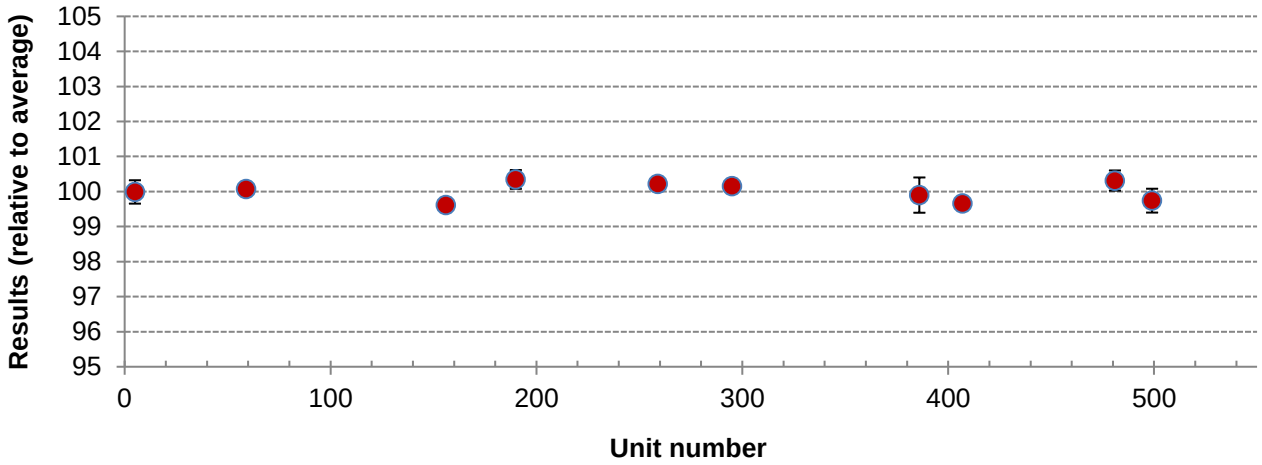


Figure A2.6. Between units homogeneity plot for Total Glycerol by BRML

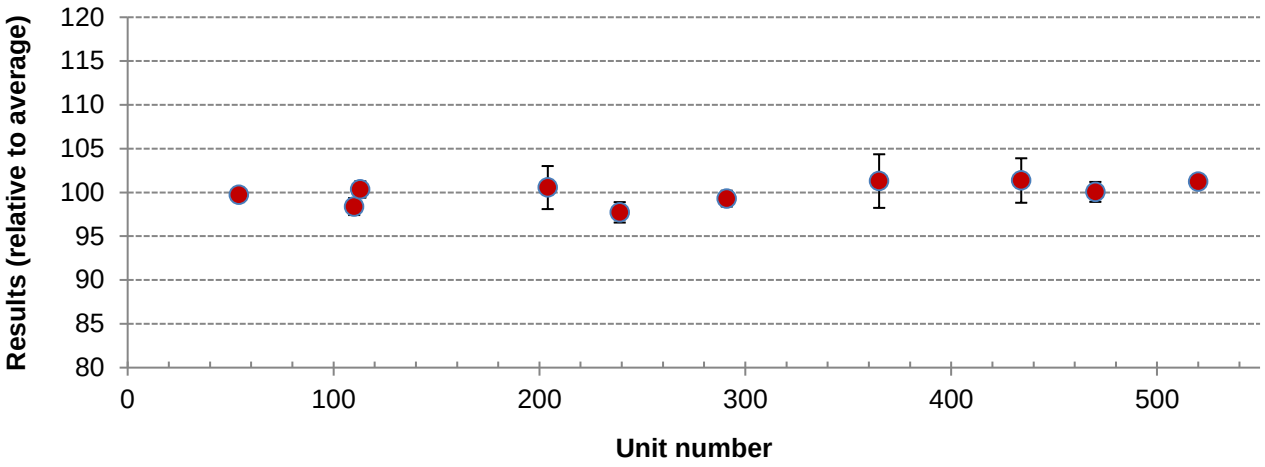


Figure A2.7. Between units homogeneity plot for Methyl Linoleate by IMBIH

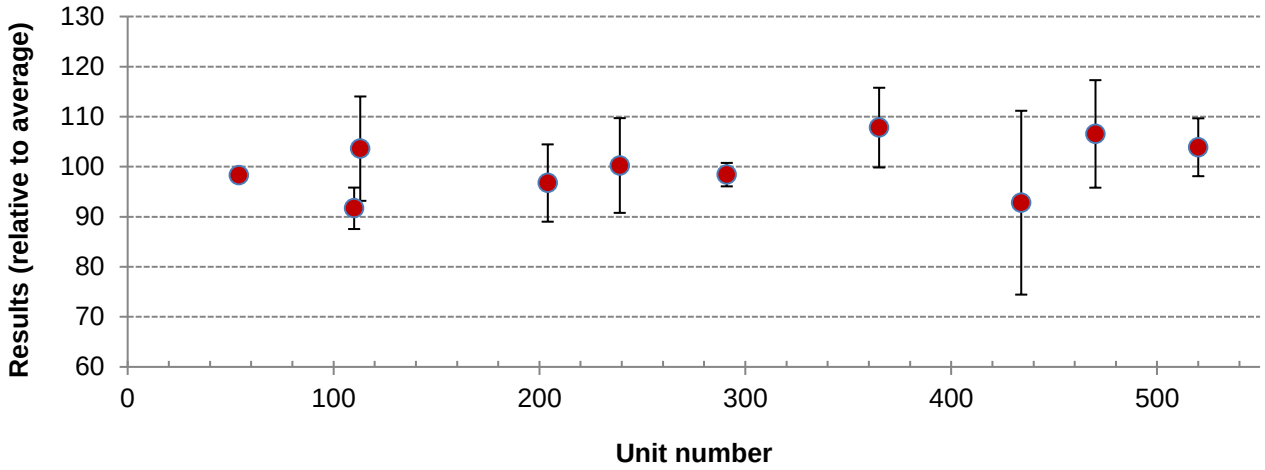


Figure A2.8. Between units homogeneity plot for Methyl Palmiteolate by IMBIH

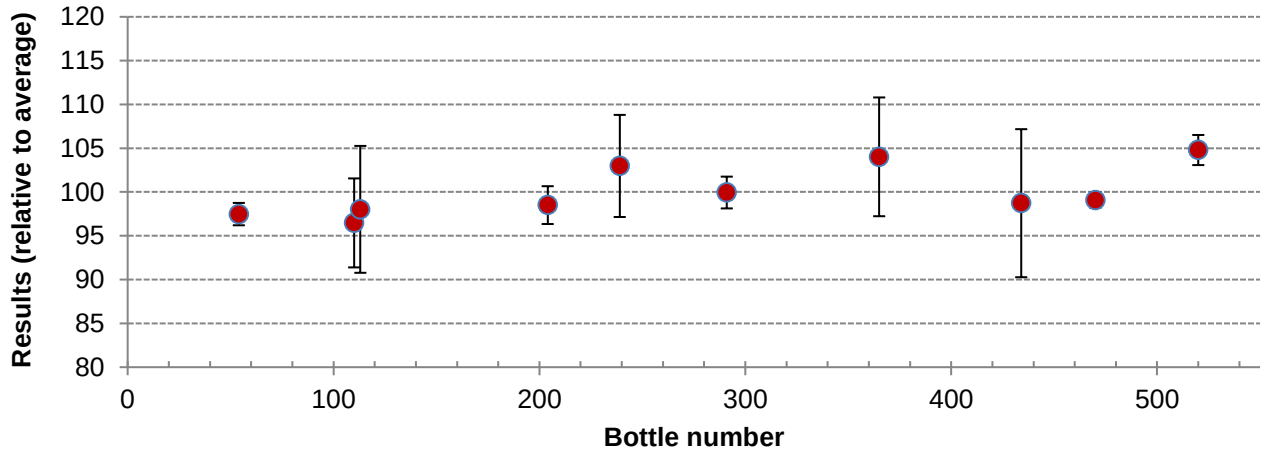


Figure A2.9. Between units homogeneity plot for Methyl Palmitate by IMBIH

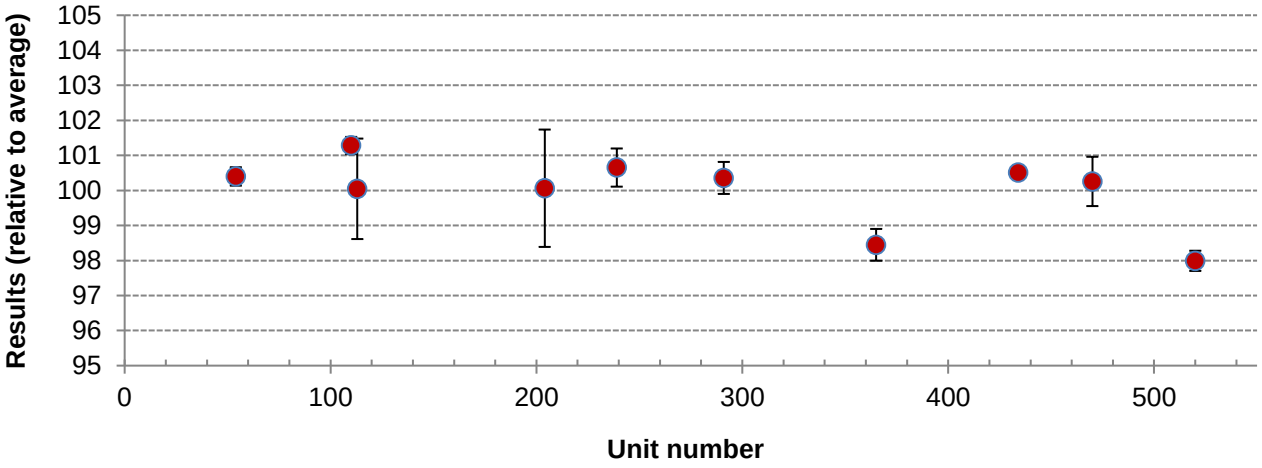


Figure A2.10. Between units homogeneity plot for Methyl 11-Octadecenoate by IMBIH

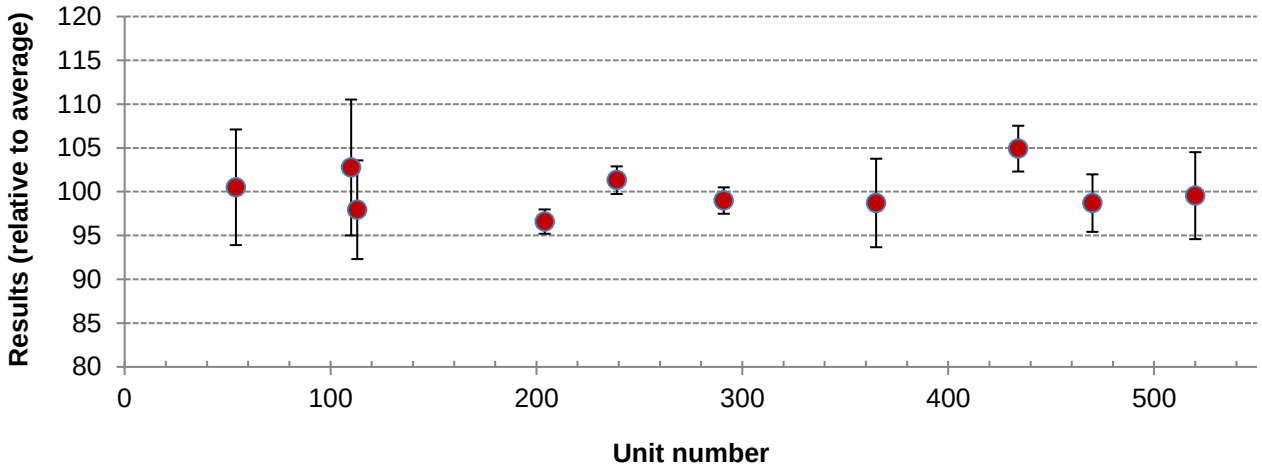


Figure A2.11. Between units homogeneity plot for Methyl Stearate by IMBIH

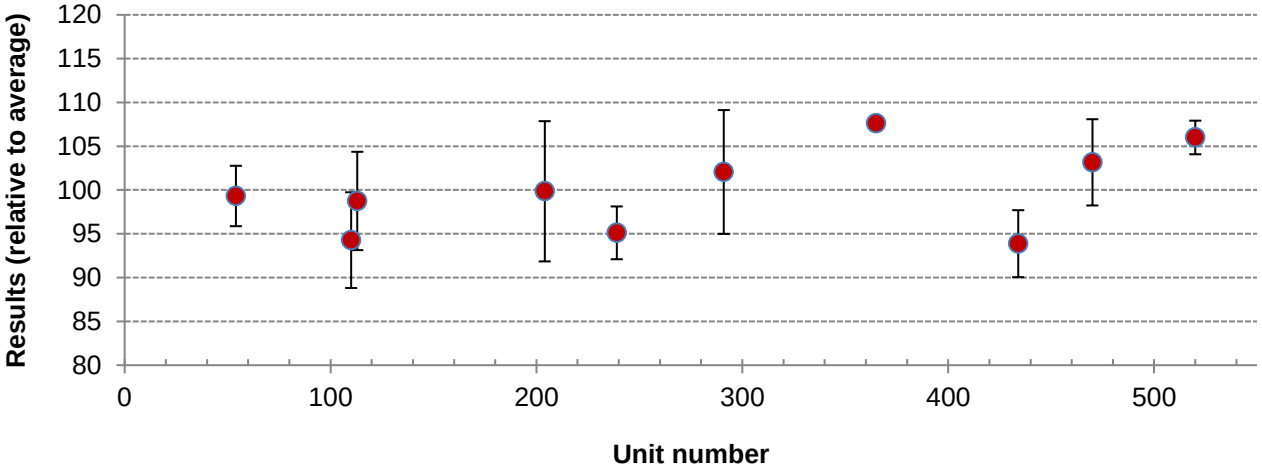


Figure A2.12. Between units homogeneity plot for Methyl cis-11-Eicosenoate by IMBIH

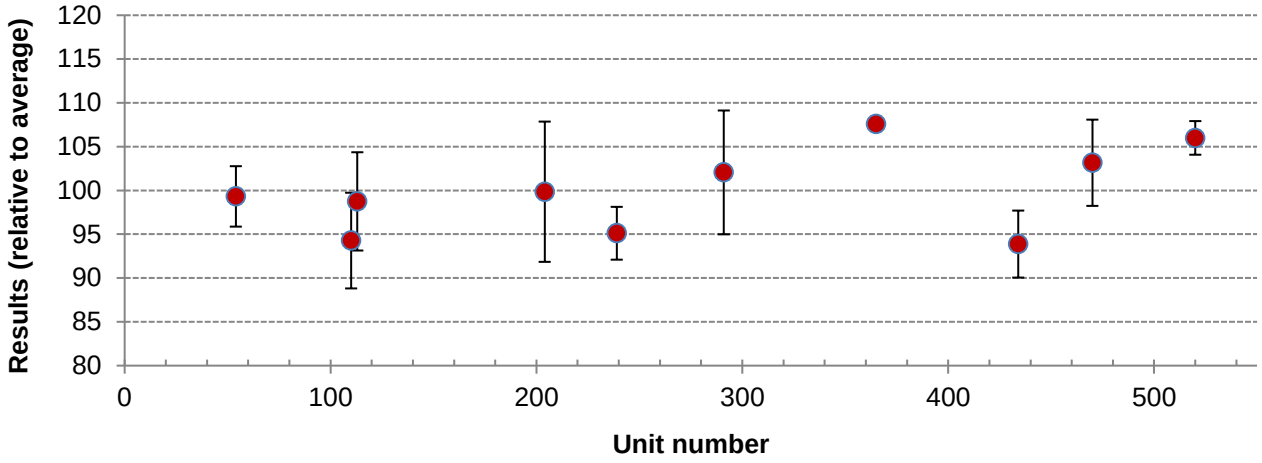


Figure A2.13. Between units homogeneity plot for Calcium by BRML

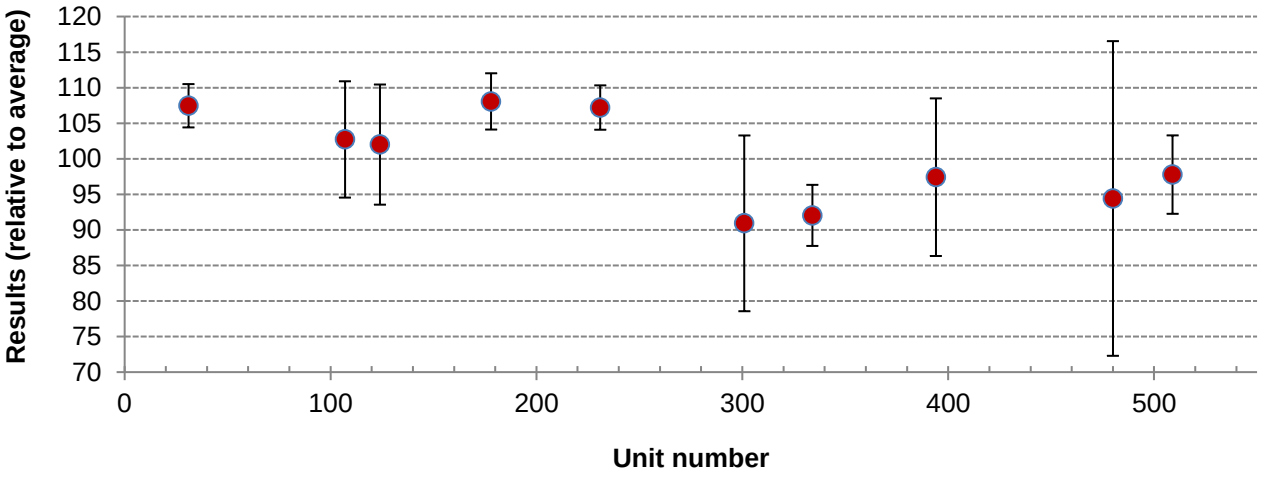


Figure A2.14. Between units homogeneity plot for Magnesium by BRML

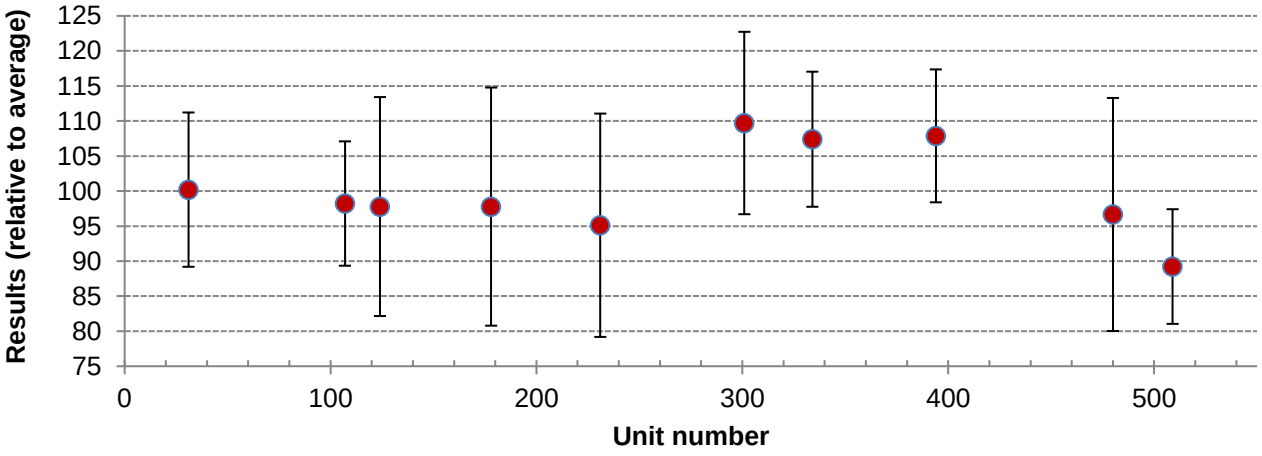


Figure A2.15. Between units homogeneity plot for Phosphorus by BRML

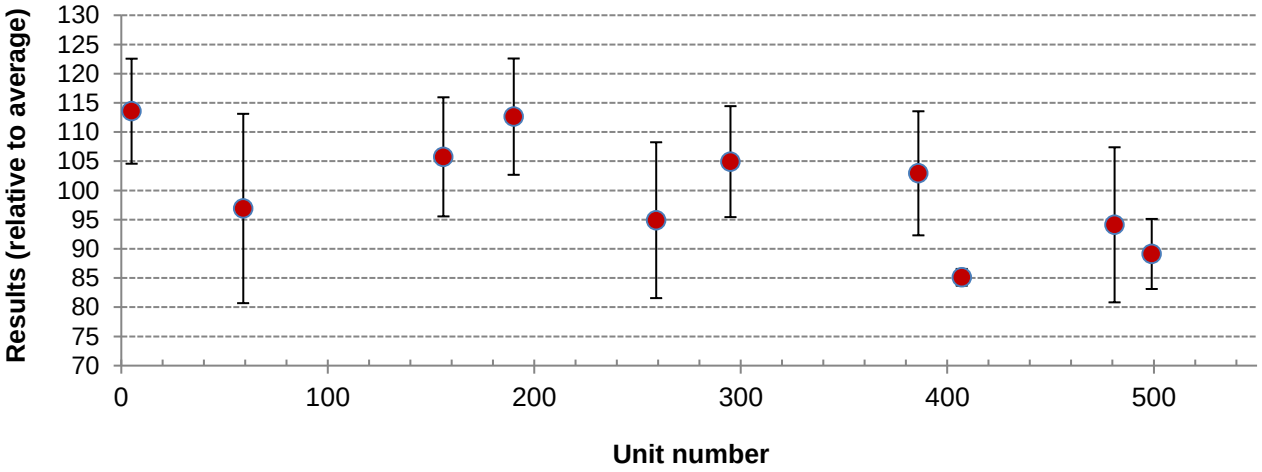


Figure A2.16. Between units homogeneity plot for Potassium by BRML

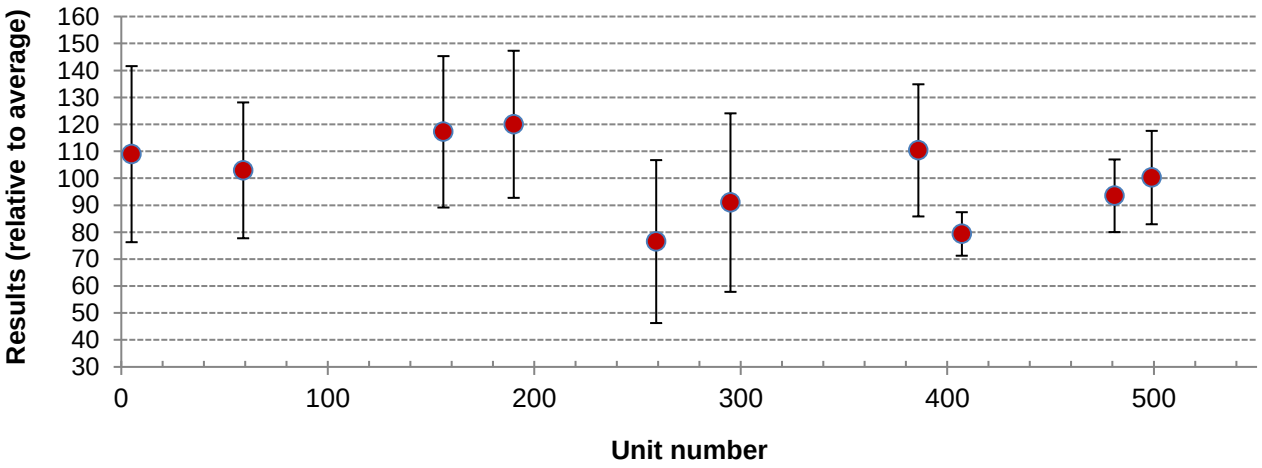


Figure A2.17. Between units homogeneity plot for Sodium by BRML

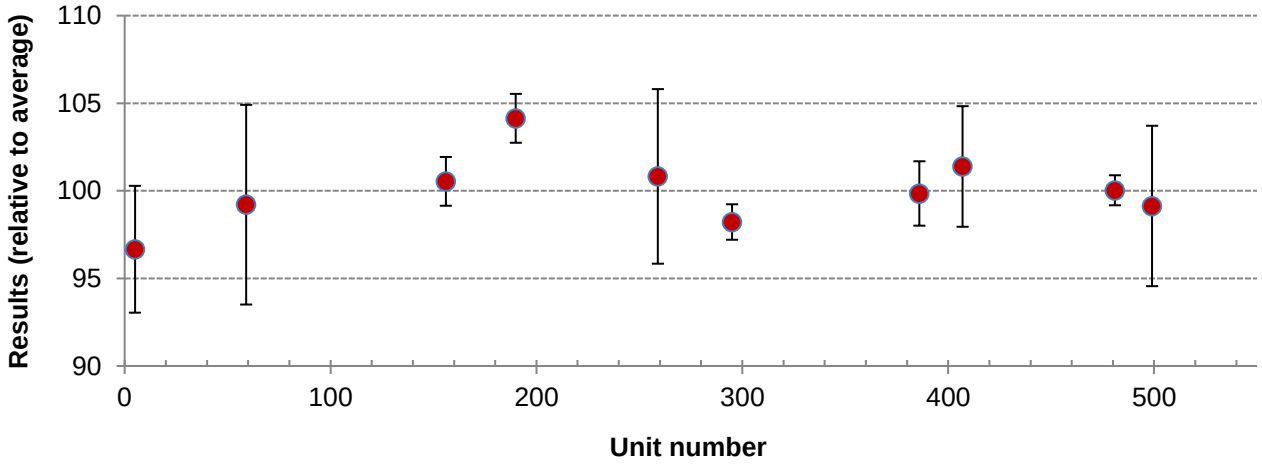


Figure A2.18. Between units homogeneity plot for Sulfur by BRML

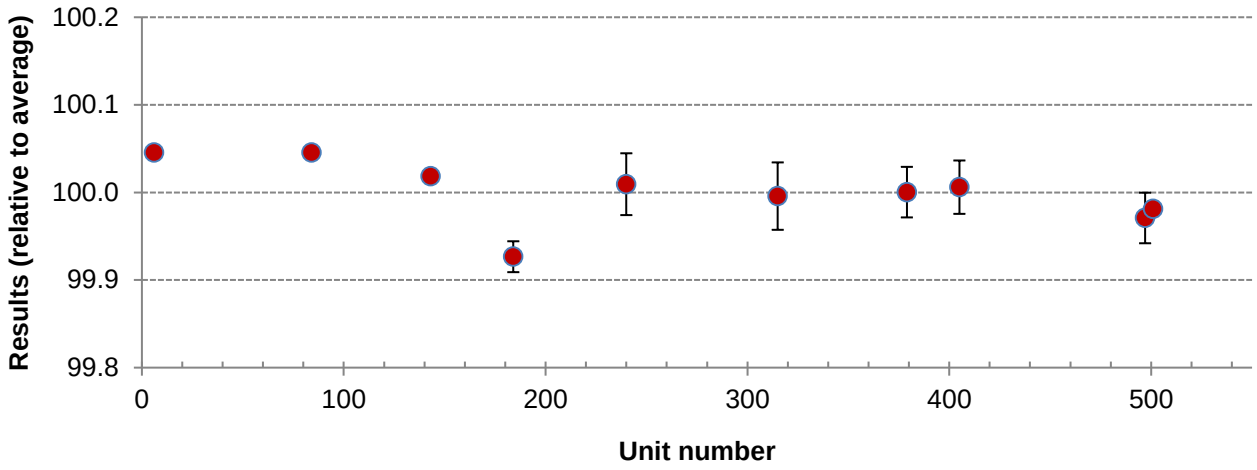


Figure A2.19. Between units homogeneity plot for Viscosity by PTB

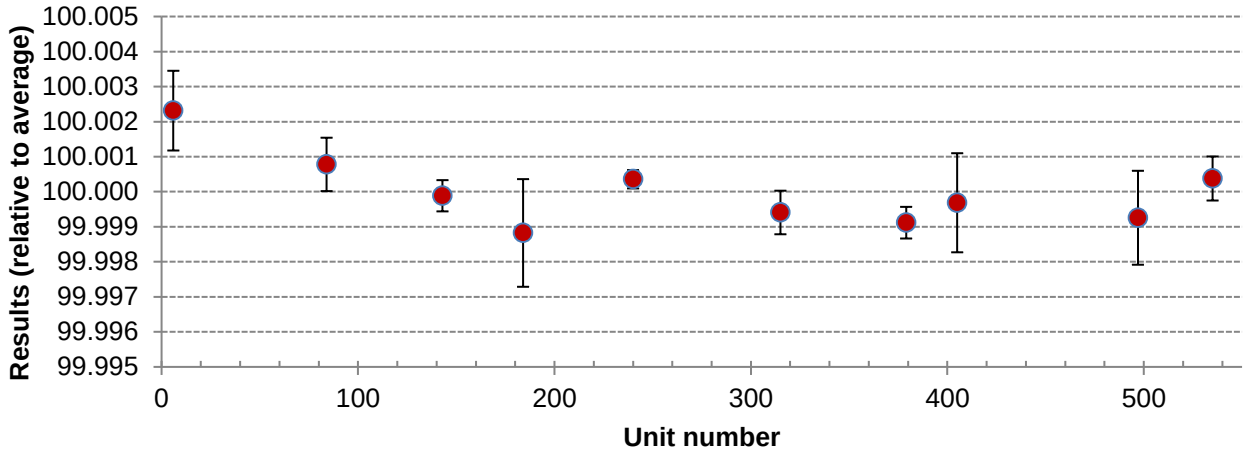


Figure A2.20. Between units homogeneity plot for Density by PTB

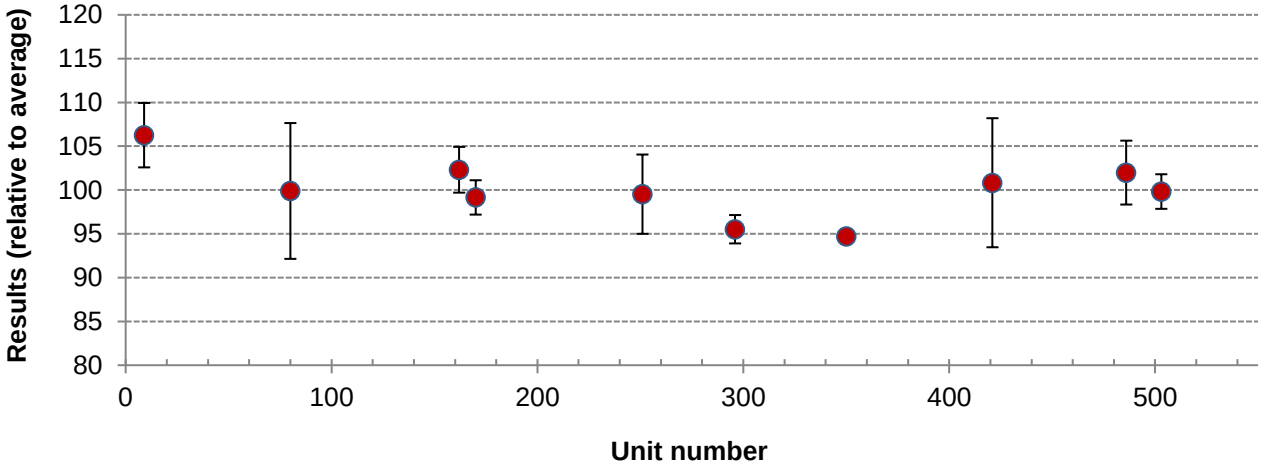


Figure A2.21. Between units homogeneity plot for Methanol by UME

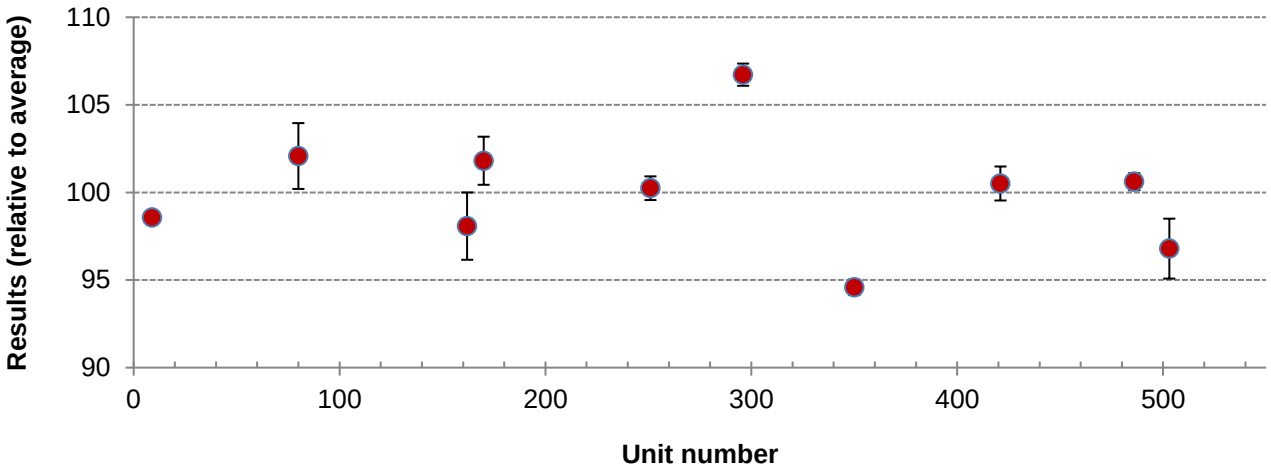


Figure A2.22. Between units homogeneity plot for Water by UME

ANNEX 3. Graphs for Short Term Stability Studies

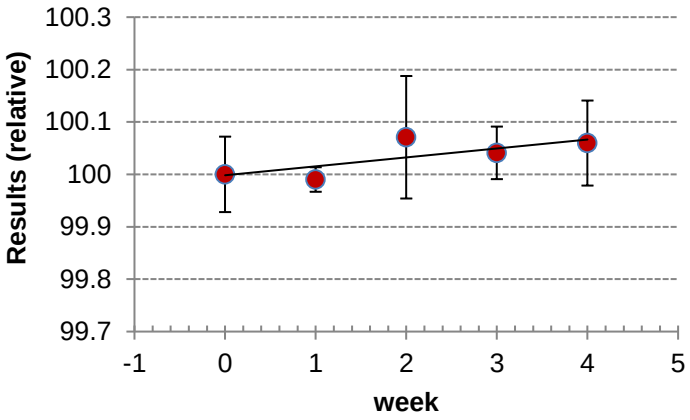


Figure A3.1. Short Term Stability Plot for Calorific Value at 45 °C by PTB

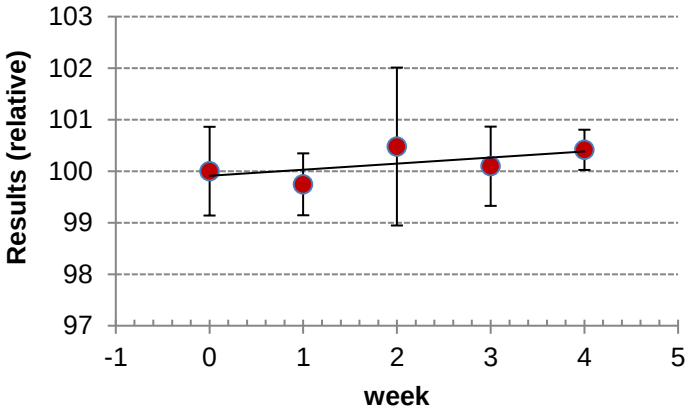


Figure A3.2. Short Term Stability Plot for Mono-Glyceride at 45 °C by BRML

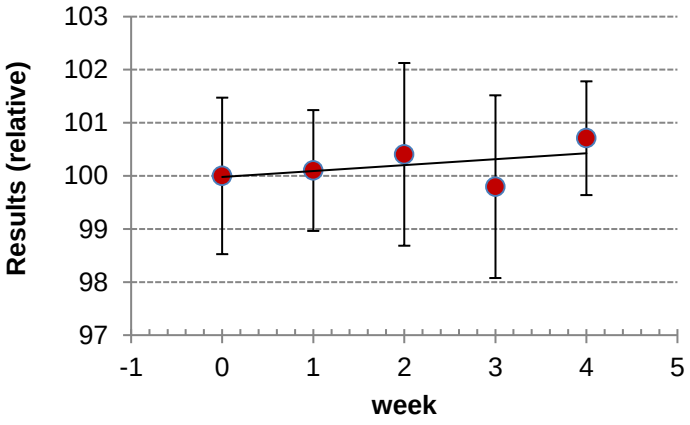


Figure A3.3. Short Term Stability Plot for Di-Glyceride at 45 °C by BRML

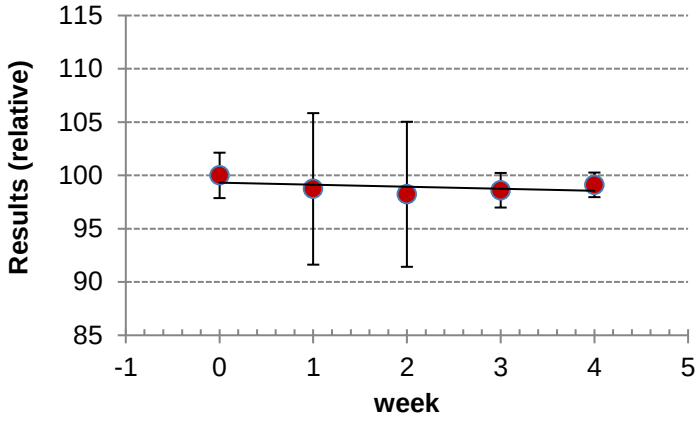


Figure A3.4. Short Term Stability Plot for Tri-Glyceride at 45 °C by BRML

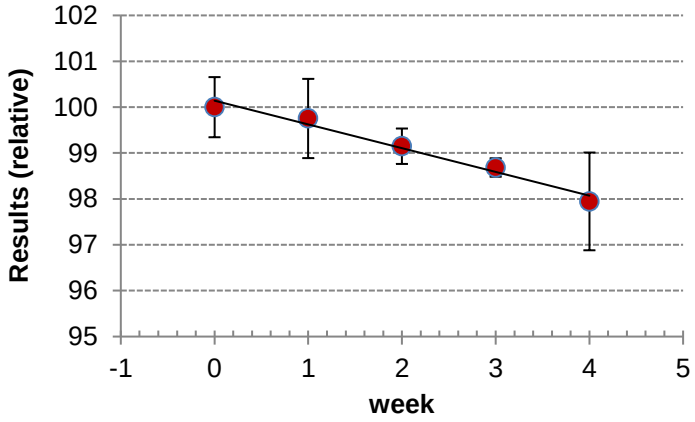


Figure A3.5. Short Term Stability Plot for Free Glycerol at 45 °C by BRML

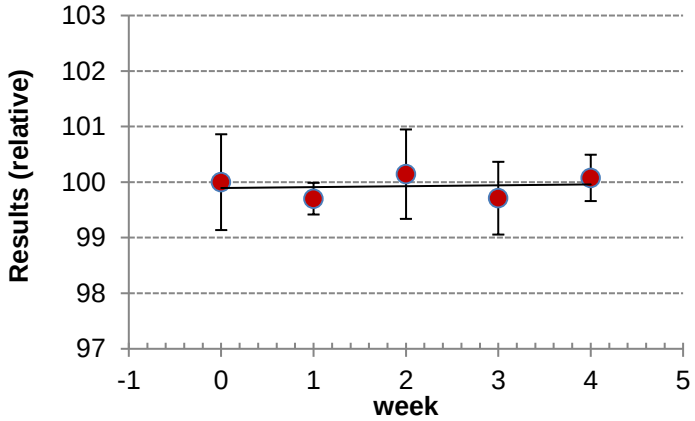


Figure A3.6. Short Term Stability Plot for Total Glycerol at 45 °C by BRML

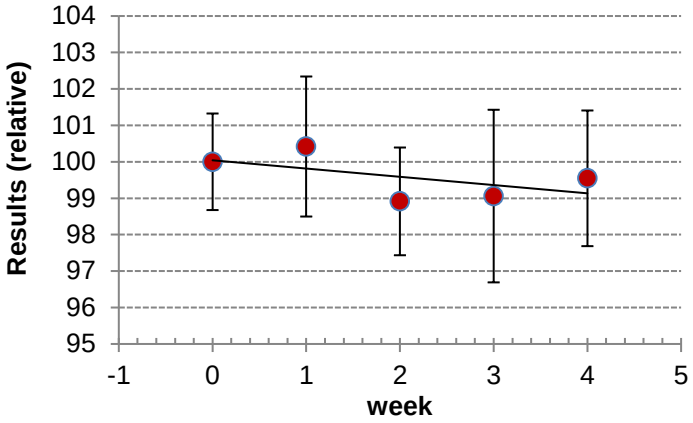


Figure A3.7. Short Term Stability Plot for Methyl Linoleate at 45 °C by IMBIH

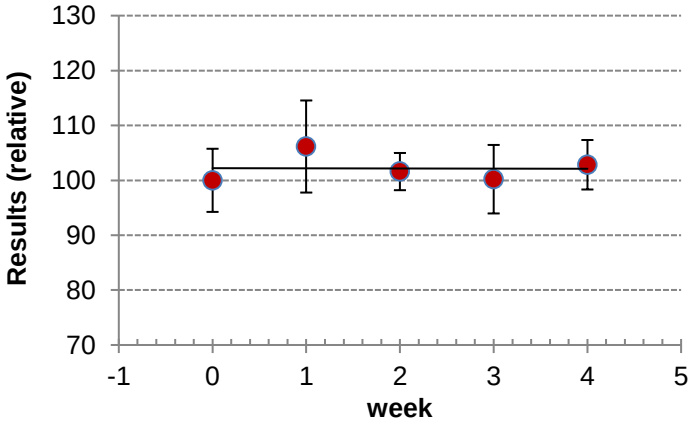


Figure A3.8. Short Term Stability Plot for Methyl Palmitoleate at 45 °C by IMBIH

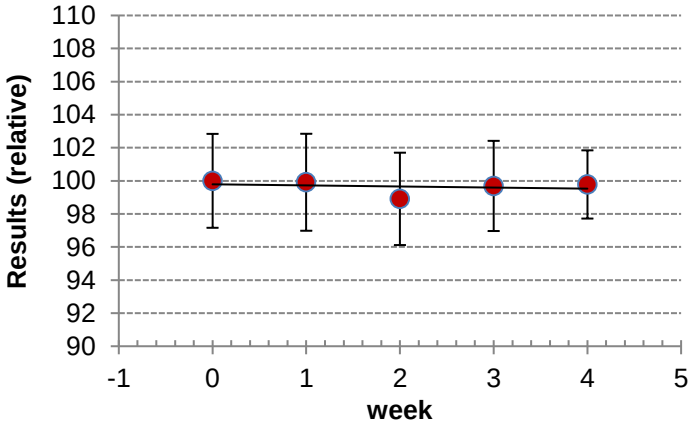


Figure A3.9. Short Term Stability Plot for Methyl Palmitate at 45 °C by IMBIH

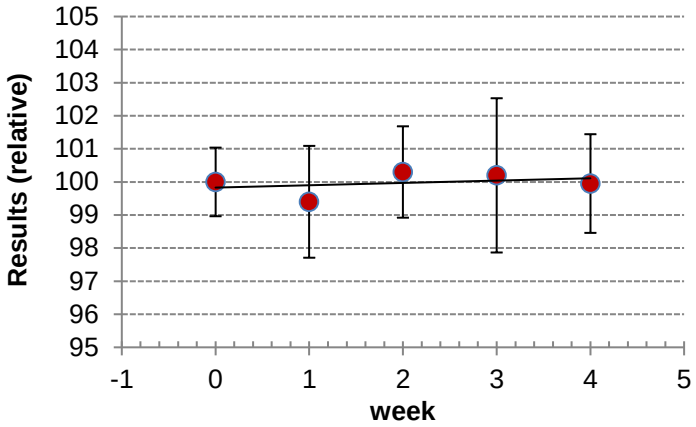


Figure A3.10. Short Term Stability Plot for Methyl 11-Octadecenoate at 45 °C by IMBIH

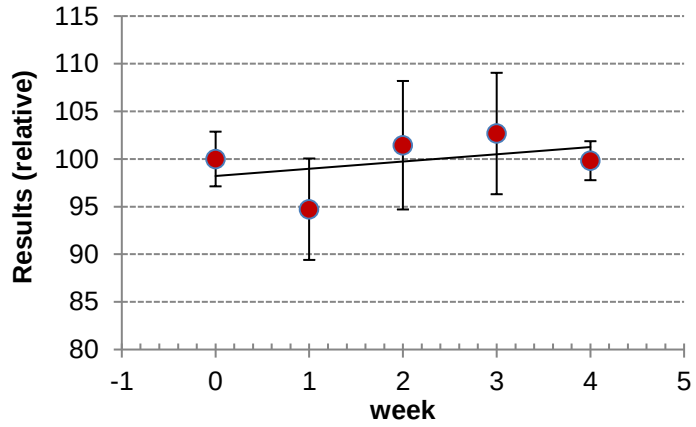


Figure A3.11. Short Term Stability Plot for Methyl Stearate at 45 °C by IMBIH

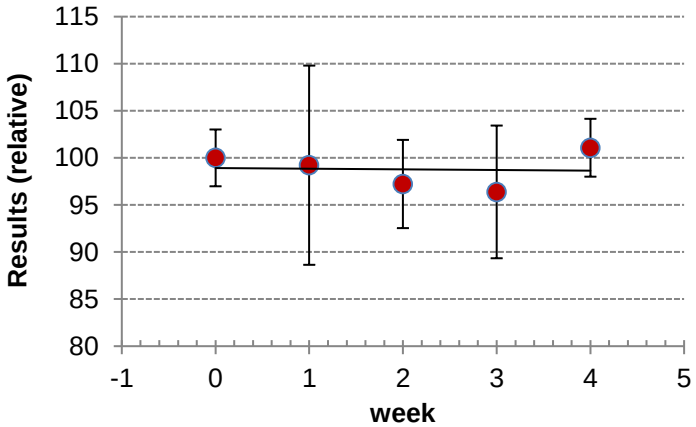


Figure A3.12. Short Term Stability Plot for Methyl cis-11-Eicosenoate at 45 °C by IMBIH

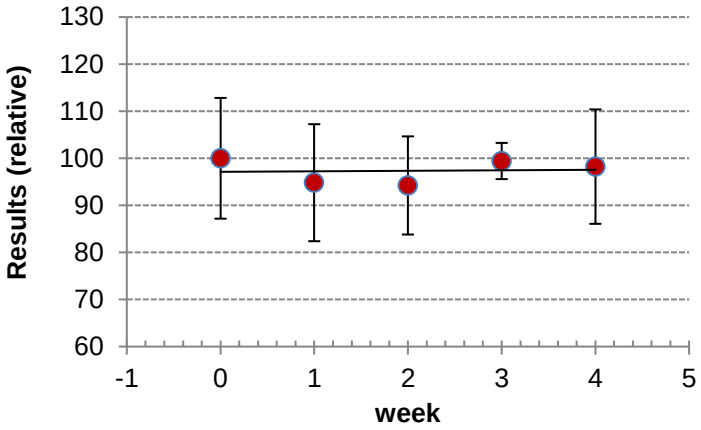


Figure A3.13. Short Term Stability Plot for Calcium at 45 °C by BRML

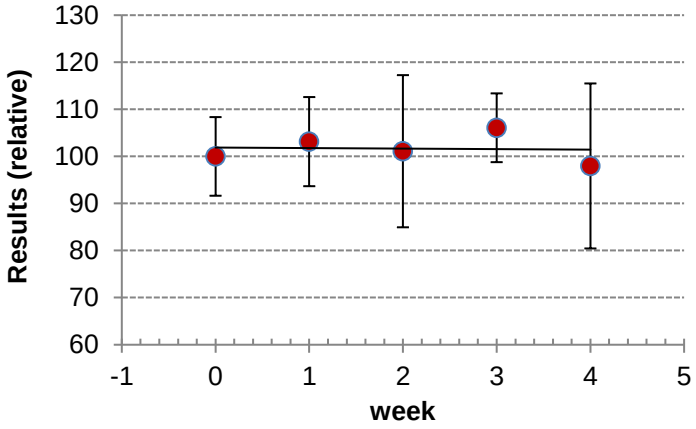


Figure A3.14. Short Term Stability Plot for Magnesium at 45 °C by BRML

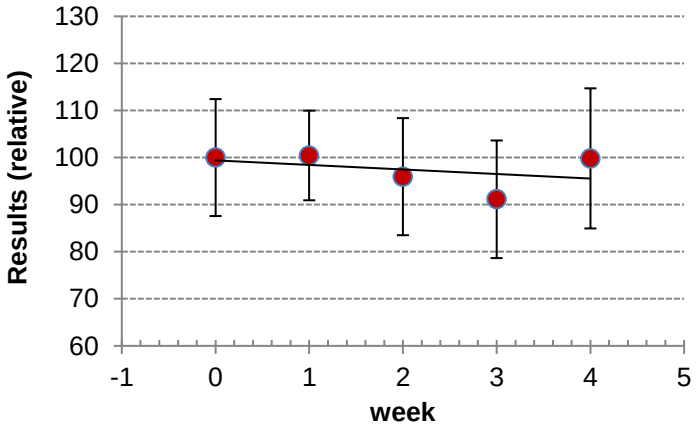


Figure A3.15. Short Term Stability Plot for Phosphorus at 45 °C by BRML

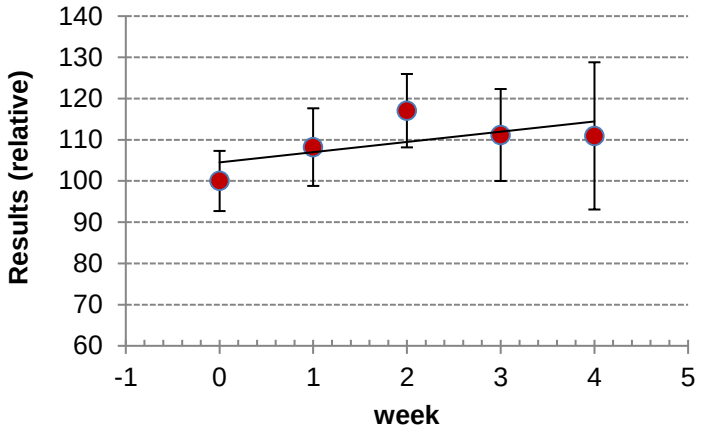


Figure A3.16. Short Term Stability Plot for Potassium at 45 °C by BRML

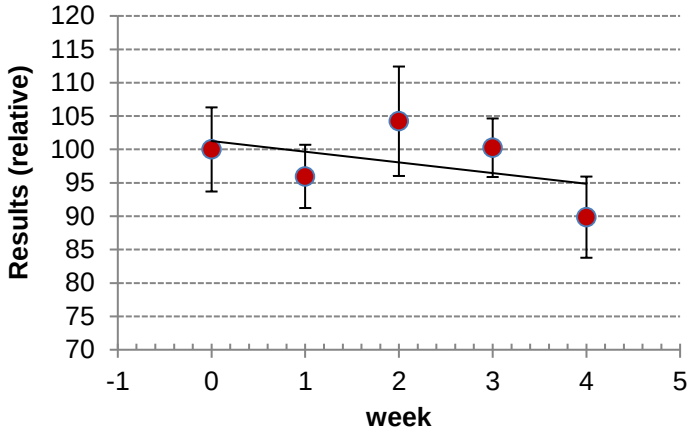


Figure A3.17. Short Term Stability Plot for Sodium at 45 °C by BRML

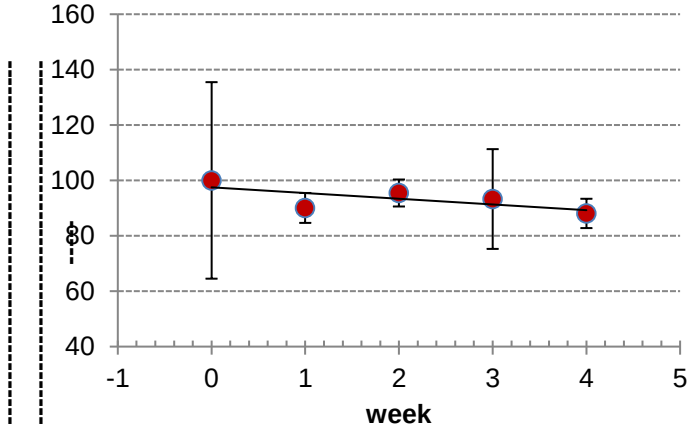


Figure A3.18. Short Term Stability Plot for Sulfur at 45 °C by BRML

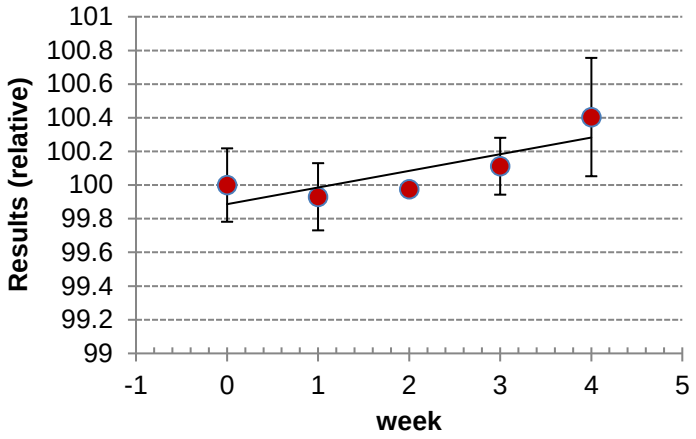


Figure A3.19. Short Term Stability Plot for Viscosity at 45 °C by PTB

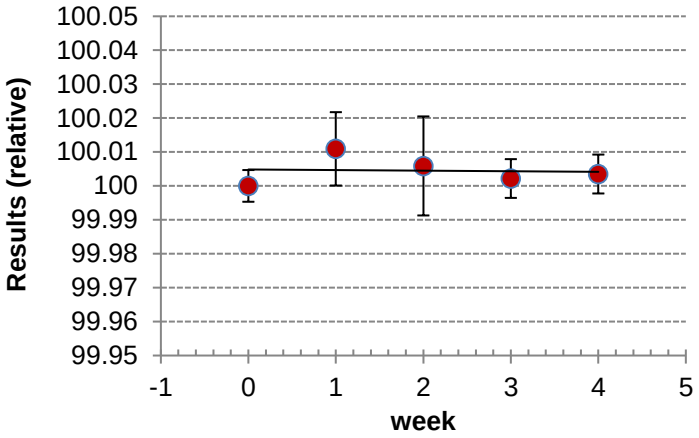


Figure A3.20. Short Term Stability Plot for Density at 45 °C by PTB

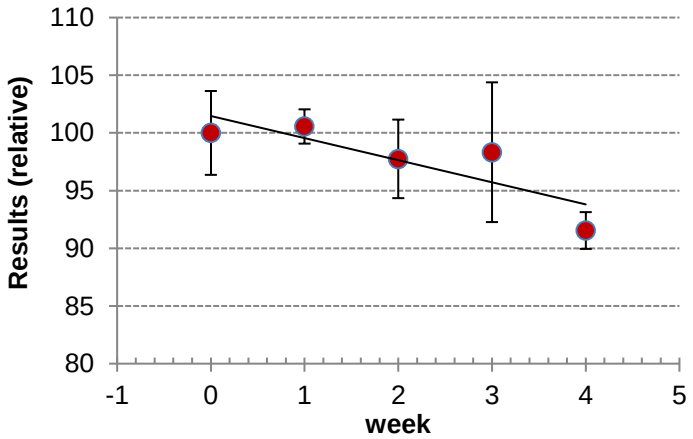


Figure A3.21. Short Term Stability Plot for Methanol at 45 °C by UME

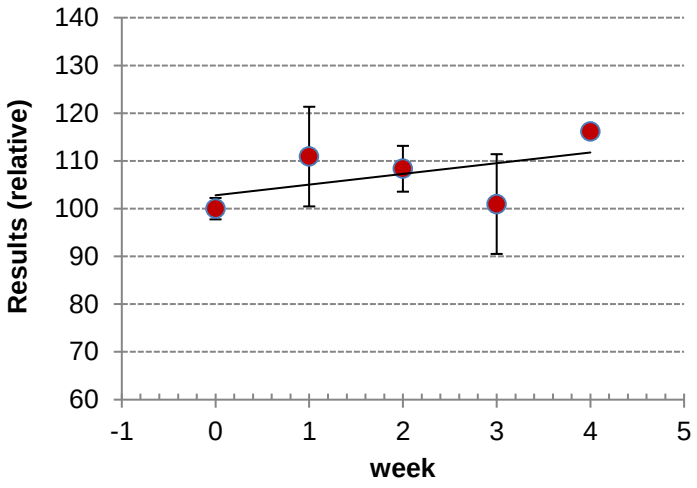


Figure A3.22. Short Term Stability Plot for Water at 45 °C by UME

ANNEX 4. Graphs for Long Term Stability Studies

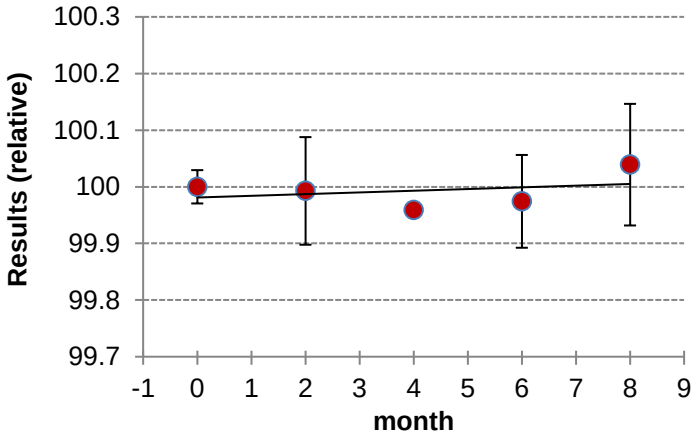


Figure A4.1. Long Term Stability Plot for Calorific Value at 22 °C by PTB

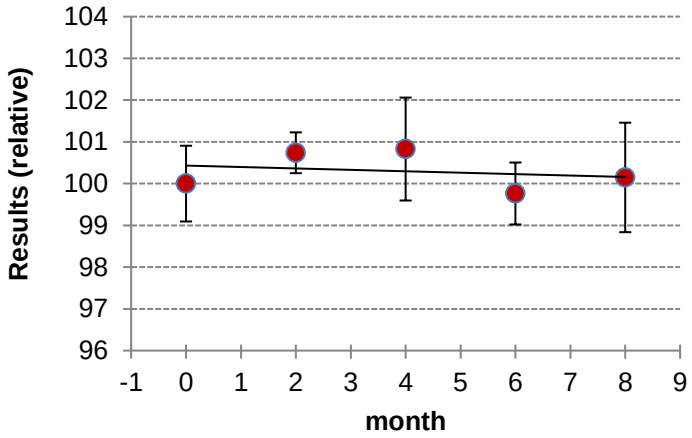


Figure A4.2. Long Term Stability Plot for Mono-Glyceride at 22 °C by BRML

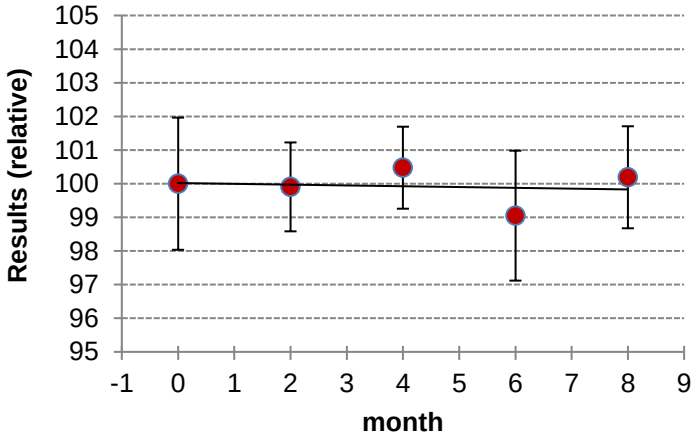


Figure A4.3. Long Term Stability Plot for Di-Glyceride at 22 °C by BRML

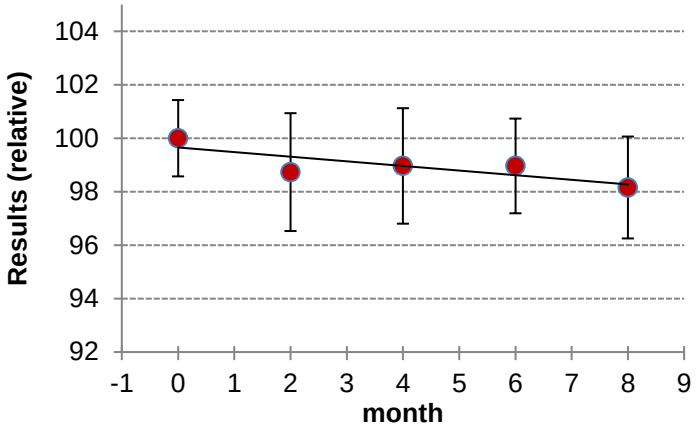


Figure A4.4. Long Term Stability Plot for Tri-Glyceride at 22 °C by BRML

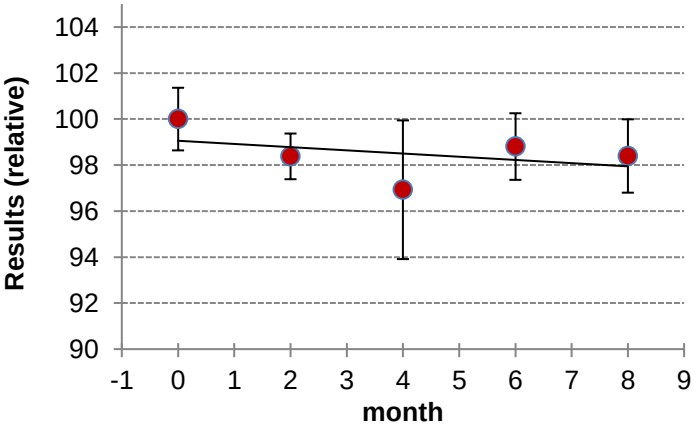


Figure A4.5. Long Term Stability Plot for Free Glycerol at 22 °C by BRML

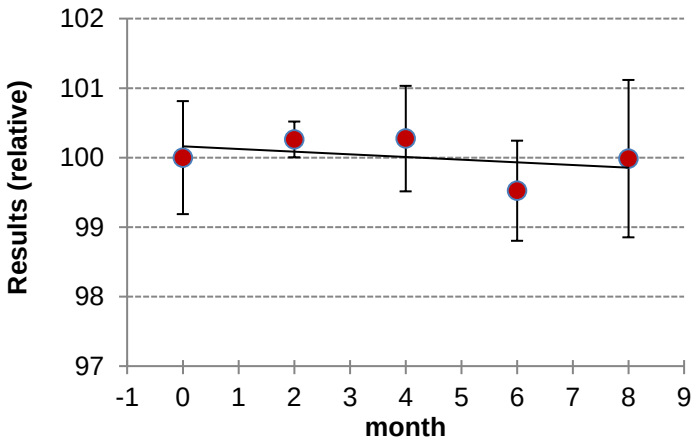


Figure A4.6. Long Term Stability Plot for Total Glycerol at 22 °C by BRML

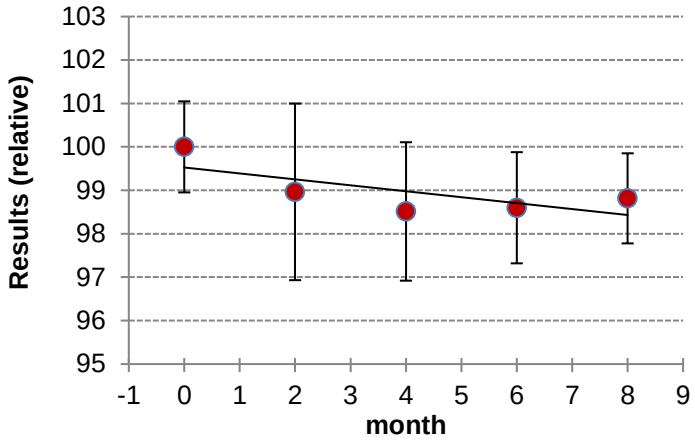


Figure A4.7. Long Term Stability Plot for Methyl Linoleate at 22 °C by IMBIH

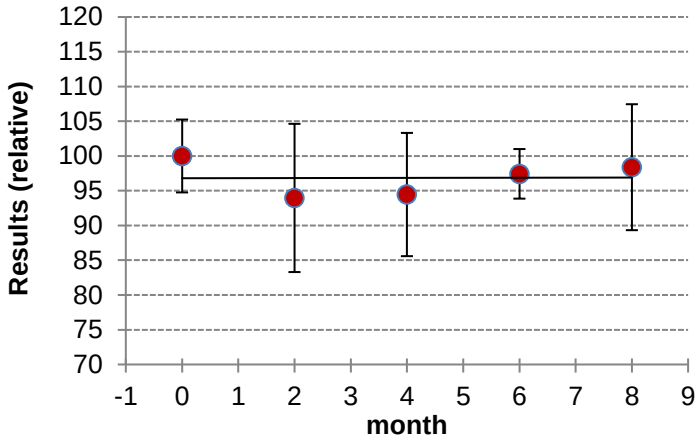


Figure A4.8. Long Term Stability Plot for Methyl Palmitoleate at 22 °C by IMBIH

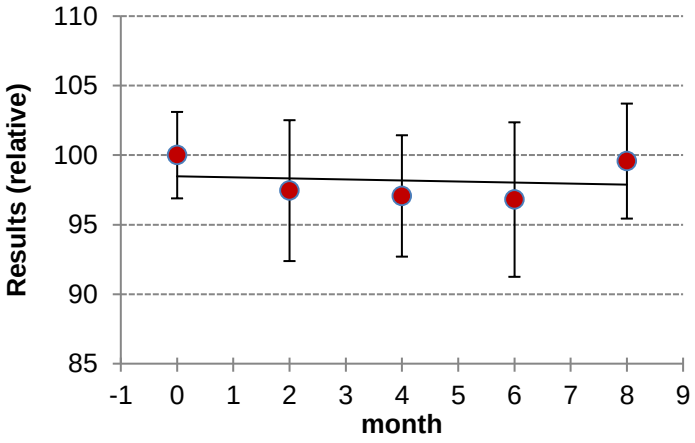


Figure A4.9. Long Term Stability Plot for Methyl Palmitate at 22 °C by IMBIH

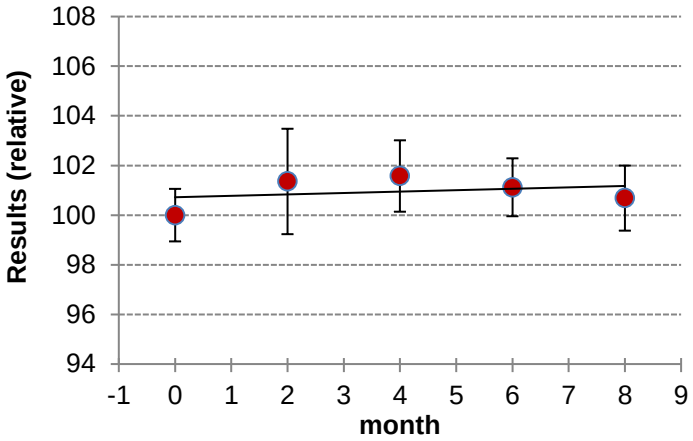


Figure A4.10. Long Term Stability Plot for Methyl 11-Octadecenoate at 22 °C by IMBIH

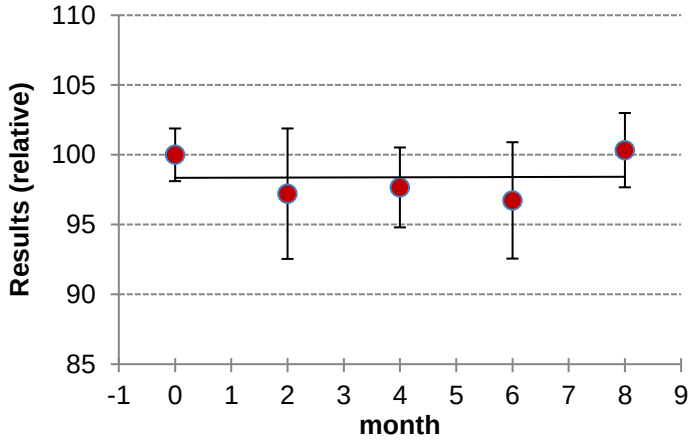


Figure A4.11. Long Term Stability Plot for Methyl Stearate at 22 °C by IMBIH

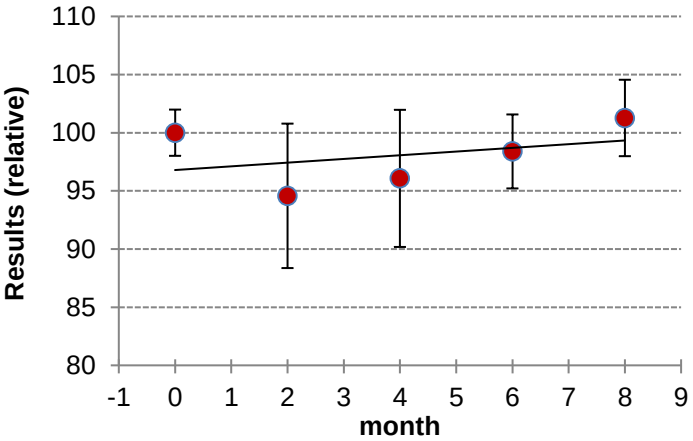


Figure A4.12. Long Term Stability Plot for Methyl cis-11-Eicosenoate at 22 °C by IMBIH

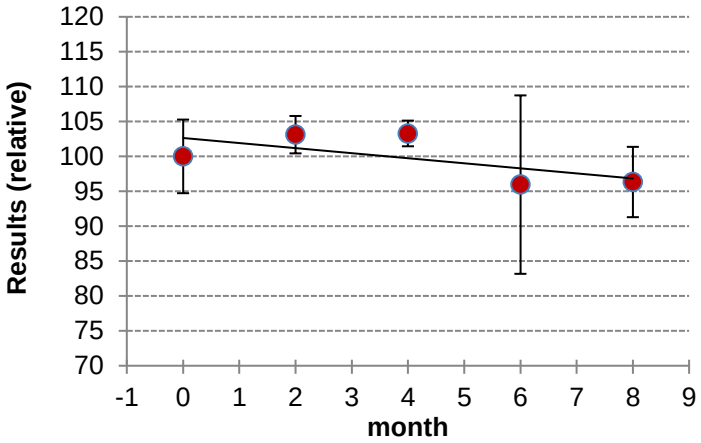


Figure A4.13. Long Term Stability Plot for Calcium at 22 °C by BRML

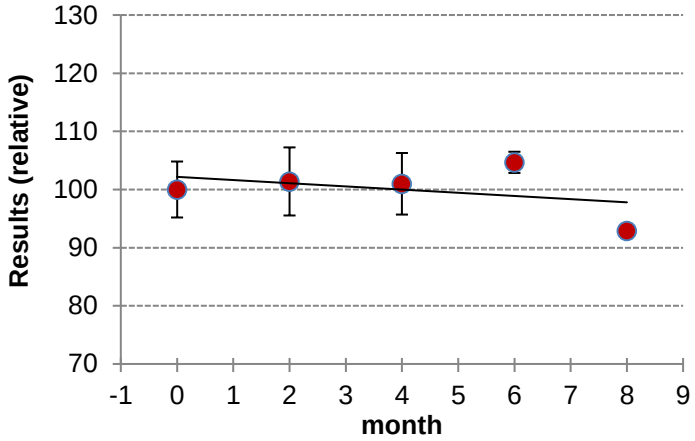


Figure A4.14. Long Term Stability Plot for Magnesium at 22 °C by BRML

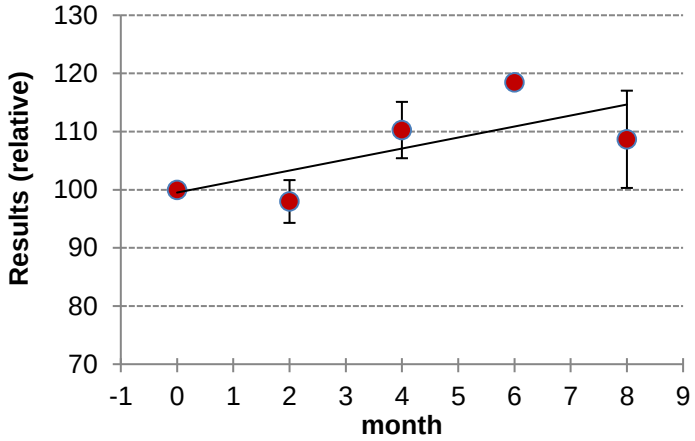


Figure A4.15. Long Term Stability Plot for Phosphorus at 22 °C by BRML

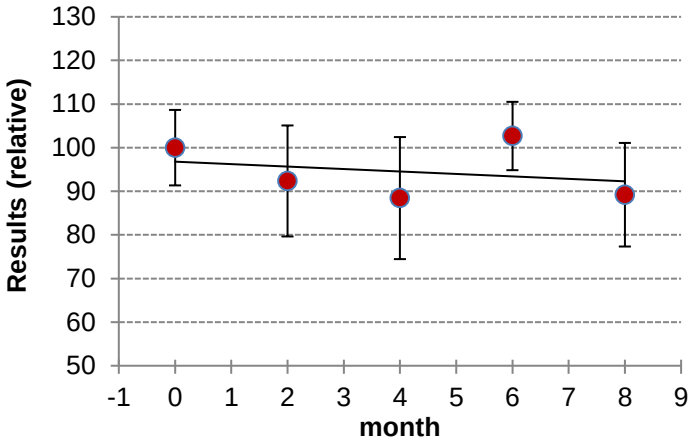


Figure A4.16. Long Term Stability Plot for Potassium at 22 °C by BRML

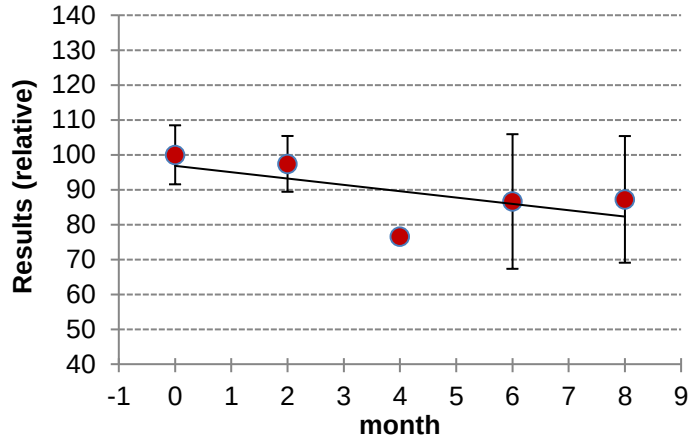


Figure A4.17. Long Term Stability Plot for Sodium at 22 °C by BRML

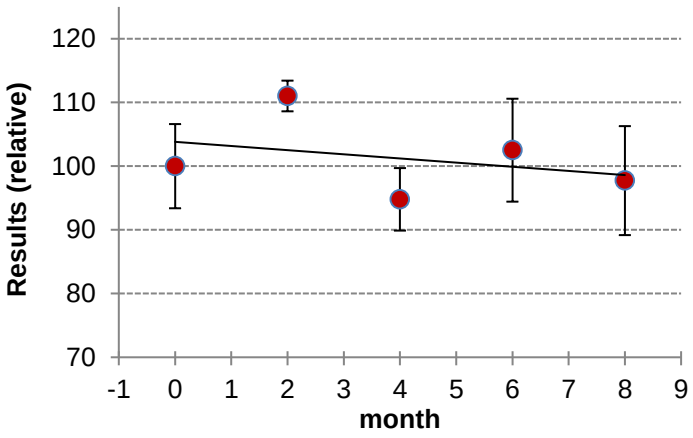


Figure A4.18. Long Term Stability Plot for Sulfur at 22 °C by BRML

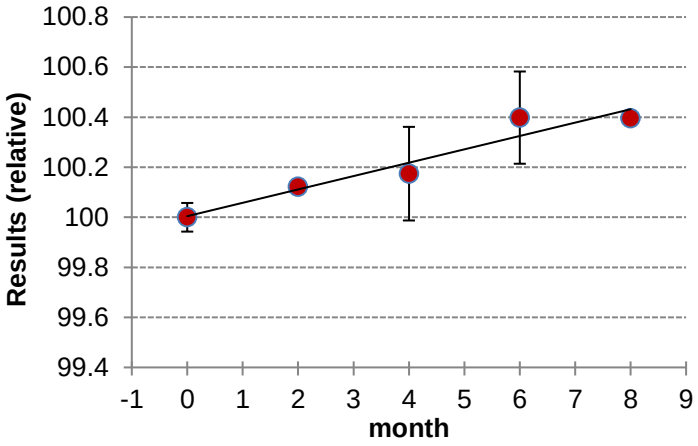


Figure A4.19. Long Term Stability Plot for Viscosity at 22 °C by PTB

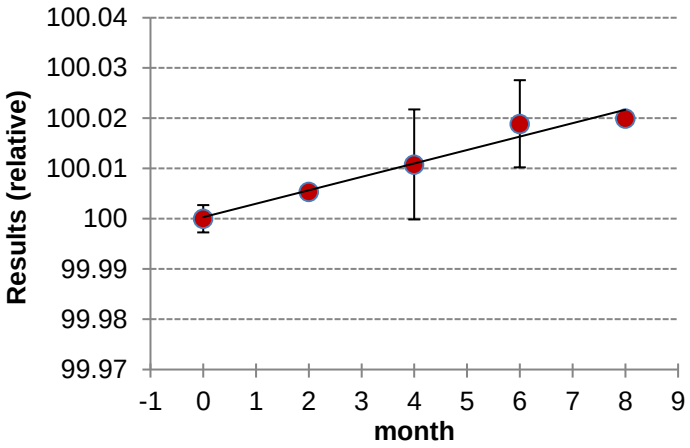


Figure A4.20. Long Term Stability Plot for Density at 22 °C by PTB

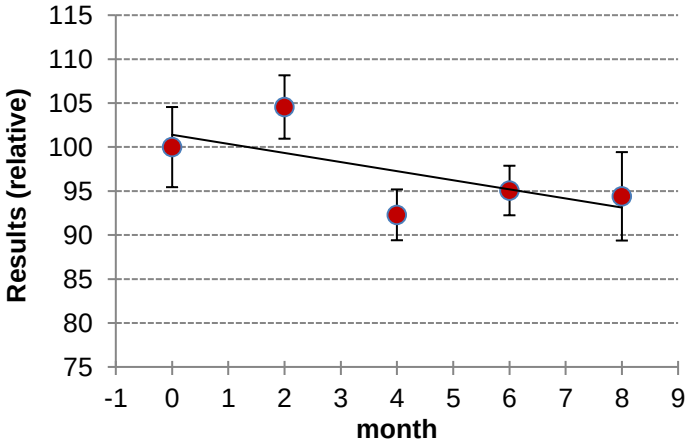


Figure A4.21. Long Term Stability Plot for Methanol at 22 °C by UME

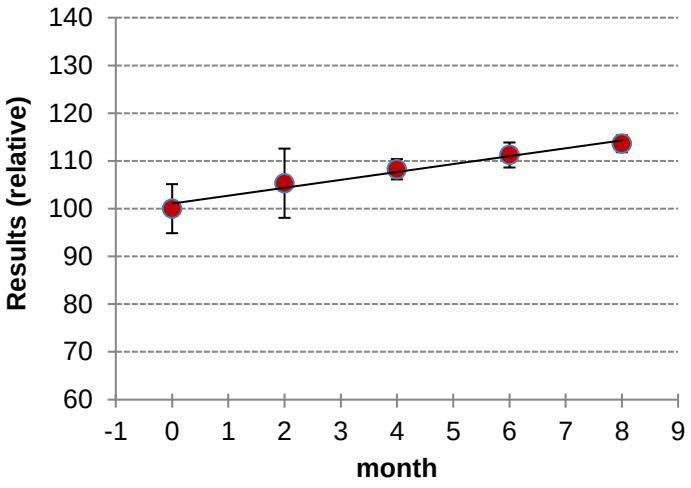


Figure A4.22. Long Term Stability Plot for Water at 22 °C by BRML

ANNEX 5. Information about the Methods Used for the Characterization Study

Lab	Parameter(s)	Sample Preparation	Calibration Strategy	Method/ Technique	CRM(s) used for Calibration and Quality Control
BAM	Sulfur	Decomposition: Closed digestion with HNO_3 (5 mL) and H_2O_2 (1 mL) using a high pressure asher with T_{max} ≈ 300°C and P_{max} ≈ 130 bar Separation: Ion exchange chromatography with AG 1X8 resin filled in Eichrom columns, sample loading with dilute HNO_3 (0.028 mol/L), elution of matrix with water, and elution of S with HNO_3 (0.25 mol/L)	IDMS with inhouse calibrated ^{34}S-spike, as backspike NIST SRM 3181 was used to establish SI traceability; isotopes measured: ^{32}S & ^{34}S, Ratio: ^{32}S/^{34}S	Isotope dilution mass spectrometry	Inhouse calibrated ^{34}S-spike NIST SRM 3181 ERM-EF213 Sulfur in petrol
BRML	Calorific Value	No pretreatment	According to international standard ASTM D240-19	Isoperibol Oxygen Bomb Calorimetry	Standard Reference Material 39j Benzoic Acid (NIST)
	Calcium Magnesium Phosphorus Potassium Sodium Sulfur	500 mg of homogenized sample is mixed with 3 mL H_2O_2 30%, 8 mL HNO_3 65 % and 1 mL HF 40% in a closed Teflon digestion container. The mixture is allowed to react for 5 minutes before closing the container. The heating was done using a microwave digestion system, according to the following temperature program: heating for 15 minutes to 190 °C; holding for 20 minutes at 190 °C. After cooling to the room temperature, HF is neutralized by addition of 10 mL H_3BO_3 4 %. After neutralization, the samples are re-digested in the microwave according to the program: heating for 15 minutes to 150 °C; holding for 20 minutes at 150 °C. After cooling down to the room temperature, the digest is transferred into a 50 mL volumetric flask by gravimetric filtration.	5 point external calibration	ICP-MS with dynamic reaction cell	Multi-element ICP-MS Calibration Std. 3, 10µL/mL, Al, As, Ba, Be, Ci, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, k, Li, Mg, Mn, Ni, Pb, Rb, Se, Na, Ag, Sr, Ti, V, U, Zn, 5% HNO3, Merck, Germany; -ICP Multi-element standard solution IV, 1000 mg/L Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn 6.5% HNO3, Merck, Germany; -Multi-element calibration standard 5, 10µL/mL B, Ge, Mo, Nb, P, Re, S, Si, Ta, Ti, W, Zr H2O / 0.2% HF / Tr. HNO3, PerkinElmer, United States.
	-Mono-Glycerides -Di-Glycerides -Tri-Glycerides	Weigh approximately 100 mg of the homogenized sample into a 10 mL volumetric flask. Add 80 µL of the 1,2,4-butanetriol stock solution, 200 µL of the internal standard stock solution for glycerides, 200 µL of pyridine and 200 µL of MSTFA. Contact with moisture must be avoided. Close the volumetric flask hermetically and shake vigorously. Keep the mixture for 15 min. at room temperature, then make up to the mark with n-heptane. 1 µL of the reaction mixture is analyzed by gas chromatography	Single point calibration by using internal standards of glycerides	GC-FID EN 14105	For mono-, di-, and triglycerides, it is considered that, within the concentration range, the response of the detector is linear For each analysis, the relative RRF response factor of dinonadecanoate (Di C38) is evaluated according to trinonadecanoate (Tri C57) RRF<1.8

Lab	Parameter(s)	Sample Preparation	Calibration Strategy	Method/ Technique	CRM(s) used for Calibration and Quality Control
BRML	Free Glycerol Total Glycerol	Free glycerol from biodiesel is transformed into a more volatile and stable silyl derivative in the presence of pyridine and N-methyl-N-trimethylsilylfluoroa-cetamide (MSTFA). After silanization, the samples are analyzed by gas chromatography on a short capillary column with a low stationary phase deposition, with the introduction of the sample directly into the capillary column (on-column) and the detection of the compound with a flame ionization detector (FID). The quantitative determination of free glycerin is carried out in the presence of the internal standard 1,2,4-butanetriol (ISBT).	4 point external calibration	GC-FID EN 14105	For calibration: Glycerol, analytical standard,and 1,2,4-butanetriol For Quality Control: CRM4 4892 Glycerin
	Water	Measurement was done in accordance with ISO 12937:2000, ASTM240-19,EN14105/2011,EN14214/2014. Cou-Lo Formula "A" Coulometric Anode Solution, Cou-Lo Formula "C" Coulometric Cathode Solution for the diaphragm titration cell of the Karl Fischer coulometric titrator were used	N/A	Coulometric Karl Fischer Titrimetry	MRC Aquastar Water Standard 0.01 % for the intermediate control of the titrator. SRM 2890 NIST USA was used for verification of measurements.
DTI	Calorific Value	No pretreatment	Specific heat capacity of the calorimeter is determined using benzoic acid reference material.	Isoperibol oxygen bomb calorimetry	Benzoic acid IKA C723, ID nr 32 430 00 EU index 607 705 – 00-8
GUM	Sodium Potassium Magnesium	An amount of 0,5 g of the sample was weighted directly in the mineralization PFTE vessel. Then 6 mL of HNO_3 and 2,5 mL H_2O_2 were added gradually to avoid sample losses. After around 1 h vessel was capped (vessel was covered by a watch glass before) and then sample was mineralised by Anton Paar Multiwave 3000 (programme for 4 vessels: (1) ramp 525 W, 20 min; (2) hold 525 W, 40 min; (3) cooling until 40 °C reached. After mineralisation sample was quantitatively transferred into the 50 mL vessel, diluted with high-purity deionized water to 50 mL and weighted.	Calibration curve with internal standardization, the following ratios were measured $^{23}Na/^{45}Sc$, $^{24}Mg/^{45}Sc$, $^{39}K/^{45}Sc$	ICP-MS with collision gas (He) mode	Monoelemental aqueous solutions provided by Slovak Institute of Metrology: sodium SMU B23, potassium SMU B18, magnesium SMU B20. Samples were spiked with the known amount of mixed standard to determine recovery of the added element. Quality Control samples were measured to control the instrument drift.

Lab	Parameter(s)	Sample Preparation	Calibration Strategy	Method/ Technique	CRM(s) used for Calibration and Quality Control
IMBIH	-Methyl Linoleate -Methyl Palmitoleate -Methyl Palmitate -Methyl 11-Octadecenoate -Methyl Stearate -Methyl cis-11-Eicosenoate	Samples were diluted in 2 steps in pure n-hexane (HPLC/GC grade) to achieve readable concentrations of corresponding compounds step 1 - 30 µL of biodiesel sample in 3,5 mL of n-hexane step 2 - 30 µL of diluted sample from step1 in 1 mL of n-hexane	Individual peaks of chemical components biodiesel samples were identified by comparison of their retention indices (RI) with those of authentic compounds or literature data and computer matching between samples mass spectra and mass spectra from spectrometer database libraries (Wiley7NIST05 and NIST14).	GC/MS	-
LGC	Density at 15 °C	No pretreatment	Calibrated Instrument for the range of measurement is used	ASTM D4052 Density Meter	Paragon Scientific D1480 Certified sample N8.
	Kinematic Viscosity at 40 °C	No pretreatment	Calibrated Instrument for the range of measurement is used	ASTM D7042 Stabinger Viscometer	Paragon Scientific D1480 Certified sample N8.
PTB	Calorific Value	No pretreatment	Specific heat capacity of the calorimeter is determined using benzoic acid reference material.	Isoperibol oxygen bomb calorimetry	UME CRM 1504 Benzoic Acid
	Density at 15 °C	No pretreatment	Calibrated instrument is used	Oscillation-type densimeter	High Purity Water
	Kinematic Viscosity at 40 °C	No pretreatment	Calibrated instrument is used	Stabinger Viscosimeter	-

Lab	Parameter(s)	Sample Preparation	Calibration Strategy	Method/ Technique	CRM(s) used for Calibration and Quality Control
UME	Calorific Value	No pretreatment	Specific heat capacity of the calorimeter is determined using benzoic acid reference material.	Isoperibol oxygen bomb calorimetry	UME CRM 1504 was used as certified reference material (benzoic acid) in instrument calibration
	Calcium Magnesium Phosphorus Potassium Sodium	1 mL sample was diluted with 2 mL ICP solvent (Conostan) All solutions were prepared by weighing	Standard addition calibration, Conostan 15-100-115 for Na, 15-100-125 for Mg, 15-100-155 for P, 15-100-195 for K, 15-100-205 for Ca were used to establish SI traceability; Wavelengths measured: Na 589.592, Na 588.995, Mg 279.553, Mg 280.270, P 177.495, P 178.287, K 766.491, Ca 396.847, Ca 315.887, Ca 422.673, Ca 396.847	ICP-OES	Conostan 15-100-115 for Na, 15-100-125 for Mg, 15-100-155 for P, 15-100-195 for K, 15-100-205 for Ca, NMIJ 8302a Biodiesel Fuel
	Magnesium Phosphorus Sodium	0,6 mL sample was digested with 4 mL HNO_3 (Suprapur, Merck) The sample was mineralised by microwave digestion system. Temperature programme : (1) ramp 25 min. up to 150 °C; (2) hold 30 min at 150 °C . After mineralisation sample was transferred into the 50 mL PP vessel, diluted with high-purity deionized water up to 50 mL. All solutions were prepared by weighing	Standard addition calibration, NIST SRM 3152a for Na, NIST SRM 3131a for Mg, NIST SRM 3139a for P were used to establish SI Isotopes measured: 23Na, 24Mg, 31P	HR ICP-MS	, NIST SRM 3152a for Na, NIST SRM 3131a for Mg, NIST SRM 3139a for P, NMIJ 8302a Biodiesel Fuel
	Sulfur	99.26 % ^{34}S enriched material (ISOFLEX) was dissolved in ICP solvent (Conostan) ^{34}S spiked solution: 65 mg/kg sulfur Sample blend : 300 µL sample was mixed with 70 µL ^{34}Spiked solution Calibration blend 1 : 300 µL NIST SRM 1616b was mixed with 50 µL ^{34}Spiked solution Calibration blend 2 : 300 µL NIST SRM 1616b was mixed with 80 µL ^{34}Spiked solution The blend solutions were diluted with ICP solvent up to 1,8 mL. All solutions were prepared by weighing	IDMS with in-house ^{34}S-spike, NIST SRM 1616b was used to establish SI traceability; Isotopes measured: 32S & 34S, Ratio: 32S/34S	ID-ICP MS	NIST SRM 1616b NMIJ 8302a Biodiesel Fuel

Lab	Parameter(s)	Sample Preparation	Calibration Strategy	Method/ Technique	CRM(s) used for Calibration and Quality Control
UME	Sulfur	0,6 mL sample was digested with 4 mL HNO_3 (Suprapur, Merck). The sample was mineralized by microwave digestion system. Temperature programme: (1) ramp 25 min. up to 150 °C; (2) hold 30 min at 150 °C . After mineralization sample was transferred into the 50 mL PP vessel, diluted with high-purity deionized water up to 50 mL. All solutions were prepared by weighing	Standard addition calibration, NIST SRM 3181 was used to establish SI traceability Isotopes measured: : 32S & 34S	HR ICP-MS	NIST SRM 3181 NMIJ 8302a Biodiesel Fuel
	Sulfur	1 mL sample was diluted with 2 mL ICP solvent (Conostan). All solutions were prepared by weighing	Standard addition calibration, NIST SRM 1616b was used to establish SI traceablity; wavelenghts measured: S180.731 & S182.034	ICP OES	NIST SRM 1616b NMIJ 8302a Biodiesel Fuel
	Methanol	980 µL biodiesel sample and 20 µL IS was mixed to the headspace sample bottle.	3 point internal standard calibration. 2-propanol solution was prepared as an internal standard.	Headspace GC-FID	-
	Water	Approximately 2.5-3.0 mL sample is weighed and sealed. Oven is adjusted to 140 °C. Sample is heated for 7 min. Hydranal® 34836 - Coulomat Ag Reagent For Coulometric Kf Titration (Anolyte Solution) was used in the cell.	N/A	COU- o-KFT	NIST 2890 Water Saturated Octanol

ANNEX 6. Graphs for Characterization Study

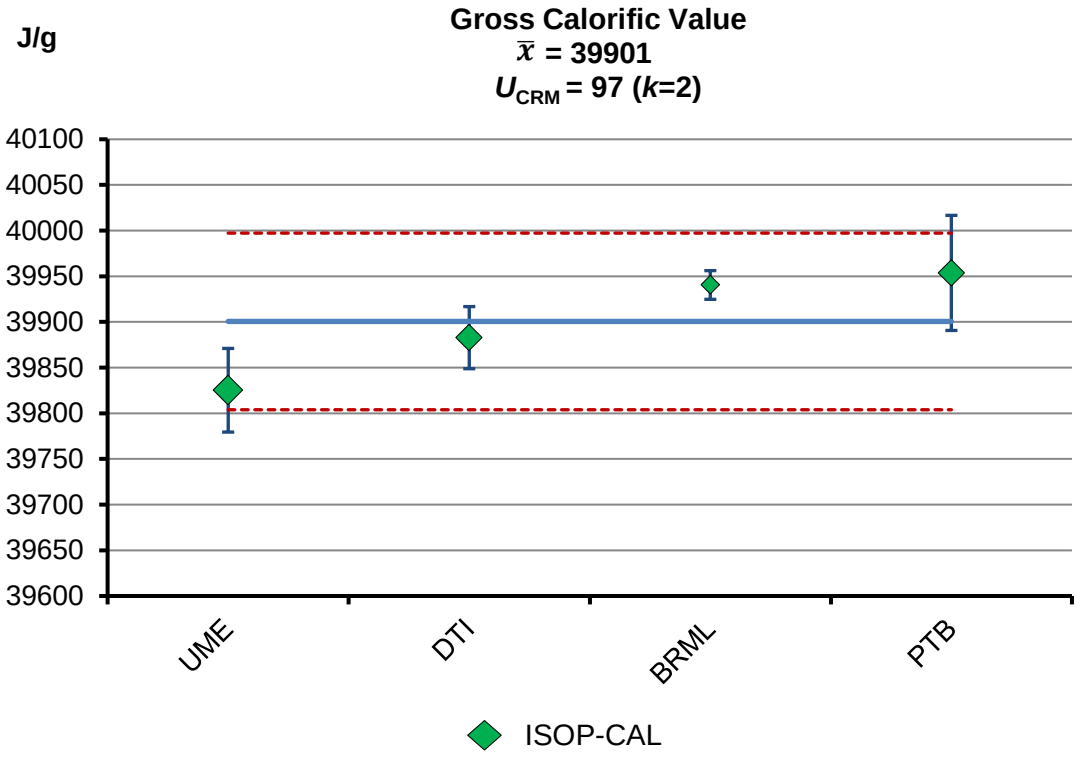


Figure A6.1. Characterization Study Plot for Calorific Value

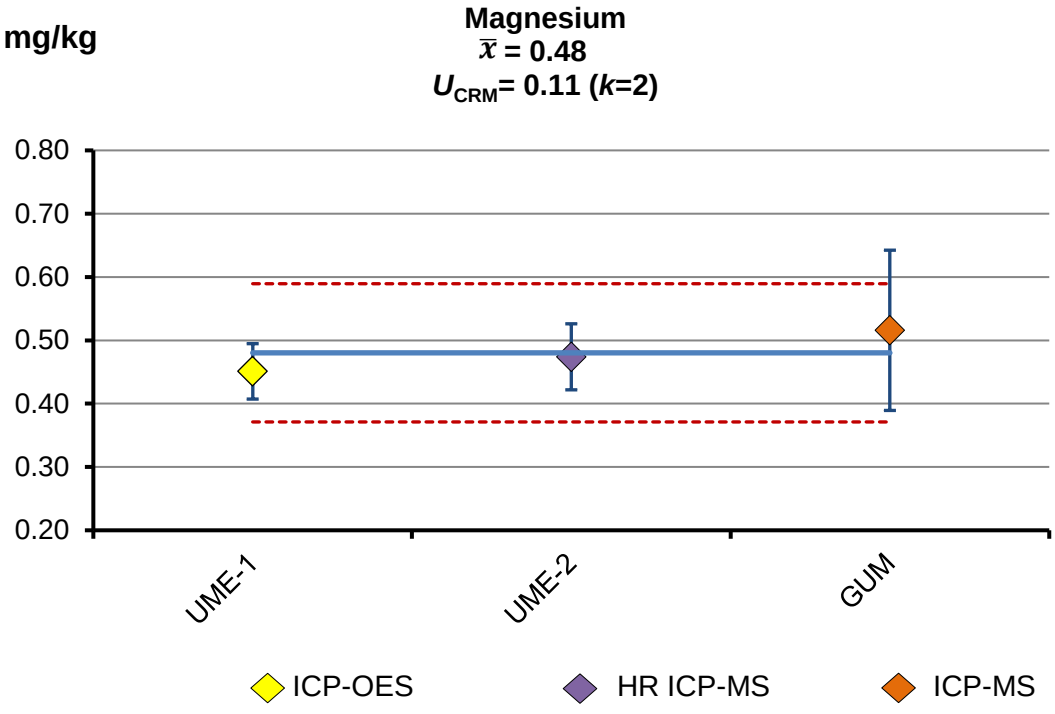


Figure A6.2. Characterization Study Plot for Magnesium

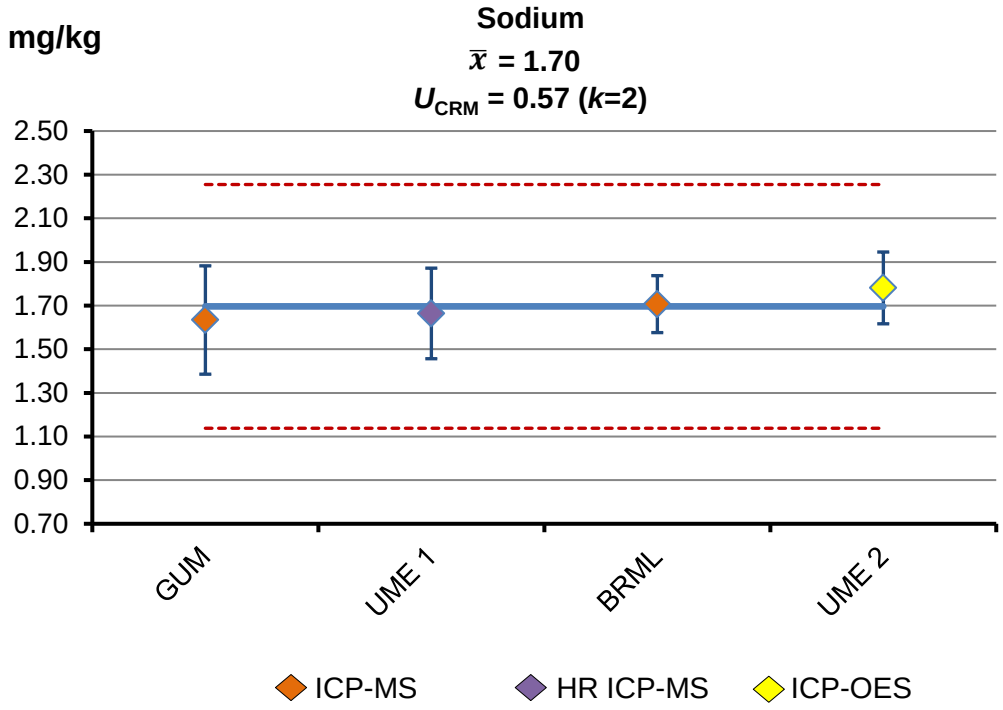


Figure A6.3. Characterization Study Plot for Sodium

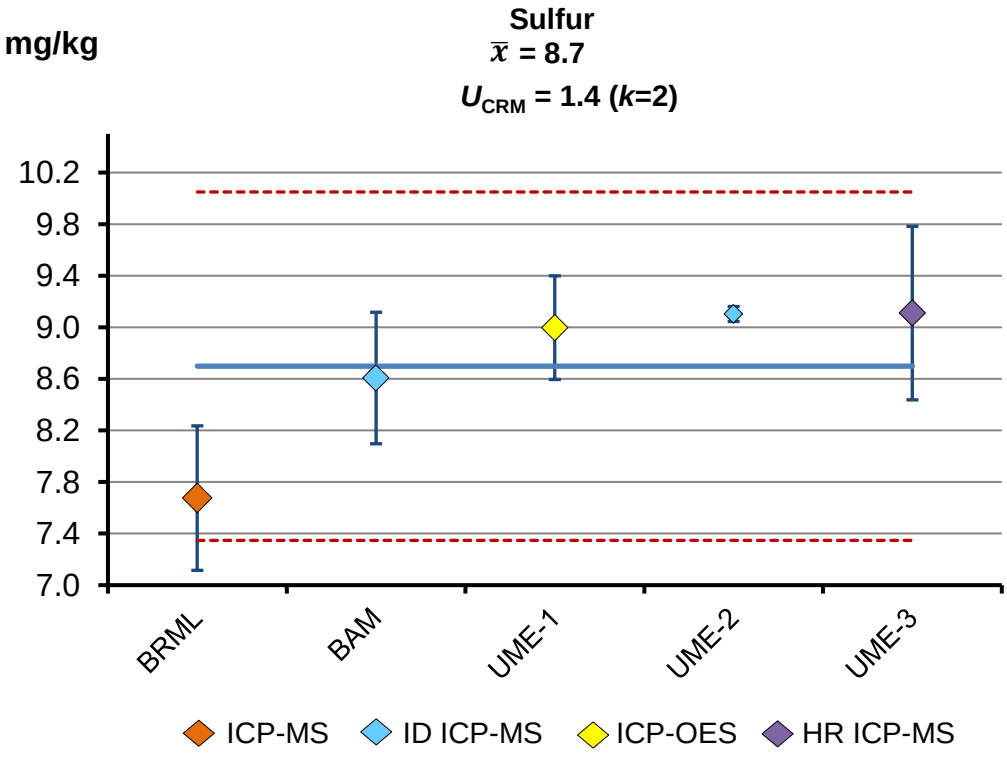


Figure A6.4. Characterization Study Plot for Sulfur