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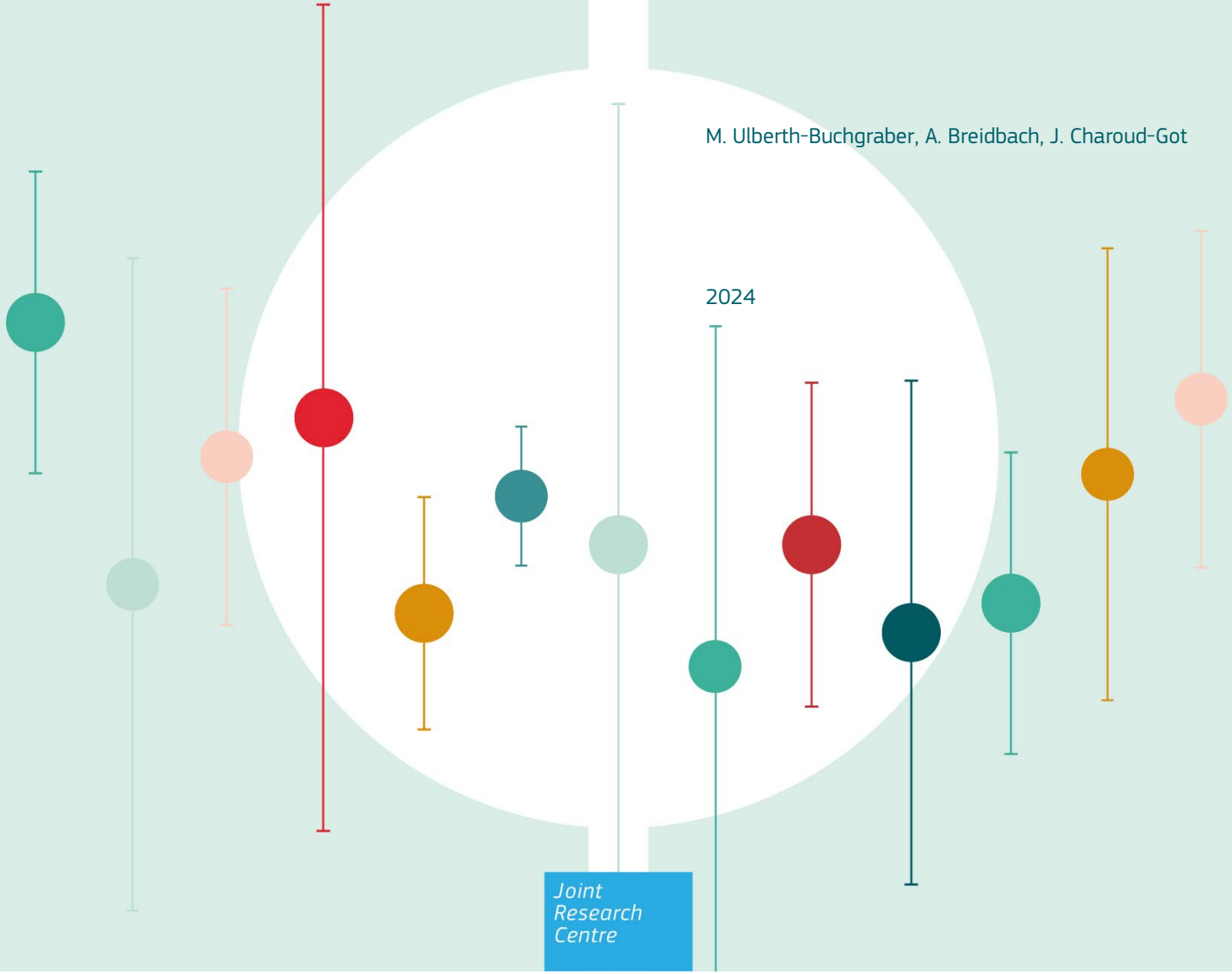
JRC REFERENCE MATERIALS REPORT

Certification of the relative mass fraction of triglycerides and cholesterol in anhydrous milk fat: ERM®-BD519

M. Ulberth-Buchgraber, A. Breidbach, J. Charoud-Got

2024



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Abstract

ERM-BD519 is an anhydrous milk fat matrix material certified for the relative mass fraction of triglycerides (C24 to C54) and cholesterol, produced within the scope of ISO 17034 accreditation [1] and ISO 33405:2024 [2].

ERM-BD519 is available in amber glass ampoules containing approximately 5 mL of anhydrous milk fat, which were sealed under an atmosphere of argon.

Between-unit homogeneity was quantified and stability during transport and storage was assessed in accordance with ISO 33405:2024 [2]. Within-unit homogeneity was quantified to determine the minimum sample size. The minimum sample size for one measurement is 10 mg.

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

Uncertainties of the certified values were estimated in accordance with ISO 17034:2016 [1] and ISO 33405:2024 [2] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended either for calibration or for the evaluation of method performance according to ISO 17678:2019/IDF 202:2019 [4].

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

Acknowledgements

The authors would like to acknowledge the support received from colleagues of JRC for the processing, organising of stability studies, measuring, reviewing of the certification project and distribution of ERM-BD519.

Furthermore, the authors would like to thank the experts of the Reference Material Review Panel Katrin Vorkamp (Aarhus University, DK) and Ángel Ríos (Castilla - La Mancha University, ES) for their constructive comments and review.

Authors

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1 Introduction

1.1. Background

Milk and milk-based products are easily subjected to food adulteration, with milk fat derived solely from cow's milk being replaced with cheaper vegetable oils or animal fats for the purpose of maximising profits. This can compromise the quality and safety of these products, leading to consumer mistrust and regulatory non-compliance.

To address this issue, the European Union has implemented a rigorous system of regulations, testing, and monitoring to prevent and detect milk fat adulteration, ensuring that dairy products sold in the EU are of high quality and safe for consumption. Commission Implementing Regulation (EU) 2018/150 defines methods for analysing and evaluating the quality of milk and milk products that are eligible for public intervention and aid for private storage [5]. It specifies that the purity of milk fat should be determined using a standardised measurement procedure based on gas chromatographic analysis of triglycerides, as laid down in the documentary standard ISO 17678 [4].

This reference method utilises the differences in the triglyceride fingerprint of milk fat from the individual triglyceride fingerprints of other fats and oils to determine samples which are outside the range normally observed for milk fat. This is achieved by using the defined triglyceride formulae based on the normalised weighted sum of individual triglyceride peaks (expressed as relative mass fractions [g/100 g], i.e. the mass fraction of each triglyceride (C24, C26, C28, C30, C32, C34, C36, C38, C40, C42, C44, C46, C48, C50, C52, C54) and cholesterol expressed as a percentage of the total triglycerides) which are sensitive to the integrity of the milk [6, 7]. The integrity of the milk fat can be determined by comparing the result of these formulae with those previously observed for a range of pure milk fat samples [8].

Even when using standardised measurement procedures, laboratories need to ensure that they are applied correctly, as their use does not per se ensure reliable results. It is widely accepted that laboratories using standardised methods must ensure that their data are reliable and have all elements that give confidence to the user [3]. Certified reference materials (CRMs) are the preferred tool to give laboratories the possibility to demonstrate their proficiency in applying the standard and proper working of their instruments.

Another common application of CRMs is their use for calibration of methods. ISO 17678:2019/IDF 202:2019 [4] specifically mentions the use of a standardised milk fat (BCR-519 [9]) with a certified triglyceride composition for determining detector response factors. Since BCR-519 is no longer available, the analytical community on milk fat (i.e. the International Organization for Standardization (ISO) and the International Dairy Federation (IDF)) asked for a suitable replacement.

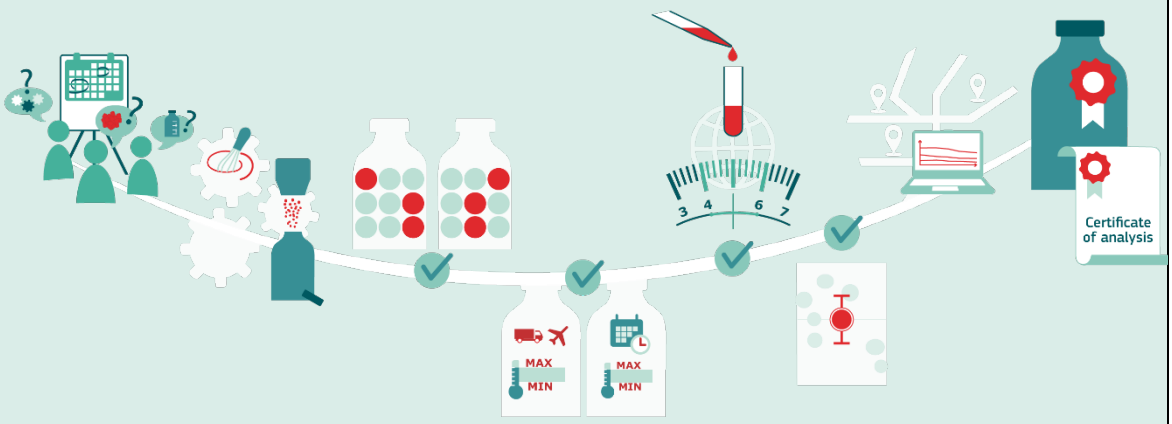
In direct response, the Joint Research Centre of the European Commission has developed a new CRM for anhydrous milk fat that supports the implementation of the ISO 17678:2019/IDF 202:2019 standard [4], replacing the previous CRMs, BCR-519 [9] and BCR-632A [10].

1.2. Choice of the material

Anhydrous milk fat (AMF) is a product prepared exclusively from milk fat with the removal of water and non-fat solids, as defined by the Codex Alimentarius Commission [11]. Due to the absence of water, AMF is a highly stable and consistent base material that is ideal for the preparation of CRMs. The stability of AMF as a base material has been demonstrated by previous CRMs, which have remained unchanged for over 15 years without any signs of degradation.

1.3. Outline of the CRM project

Box 1. Reference material production



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

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

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

The material characterisation was based on an interlaboratory comparison of different expert laboratories using all the same measurement procedure, i.e. ISO 17678:2019/IDF 202:2019 [4]. They were selected based on criteria that comprised both technical competence and quality management aspects.

Uncertainties of certified values were estimated in compliance with ISO 17034 [1], which implements the basic principles of ISO/IEC Guide 98 (GUM) [12].

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

Certain commercial equipment, instruments, and materials are identified in this report to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

2 Participants

2.1 Project management and data evaluation

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

2.2 Processing

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

2.3 Homogeneity measurements

Wageningen Food Safety Research, Wageningen, NL
(measurements under the scope of ISO/IEC 17025:2017 accreditation RvA No. L 014)

2.4 Stability measurements

Wageningen Food Safety Research, Wageningen, NL
(measurements under the scope of ISO/IEC 17025:2017 accreditation RvA No. L 014)

2.5 Characterisation measurements

AsureQuality, Auckland, NZ

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

L'Institut des Corps Gras et Produits apparentés, Pessac, FR

Landwirtschaftliches Zentrum für Rinderhaltung, Grünlandwirtschaft, Milchwirtschaft, Wild und Fischerei, Baden-Württemberg, DE
(measurements under the scope of ISO/IEC 17025:2017 accreditation DAkkS No. D-PL-14491-01-00)

Max Rubner-Institut, Kiel, DE

Milchwirtschaftliche Lehr- und Untersuchungsanstalt Oranienburg e.V., Oranienburg, DE
(measurements under the scope of ISO/IEC 17025:2017 accreditation DAkkS No. D-PL-14007-01-00)

Muva Kempten GmbH, Kempten, DE
(measurements under the scope of ISO/IEC 17025:2017 accreditation DAkkS No. D-PL-20469-01-00)

State Veterinary and Food Institute, Bratislava, SK

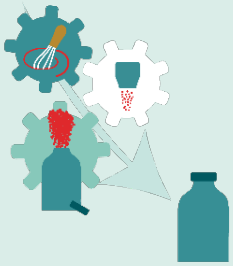
State Veterinary Institute Jihlava, Jihlava, CZ
(measurements under the scope of ISO/IEC 17025:2017 accreditation CAI No. L1129)

Wageningen Food Safety Research, Wageningen, NL
(measurements under the scope of ISO/IEC 17025:2017 accreditation RvA No. L 014)

All datasets are identified by a code (e.g. D01). The numbering is not related to the order of the laboratories presented above.

3 Material processing and processing control

Box 2. Reference material processing



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

3.1 Origin of the starting material

AMF was selected as base material and provided by S.A. Corman (Limbourg, BE). It had been prepared from premium cream and butter made exclusively from cow's milk. AMF was isolated by means of physical separation processes during which virtually all water and non-fat solids were removed. JRC received 50 kg of AMF in five cardboard boxes, each containing 10 kg of AMF. The material was delivered with a certificate of analysis proving the suitability of the material in terms of freshness by testing the free fatty acids, peroxide value, fat content and water content. Its triglyceride profile was analysed by Wageningen Food Safety Research (Wageningen, NL) according to ISO 17678:2019/IDF 202:2019 [4]. The results confirmed the purity of the AMF. The triglyceride profile was found adequate, reflecting a raw material from the transition of feeding conditions between barn and pasture feeding. Upon arrival at JRC (Geel, BE) the material was immediately stored at -20 °C in darkness until further treatment.

3.2 Processing

About 30 kg of the material were moved from -20 °C to 4 °C, stored there for 24 h and then placed in a drying oven at 50 °C until complete melting of AMF.

The main means of stabilisation were the addition of an antioxidant and creation of an inert atmosphere. The antioxidant t-butylhydroquinone (TBHQ) was added in a two-step dilution process as follows: 3 g of TBHQ was added to 6 kg of liquefied AMF. After vigorous manual mixing for 5 min, the mixture was then added to the remaining liquefied AMF (to reach a target concentration of TBHQ of 0.1 g/kg). The AMF was transferred into a 70 L double-mantled glass vessel, which was heated to 50 °C. All tubing, needles and devices into contact with the liquid AMF were also heated to 50 °C to keep the fat liquid during filling. The 30 kg of liquefied AMF were constantly flushed with nitrogen till the end of the filling to homogenise and stabilise the material.

Ampoules were filled on a ROTA automatic ampoule filling machine, model R910/PA (ROTA Verpackungstechnik GmbH & Co.KG, Wehr, DE). 10 mL amber glass ampoules were filled with 5 mL of AMF. To remove most of the oxygen from the amber glass ampoules, they were flushed with argon before the filling. Afterwards, the ampoules were flame-sealed, labelled and stored at –20 °C.

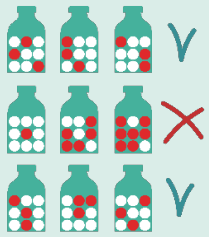
In total, 4000 ampoules were produced under the conditions described above. For the purpose of this report, the term 'unit' refers to one ampoule of ERM-BD519. One unit of ERM-BD519 is shown in Figure 1.

Figure 1. ERM-BD519.



4 Homogeneity

Box 3. Homogeneity assessment



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

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

4.1 Between-unit homogeneity

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

The number of units selected corresponds to approximately the cube root of the total number of units produced. Sixteen units were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. Random stratified sampling involves dividing the batch into 16 groups (with a similar number of units in each group) and selecting one unit randomly from each group. Two independent samples were taken from each selected unit and analysed for triglycerides and cholesterol according to ISO 17678:2019/IDF 202:2019 [4]. The measurements were performed under repeatability conditions, and in a randomised manner to separate a potential drift in the measurement results from a potential trend in the filling sequence. The results are shown as graphs in Annex 1.

Regression analyses were performed to evaluate potential trends in the measurement sequence as well as trends in the filling sequence. No trends in the filling sequence were observed at a 95 % confidence level, except for C42. However, it is technically almost impossible to obtain a trend in the filling sequence for only one triglyceride. Therefore, it was decided not to correct for the filling trend of C42. Moreover, the obtained values of the individual units differ only marginally from each other, which indicate a statistical phenomenon rather than a technical issue.

For half of the parameters significant (95 % confidence level) trends in the measurement sequence were visible, pointing at a changing parameter, e.g. a signal drift in the measurement system. Research has shown that the correction of even non-significant biases is beneficial, for two reasons: firstly, the resulting uncertainties are smaller than for non-corrected biases. Secondly, after correction the uncertainty interval has the highest probability of including the true value [13]. A correction of trends is therefore expected to improve the sensitivity of the subsequent statistical analysis through a reduction in the variation of measurement results without masking potential between-unit heterogeneities. As the measurement sequence and the unit numbers were not correlated, trends significant on at least a 95 % confidence level were corrected as shown below:

$$x_{i_corr} = x_i - b \cdot i \qquad \textbf{Equation 1}$$

b slope of the linear regression
i position of the result in the measurement sequence

The original dataset or, if applicable, the trend-corrected dataset was assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the unit means. No outlying individual

results but one outlying unit mean (C36) was detected. Since no technical reason for the outlier could be found, all the data were retained for statistical analysis.

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

Evaluation by ANOVA requires mean values per unit, which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per unit was visually tested using histograms and normal probability plots. 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 1.

Table 1. Results of the statistical evaluation of the homogeneity study. In the event of trends in the measurement sequence, these were corrected prior to further data treatment, addressing trends in filling order, outliers, and distribution.

Property	Trends [1)]		Outliers [2)]		Distribution	
	Measurement sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
C24	yes	no	none	none	unimodal	unimodal
Cholesterol	yes	no	none	none	unimodal	unimodal
C26	no	no	none	none	unimodal	unimodal
C28	no	no	none	none	unimodal	unimodal
C30	no	no	none	none	unimodal	unimodal
C32	no	no	none	none	unimodal	unimodal
C34	yes	no	none	none	unimodal	unimodal
C36	no	no	none	one–statistical reason (retained)	unimodal	unimodal
C38	no	no	none	none	unimodal	unimodal
C40	no	no	none	none	unimodal	unimodal
C42	yes	yes	none	none	unimodal	unimodal
C44	yes	no	none	none	unimodal	unimodal
C46	yes	no	none	none	unimodal	unimodal
C48	no	no	none	none	unimodal	unimodal
C50	yes	no	none	none	unimodal	unimodal
C52	yes	no	none	none	unimodal	unimodal
C54	no	no	none	none	unimodal	unimodal

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

It should be noted that $s_{bb,\,rel}$ and $s_{wb,\,rel}$ are estimates of the standard deviations and are therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in a negative number under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be less than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as shown in equation 4 and described by Linsinger *et al.* [14]. u^*_{bb} is comparable to the limit of detection of a measurement method yielding the maximum degree of inhomogeneity that might be undetected by the given study setup.

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

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

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

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

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

However, a different approach was adopted for the substance C36 for which one outlying unit mean was detected. In this case between-unit inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit mean [12], and the rectangular standard uncertainty of homogeneity (u_{rec}) was estimated by:

$$u_{rec} = \frac{|\bar{x}_{outlier} - \bar{y}|}{\sqrt{3} \cdot \bar{y}} \qquad \textbf{Equation 5}$$

$\bar{y}$	mean of all results of the homogeneity study
$\bar{x}_{outlier}$	largest outlying unit mean of the homogeneity study

The results of the evaluation of the between-unit variation are summarised in Table 2.

Table 2. Results of the homogeneity study.

Property	$s_{wb,\ rel}$ [%]	$s_{bb,\ rel}$ [%]	$u^*_{bb,\ rel}$ [%]	$u_{rec,\ rel}$ [%]	$u_{bb,\ rel}$ [%]
C24	1.43	0.89	0.60	n.a.	0.89
Cholesterol	0.63	0.42	0.26	n.a.	0.42
C26	1.89	n.c.	0.80	n.a.	0.80
C28	0.89	0.15	0.37	n.a.	0.37
C30	0.64	n.c.	0.27	n.a.	0.27
C32	0.26	0.15	0.11	n.a.	0.15
C34	0.14	0.18	0.06	n.a.	0.18
C36	0.18	0.11	0.08	0.27	0.27
C38	0.18	0.08	0.08	n.a.	0.08
C40	0.10	0.11	0.04	n.a.	0.11
C42	0.15	n.c.	0.06	n.a.	0.06
C44	0.16	n.c.	0.07	n.a.	0.07
C46	0.20	n.c.	0.09	n.a.	0.09
C48	0.24	0.19	0.10	n.a.	0.19
C50	0.23	0.11	0.10	n.a.	0.11
C52	0.19	n.c.	0.08	n.a.	0.08
C54	0.28	0.17	0.12	n.a.	0.17

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

2) n.a.: not applicable.

The homogeneity studies showed no outlying unit means (except for C36) or trends in the filling sequence (except for C42). Therefore, the between-unit standard deviation can be used as an estimate of u_{bb}. As u^*_{bb} sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

This filling trend detected for C42 was disregarded, as it is considered a statistical phenomenon rather than a technical issue.

One outlying unit mean was found for C36. However, taking this outlying value into account, the inhomogeneity, quantified as u_{rec}, is still sufficiently small to make the material suitable for its intended use. Therefore, u_{rec} was used as estimate of u_{bb} for C36.

4.2 Within-unit homogeneity and minimum sample size

Homogeneity and stability experiments were performed using a 10 mg sample intake. This sample intake gave acceptable repeatability, demonstrating that the within-unit inhomogeneity no longer contributes to variation of measurement results at this sample intake. Hence, the minimum amount of sample for one measurement is 10 mg.

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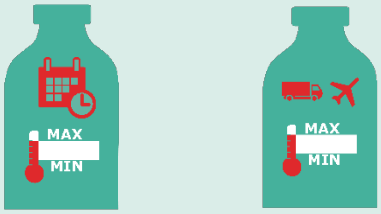
5 Stability

Box 4. Stability assessment



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

Time, temperature, light (including ultraviolet radiation) and the presence of oxygen were regarded as the most relevant parameters potentially influencing the stability of the material. The influence of ultraviolet and visible light was minimised by storing the material in amber glass ampoules, which reduce light exposure. In addition, materials are stored in the dark and transported in boxes, thus removing any possibility of degradation by light. Principal means of stabilisation were the addition of an antioxidant (TBHQ), and creation of an inert atmosphere by flushing argon into the ampoules just before filling, removing the oxygen present, and by bubbling the material with nitrogen throughout the filling. Therefore, only the influences of time and temperature needed to be investigated.

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

Storage stability was assessed using stability data from the post-certification monitoring of a similarly produced CRM (BCR-632A) [10]. ERM-BD519 is an anhydrous milk fat like BCR-632A [10]. AMF consists of 99.8 g/100 g milk fat [11], which is mainly composed of triglycerides. It is a highly concentrated fat product with minimal water and non-fat solids, representing a very stable matrix. Since the measurands of the new material are identical to those of BCR-632A [10], the stability data collected for the previous material should provide reliable and valid results for the new material.

5.1 Transport stability

The conditions for the transport of the material to the customers were established in a short-term stability study. To this end, units were stored at 60 °C for 0, 1, 2 and 4 weeks. The reference temperature was set to -70 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, triglycerides and cholesterol were measured in two samples according to ISO 17678:2019/IDF 202:2019 [4]. The measurements were performed under repeatability conditions, and a randomised sequence was used to differentiate any potential drift in the measurement results from a potential trend over storage time.

Regression analyses were performed to evaluate potential trends in the measurement sequence. For half of the parameters significant (95 % confidence level) trends in the measurement sequence were visible, pointing at a changing parameter, e.g. a signal drift in the measurement system. Trends significant on at least a 95 % confidence level were corrected as given in Equation 1.

The original results or, if applicable, the trend-corrected results were screened for outliers using the single and double Grubbs test on a confidence level of 99 %.

The data were evaluated against storage time, and regression lines of relative mass fraction versus time were calculated, to test for potential increases or decreases of the measurands under conditions that could occur during transport. The slopes of the regression lines were tested for statistical significance.

The results of the measurements are shown in Annex 2. The results of the statistical evaluation of the short-term stability are summarised in Table 3.

Table 3. Results of the short-term stability tests at 60 °C.

Property	Number of individual outlying results [1)]	Significance of the trend [2)]
C24	none	no
Cholesterol	none	yes
C26	none	no
C28	none	no
C30	none	no
C32	none	no
C34	none	no
C36	none	no
C38	none	no
C40	none	no
C42	none	no
C44	none	no
C46	none	no
C48	none	no
C50	none	no
C52	none	no
C54	none	no

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

No outliers were identified at a 99 % confidence level, and a significant trend over time at 60 °C was observed solely for cholesterol. No technical explanation could be found for its decrease. Therefore, a combined uncertainty is given for cholesterol with u_{sts} including potential degradation of the material (see Equation 8 – 10). This uncertainty is significantly smaller than the other uncertainties associated with U_{CRM} (Table 13), rendering it insignificant.

Hence, the material can be dispatched without further precautions under ambient conditions.

5.2 Storage stability

Long-term storage conditions and shelf life guaranteeing the stability of the material and the certified values were established using data from the stability monitoring programme for a similar produced CRM, namely BCR-632A [10].

This previously released anhydrous milk fat CRM was analysed for the same measurands as those certified in ERM-BD519 on four occasions over a period of 181 months (for C24, 144 months) with no sign of degradation for any of the certified properties. At each time point measurements were performed on units stored at normal storage temperature (-20 °C) and at a reference temperature (-70 °C). Each of these studies can be viewed as a two-point isochronous study. The evaluation was based on the ratio of samples from -20 °C and -70 °C.

The stability monitoring data were screened for outliers using the single and double Grubbs test at a confidence level of 99 %.

In addition, the data were plotted against storage time and linear regression lines of relative mass fraction versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to storage).

The results of the stability monitoring measurements of BCR-632A are shown in Annex 3. The results of the statistical evaluation of the stability monitoring study of BCR-632A are summarised in Table 4.

Table 4. Results of the long-term stability tests of BCR-632A at -20 °C.

Property	Number of individual outlying results [1)]	Significance of the trend [2)]
C24	none	no
Cholesterol	two-statistical (retained)	no
C26	one-statistical (retained)	no
C28	one-statistical (retained)	no
C30	one-statistical (retained)	no
C32	one-statistical (retained)	no
C34	none	no
C36	one-statistical (retained)	no
C38	one-statistical (retained)	no
C40	one-statistical (retained)	no
C42	one-statistical (retained)	no
C44	two-statistical (retained)	no
C46	none	no
C48	one-statistical (retained)	no
C50	one-statistical (retained)	no
C52	one-statistical (retained)	no
C54	one-statistical (retained)	no

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

Statistical outliers were detected for almost all measurands. Since removing these outliers shows only a negligible impact on the estimated uncertainty of stability during storage, all data were retained for the estimation of u_{lts}. None of the trends was statistically significant at a 95 % confidence level. The material can therefore be stored at -20 °C.

5.3 Estimation of uncertainties

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

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

$$u_{sts,\,rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt}$$ **Equation 6**

$$u_{lts,\,rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl}$$ **Equation 7**

s_{rel} relative standard deviation of all results of the stability study
t_i time elapsed at time point *i*
$\bar{t}$ mean of all t_i
t_{tt} chosen transport time (one week at 60 °C)
t_{sl} chosen shelf life (72 months at -20 °C)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of stability during transport. This was estimated from the 60 °C study. The uncertainty describes the possible change during a transport at 60 °C lasting for one week.
- $u_{lts,rel}$, the uncertainty of stability during storage. This uncertainty contribution was estimated from the stability monitoring programme for CRM BCR-632A [10]. The uncertainty contribution describes the possible degradation during storage for 72 months at -20 °C.

The u_{sts} for cholesterol, which exhibits a significant trend in the short term stability study, consists of two main contributions. The first term accounts for to the potential degradation of the measurand mentioned in 5.1 corresponding to a bias (u_{a1}) and is calculated as a rectangular distribution of the slope (*b*). The second term captures the uncertainty associated with this bias (u_{a2}) including potential material degradation. For the chosen transport time of the material (t_{tt} = 1 week at 60 °C), the u_{sts} for cholesterol is estimated as follows:

$$u_{sts,\,rel} = \sqrt{u_{a1}^2 + u_{a2}^2} \cdot t_{tt}$$ **Equation 8**

where,

$$u_{a1} = \frac{b}{\sqrt{3}}$$ **Equation 9**

$$u_{a2} = \frac{s_{rel}}{\sqrt{(t_i - \bar{t})}}$$ **Equation 10**

The results of these calculations are summarised in Table 5.

Table 5. Uncertainties of stability during transport and storage. $u_{sts,rel}$ was calculated for a temperature of 60 °C and one week; $u_{lts,rel}$ was calculated for a storage temperature of -20 °C and 72 months.

Property	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
C24	0.22	2.86
Cholesterol	0.30	0.46
C26	0.18	0.53
C28	0.13	0.39
C30	0.07	0.30
C32	0.08	0.18
C34	0.06	0.10
C36	0.04	0.19
C38	0.04	0.23
C40	0.03	0.15
C42	0.02	0.13
C44	0.03	0.10
C46	0.03	0.10
C48	0.06	0.20
C50	0.04	0.22
C52	0.04	0.24
C54	0.04	0.19

No significant degradation was observed at 60 °C apart from cholesterol. The combined uncertainty given for cholesterol with u_{sts} including potential degradation of the material, is significantly smaller than the other uncertainty sources of U_{CRM} (Table 13). Therefore, the trend can be considered insignificant and the material can be shipped at ambient conditions without special precautions.

No significant degradation is observed for storage at -20 °C.

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

Box 5. Stability monitoring



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

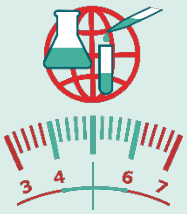
Therefore, the stability of RMs whose assigned values might change is regularly monitored. The monitoring frequency depends on the outcome of the storage stability assessment.

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

The first measurement in the further monitoring was already done before the release of the material and confirmed the assigned values.

6 Characterisation

Box 6. Reference material characterisation



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

The material characterisation was based on an interlaboratory comparison, i.e. the properties of the material were determined by combining independent datasets obtained in different laboratories. Due to the nature of the measurands the same method was applied for all datasets. This approach converts the potential bias of each dataset into a random variable, the combined effect of which is reduced by averaging over several datasets.

6.1 Selection of participants

Ten laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participating laboratory was required to operate a quality system and to deliver documented evidence of its laboratory proficiency for the measurements of cholesterol and the triglyceride profile in anhydrous milk fat according to ISO 17678:2019/IDF 202:2019 [4] by submitting results for interlaboratory comparison exercises or method validation reports. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 [3] was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2.5).

6.2 Study setup

Laboratories received three units of ERM-BD519 per dataset and were requested to provide for each dataset six independent results, two 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 done on three days to ensure intermediate precision conditions.

An independent calibration was conducted by the expert laboratories for each day of the instrumental analysis of ERM-BD519 using separate units of the common calibrant provided by JRC (see Section 6.3 and Annex 4 for details).

For each dataset, one unit of BCR-632A [10] was provided as a quality control (QC) sample. The results for this material were used to support the evaluation of the characterisation results.

Laboratories were required to strictly adhere to ISO 17678:2019/IDF 202:2019 [4] for the measurements and meet the specified performance criteria.

To estimate the expanded uncertainties of the mean value of the six results from each laboratory, two times the standard deviations obtained for the six results were used.

6.3 Production and characterisation of the calibrant

6.3.1 Processing of the calibrant

A common calibrant prepared by JRC (Geel, BE) was distributed to the participants of the characterisation study to determine the response factors of cholesterol and individual triglycerides for the quantification of these substances in anhydrous milk fat. The calibrant was a gravimetrically formulated mixture containing the following substances (see Table 6): glyceryl trioctanoate (CCC), cholesterol, glyceryl tricaprinate (DDD), glyceryl trilaurate (LLL), glyceryl trimyristate (MMM), glyceryl tripalmitate (PPP), 1,3-distearate-2-myristate-glycerol

(SMS), 1,3-distearate-2-palmitate-glycerol (SPS), and glyceryl tristearate (SSS). They were all obtained from Larodan AB (Solna, SE).

Table 6. Substances used to formulate the common calibrant.

Substance	Molecular formula	Molecular weight [g/mol]	CAS number	Physical state [1)]
CCC [C24]	C_{27} H_{50} O_6	470.7	538-23-8	Liquid
Cholesterol	C_{27} H_{46} O	386.7	57-88-5	Solid
DDD [C30]	C_{33} H_{62} O_6	554.8	621-71-6	Solid
LLL [C36]	C_{39} H_{74} O_6	639.0	538-24-9	Solid
MMM [C42]	C_{45} H_{86} O_6	723.2	555-45-3	Solid
PPP [C48]	C_{51} H_{98} O_6	807.3	555-44-2	Solid
SMS [C50]	C_{53} H_{102} O_6	835.4	57396-99-3	Solid
SPS [C52]	C_{55} H_{106} O_6	863.4	2190-24-1	Solid
SSS [C54]	C_{57} H_{110} O_6	891.5	555-43-1	Solid

1) at ambient temperature

Four solutions were prepared by two operators, containing all compounds in Table 6 in a cumulative mass of approximately 2 g in each solution. They were dissolved in 200 g of *n*-heptane. The relative mass fractions of triglycerides to the sum of triglycerides are resembling those found in anhydrous milk fat (see Annex 4 Table 4.1). Each solution was analysed ten times using gas chromatography-flame ionisation detection (GC-FID) following ISO 17678:2019/IDF 202:2019 [4], with no differences in obtained response factors observed (see Annex 4 Figures 4.1 to 4.9).

Using the response factors from calibration solution 4 for quantification of the other three calibration solutions, the obtained relative mass fractions for the three solutions were in agreement with the relative mass fractions from the weighing results. Hence, the gravimetric mixing to be considered as primary method was confirmed by independent analysis.

Calibration solution 2 was selected as the common calibrant for the characterisation study of ERM-BD519. Amber glass ampoules (10 mL) were filled with 4 mL of this solution, flame-sealed, and labelled. In total, 51 ampoules were produced under these conditions, divided into three lots (day 1 Unit 1-17, day 2 Unit 18-34, day 3 Unit 35-51) following the filling sequence. Six units were randomly selected for homogeneity testing, and each laboratory received three randomly selected units (one for each analysis day) for the characterisation study.

6.3.2 Characterisation of the calibrant

6.3.2.1 Purity assessment

The purity determination of the nine substances used for the preparation of the common calibrant was carried out by JRC (Geel, BE). The identity of the substances was confirmed by the standard manufacturer using Nuclear Magnetic Resonance and by liquid chromatography-mass spectrometry (LC-MS) and GC-FID. The purity of the eight individual triglycerides (CCC, DDD, LLL, MMM, PPP, SMS, SPS, SSS) and cholesterol was assessed via a mass balance approach. Implementing the mass balance method for purity assignment requires the application of an ensemble of orthogonal techniques capable of quantifying each impurity class present in the substance [17]. The mass fractions of the non-volatile/inorganic content (w_{NV}) and residual organic solvent/volatile organic impurities (w_{OS}; including possible residues of water) were determined by thermogravimetric analysis. The mass fractions of related-structure impurities (w_{RS}) were determined using LC-MS. Major components of impurities were identified as triglycerides, which are structurally related to the principal components. Mass fractions were calculated using response factors. For the main components, response factors were determined experimentally, while for impurities eluting between two main components, response factors were mathematically calculated applying interpolation. The results obtained by LC-MS were confirmed by GC-FID analyses following ISO 17678:2019/IDF 202:2019 [4].

The mass fraction of the primary component in the substance, w_P, reported in units of mg/g, is given by:

$$w_P = 1000\ \text{mg/g} - (w_{RS} + w_{OS} + w_{NV})\ \text{mg/g} \qquad \textbf{Equation 11}$$

The combined standard uncertainty u_P of the substance is obtained by a quadratic combination of the uncertainties associated with each contributing impurity assignment:

$$u_P = \sqrt{u_{RS}^2 + u_{OS}^2 + u_{NV}^2} \qquad \textbf{\textit{Equation 12}}$$

The assigned purities of the individual substances are reported in Table 7.

Table 7. Purity of substances and associated uncertainties in mg/g used for the common calibrant.

Substance	w_{RS}	u_{RS}	w_{OS}	u_{OS}	w_{NV}	u_{NV}	w_P	u_P
CCC	7.4907	0.7087	9.0	2.4	9.30	0.98	974.2093	2.6875
Cholesterol	8.5033	1.1958	6.8	2.4	3.80	0.98	980.8967	2.8549
DDD	11.7251	0.3435	0.0	2.4	0.00	0.98	988.2749	2.6150
LLL	25.4905	11.9025	0.0	2.4	5.02	0.98	969.4895	12.1816
MMM	23.6512	0.8687	0.0	2.4	0.00	0.98	976.3488	2.7341
PPP	0.0000	1.6151	0.0	2.4	0.00	0.98	1000.0	3.0543
SMS	22.5515	0.8749	0.0	2.4	2.62	0.98	974.8285	2.7360
SPS	2.9623	0.1673	0.0	2.4	0.00	0.98	997.0377	2.5978
SSS	2.7024	0.2019	0.0	2.4	0.00	0.98	997.2976	2.6002

6.3.2.2 Value assignment

For value assignment of the common calibrant, the quantities of each substance were corrected first for their purities as given in Table 7. Secondly, each quantity was adjusted to account for contributions from other substances in the calibration solution. This involved considering impurities from the different substances that have the same retention times as other substances in the common calibrant, and excluding impurities that elute between them. The results are given in Annex 4, Table 4.2 and are summarised with the associated uncertainties in Table 8.

The assigned uncertainties to the substances in the common calibrant (u_{cal}) consist of uncertainties relating to the purity determination (u_p) that already contains the uncertainty contribution related to the weighing, and potential between-unit inhomogeneity ($u_{bb\ cal}$).

$$u_{cal} = \sqrt{u_p^2 + u_{bb\ cal}^2} \qquad \textbf{Equation 13}$$

For homogeneity testing, six units were randomly selected from the prepared batch and tested by GC-FID following ISO 17678:2019/IDF 202:2019 [4]. Two independent samples were taken from each selected unit. The results are shown in Annex 4. The homogeneity studies showed no trends in the filling sequence, except for C48. Furthermore, an individual outlier was found for C50 and one outlying unit mean for cholesterol. Upon reviewing those cases, it was noted that these results did not have any technical reasons and were deemed to be a statistical anomaly rather than a technical issue (Annex 4, Table 4.4). The results of the evaluation of the between-unit variation are summarised in Annex 4, Table 4.5. In most cases, the uncertainty contribution for homogeneity was determined by the method repeatability.

Table 8. The masses (m_i) of the substances and the associated uncertainties in the common calibrant.

Substance	m_i [mg]	u_p [mg]	$u_{bb\ cal}$ [mg]	u_{cal} [mg]
C24	2.4677	0.0164	0.0025	0.0166
Cholesterol	9.5392	0.0316	0.0155	0.0352
C30	47.0666	0.1322	0.0437	0.1392
C36	377.4274	4.7420	0.3491	4.7548
C42	247.7907	0.6953	0.3026	0.7583
C48	325.4126	1.0090	0.2496	1.0394
C50	394.7778	1.1550	0.1717	1.1677
C52	387.8717	1.0330	0.2599	1.0652
C54	199.0107	0.5209	0.2034	0.5592

Finally, the relative mass fraction (w_i; expressed as g triglyceride/100 g of the total triglyceride content of common calibrant) and associated uncertainty (u_{wi}) of each substance in the common calibrant are summarised in Table 9.

Table 9. The relative mass fraction (w_i) of each substance and the associated uncertainty (u_{wi}; absolute and relative) in the common calibrant.

Substance	w_i [g/100 g]	u_{wi} [g/100 g]	$u_{wi,\ rel}$ [%]
C24	0.1239	0.0009	0.73
Cholesterol	0.4790	0.0021	0.44
C30	2.364	0.009	0.38
C36	18.95	0.20	1.06
C42	12.44	0.05	0.40
C48	16.34	0.06	0.37
C50	19.82	0.07	0.35
C52	19.48	0.07	0.36
C54	9.99	0.04	0.40

The approach applied in the purity assessment of the individual substances and the preparation of the common calibrant ensures that the assigned values and uncertainties are traceable to the International System of Units (SI).

6.4 Measurement procedure used

All laboratories used the same measurement procedure for the determination of the cholesterol content and triglyceride composition in anhydrous milk fat as outlined in ISO 17678:2019/IDF 202:2019 [4].

It employs GC-FID analysis on non-polar columns for triglyceride profiling, separating them based on their total carbon number. Triglycerides (TGs) elute in ascending order based on molecular weight, corresponding to the increasing number of carbon atoms per triglyceride. Odd-numbered acyl-carbon triglycerides (2n+1) are combined with the preceding even-numbered TGs (2n). Results are expressed as relative mass fractions [g/100 g], i.e. the mass fraction of each triglyceride (C24, C26, C28, C30, C32, C34, C36, C38, C40, C42, C44, C46, C48, C50, C52, C54) and cholesterol is expressed as a percentage of the total triglycerides.

Method specifics not explicitly defined in ISO 17678:2019/IDF 202:2019 [4] are detailed in Annex 5. All participating laboratories employed capillary columns, utilizing various suppliers, lengths, diameters, and coating film thicknesses. The methods varied in terms of temperature gradients, carrier gases, injection techniques, and applied FID temperatures.

6.5 Evaluation of results

The characterisation study resulted in ten datasets per measurand. All individual datasets of the participating laboratories, grouped per measurand, are displayed in tabular and graphical form in Annex 6.

6.5.1 Technical evaluation

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

- compliance with the instructions given: measurements performed on three days following the specified measurement sequence.
- agreement with performance criteria of documentary standard ISO 17678:2019/IDF 202:2019 [4] for variation coefficients (VCs), response factors (RFs), repeatability (r) limit, reproducibility (R) limit, and S-values (results obtained using the triglyceride formulae).
    - The specified VCs values are not mandatory. Hence datasets are only rejected if the VCs are consistently higher for multiple triglycerides (TGs). If higher VCs are accepted, the repeatability and reproducibility limits shall nonetheless be complied with.
    - Datasets are rejected if the RFs are higher than 1.25. Because of variable overlap between C24 and cholesterol, a higher (RF) is accepted for C24.
    - Datasets are rejected if the absolute difference between two independent test results from the same unit exceeds the repeatability limit (r).
    - Datasets are rejected if the absolute difference between two independent test results from two different units exceeds the reproducibility limit (R).
    - Datasets are rejected if the obtained S-values are higher than the specified S-values.
- agreement of the measurement results with the certified values of the QC sample.
    - Datasets are rejected if the QC results for multiple TGs in an analysis consistently do not agree with the assigned values. However, datasets are not rejected if the QC results deviate only for one of the TGs in an analysis.

Based on these criteria, the following datasets were rejected as not technically valid (Table 10).

Table 10. Datasets that showed non-compliance with the instructions given and technical specifications, and action taken.

Dataset code	Description of problem	Action taken
D01	• VC for 2 TGs higher but still acceptable as r and R limits are met	retained
D02	• VC for 1 TG higher but still acceptable as r and R limits are met • QC measurements: 1 TG did not match the assigned value	retained
D03	• RFs for C24 and cholesterol outside given range • R limit not fulfilled • S-values higher than specified S-values • QC measurements: 6 TGs did not match the assigned values	dataset not used for evaluation
D04	• QC measurements: 1 TG did not match the assigned value	retained
D06	• VC for 1 TG higher but still acceptable as r and R limits are met	retained
D07	• RFs for C24 and cholesterol outside given range • QC measurements: 11 TGs did not match the assigned values	dataset not used for evaluation
D10	• QC measurements: 1 TG did not match the assigned value	retained

All laboratories complied with the instructions.

Some laboratories deviated from the specified VCs in ISO 17678:2019/IDF 202:2019 [4] for a few triglycerides (D01, D02, and D06). This is an exception rather than a consistent pattern for all triglycerides, as it only occurs in one (D02, D06) or two (D01) triglycerides. Additionally, the documentary standard allows for a certain deviation for this performance criterion. Since it is not mandatory to determine VCs and the VCs obtained were not considerably higher than the specified values, the chromatographic conditions were considered appropriate. Moreover, ISO 17678:2019/IDF 202:2019 [4] specifies, if higher VCs are accepted, the repeatability and reproducibility limits shall nonetheless be complied with, which is the case for all three laboratories.

One laboratory (D03) did not meet the reproducibility limit and S-values. The lab did not achieve the expected RFs for some triglycerides and 6 out of 17 triglycerides of the QC sample did not align with the assigned values. As the laboratory confirmed that this was not a transcription error, the complete dataset (D03) was rejected.

The complete dataset (D07) from laboratory 7 was rejected as the results for 11 out of 17 triglycerides did not align with the assigned values of the QC sample. In addition, the expected RFs were not achieved for some triglycerides. As the laboratory confirmed that this was not a transcription error, the complete dataset (D07) was rejected.

Three laboratories (D02, D04, D10) had results that did not align with the assigned value for one triglyceride of the QC sample. This can occur occasionally when normalising 17 measurands to 100 and does not necessarily indicate an issue with the analytical quality. Therefore, this deviation was deemed an exception and the data were retained.

6.5.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis and skewness tests and normal probability plots. They were also tested for outlying means using the Grubbs test and for outlying standard deviations using the Cochran test (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 11.

Table 11. Statistical evaluation of the technically accepted datasets for ERM-BD519. *p*: number of technically valid datasets.

Measurand	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [g/100 g]	*s* [g/100 g]	$s_{between}$ [g/100 g]	s_{within} [g/100 g]
C24	8	No	No	Yes	0.0782	0.0098	0.0096	0.0045
Cholesterol	8	No	No	Yes	0.3031	0.0290	0.0287	0.0097
C26	8	No	Yes (D06, D02)	Yes	0.3383	0.0338	0.0330	0.0183
C28	8	No	Yes (D06, D02)	Yes	0.7634	0.0390	0.0376	0.0252
C30	8	No	No	Yes	1.4525	0.0447	0.0429	0.0305
C32	8	No	No	Yes	3.0313	0.0817	0.0804	0.0358
C34	8	No	No	Yes	6.5966	0.1561	0.1547	0.0506
C36	8	No	No	Yes	11.2502	0.2089	0.2075	0.0601
C38	8	No	No	Yes	12.4627	0.2164	0.2154	0.0510
C40	8	No	No	Yes	9.9080	0.1378	0.1368	0.0406
C42	8	No	No	Yes	7.4039	0.0887	0.0869	0.0440
C44	8	No	No	Yes	7.1007	0.0433	0.0413	0.0321
C46	8	No	Yes (D09)	Yes	7.7372	0.0673	0.0664	0.0272
C48	8	No	No	Yes	9.0689	0.1429	0.1416	0.0478
C50	8	No	No	Yes	10.3443	0.2297	0.2269	0.0872
C52	8	No	Yes (D09)	Yes	8.3290	0.2398	0.2361	0.1022
C54	8	No	Yes (D09)	Yes	3.8316	0.1154	0.1129	0.0591

The dataset means followed normal distributions. None of the datasets contained outlying means. The statistical evaluation flagged some datasets (D02, D06 and D09) as outlying variance for a few triglycerides. As all laboratories used the same method, this indicated that the proficiency of these laboratories in applying the measurement procedure is worse than that of the other laboratories. However, all datasets were retained, as all results still agreed well with the repeatability and reproducibility requirements of ISO 17678:2019/IDF 202:2019 [4]. The datasets were therefore consistent and the mean of means is a good estimate of the true value. Standard deviations between dataset means were larger than the standard deviations within datasets, showing that confidence intervals of replicate measurement results are unsuitable as estimates of measurement uncertainty.

The uncertainties for the characterisation are a combination of uncertainties, which are laboratory-dependent [$u(I)$] and uncertainties that are common to all laboratories participating in the characterisation [$u(II)$]:

$$u_{char} = \sqrt{u(I)^2 + u(II)^2}$$ **Equation 14**

$u(I)$ is estimated as the standard error of the mean of means ($s/\sqrt{p}$) and $u(II)$ is the uncertainty contribution derived from the common calibrant as described in chapter 6.3. (Table 9). For those triglycerides not present in the common calibrant, the average of the relative combined uncertainties of C24 – C48 was used to calculate the uncertainty of these compounds in the final calibrant (= 0.586 %).

Table 12. Uncertainty of characterisation for ERM-BD519.

Measurand	p	Mean [g/100 g]	s [g/100 g]	u_{char} [g/100 g]
C24	8	0.0782	0.0098	0.0035
Cholesterol	8	0.3031	0.0290	0.0103
C26	8	0.3383	0.0338	0.0121
C28	8	0.7634	0.0390	0.0145
C30	8	1.4525	0.0447	0.0167
C32	8	3.0313	0.0817	0.0339
C34	8	6.5966	0.1561	0.0674
C36	8	11.2502	0.2089	0.1398
C38	8	12.4627	0.2164	0.1058
C40	8	9.9080	0.1378	0.0758
C42	8	7.4039	0.0887	0.0432
C44	8	7.1007	0.0433	0.0444
C46	8	7.7372	0.0673	0.0512
C48	8	9.0689	0.1429	0.0605
C50	8	10.3443	0.2297	0.0891
C52	8	8.3290	0.2398	0.0899
C54	8	3.8316	0.1154	0.0436

7 Value Assignment

Box 7. Assignment of values to a reference material



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

Certified values are values that fulfil the highest standards of accuracy. Procedures at JRC Directorate F require a sufficient number of datasets to assign certified values. Full uncertainty budgets in accordance with ISO 17034 [1] and ISO 33405 [2] are required. Certified values of a CRM can be used for calibration and trueness controls as well as for quality control measures.

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

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

Certified values were assigned to all measurands in this material.

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 each parameter.

The assigned uncertainty consists of uncertainties relating to characterisation (u_{char}), potential between-unit inhomogeneity (u_{bb}), and potential degradation during transport (u_{sts}), and long-term storage (u_{lts}). These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM, rel}$) with a coverage factor k given as:

$$U_{CRM,\,rel} = k \cdot \sqrt{u^2_{bb,\,rel} + u^2_{sts,\,rel} + u^2_{lts,\,rel} + u^2_{char,\,rel}} \qquad \textbf{Equation 15}$$

- u_{char} was estimated as described in Section 6.5.2
- u_{bb} was estimated as described in Section 4.1
- u_{sts} and u_{lts} were estimated as described in Section 5.3

Following JRC's procedures for assigning uncertainties to certified values, a coverage (k) factor of 2 can be chosen if the main uncertainty component has at least five degrees of freedom. As can be seen in Table 13, the $u_{char, rel}$ is the dominant contribution to the combined uncertainty and it has seven degrees of freedom. Therefore, a k-factor of 2 was applied to obtain the expanded uncertainties.

Some datasets did not match the certified values within their uncertainties (Annex 6). The decision whether datasets agree is based on their measurement uncertainties. The uncertainties of the individual laboratories shown in Annex 6 reflect twice the standard deviation of the six results that a laboratory produced (see section 6.2). The datasets would align with the certified values and their uncertainties if the uncertainties were based on a complete uncertainty budget. Consequently, for all measurands certified values were assigned.

The certified values and their uncertainties are summarised in Table 13.

Table 13. Certified values and their uncertainties for ERM-BD519.

Measurand	Certified value [g/100 g]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [1)] [g/100 g]
C24	0.078	4.49	0.89	0.22	2.86	10.81	0.009
Cholesterol	0.303	3.41	0.42	0.30	0.46	6.96	0.022
C26	0.338	3.58	0.80	0.18	0.53	7.43	0.026
C28	0.76	1.90	0.37	0.13	0.39	3.95	0.03
C30	1.45	1.15	0.27	0.07	0.30	2.45	0.04
C32	3.03	1.12	0.15	0.08	0.18	2.29	0.07
C34	6.60	1.02	0.18	0.06	0.10	2.09	0.14
C36	11.25	1.24	0.27	0.04	0.19	2.57	0.29
C38	12.46	0.85	0.08	0.04	0.23	1.77	0.22
C40	9.91	0.77	0.11	0.03	0.15	1.58	0.16
C42	7.40	0.58	0.06	0.02	0.13	1.20	0.09
C44	7.10	0.62	0.07	0.03	0.10	1.27	0.09
C46	7.74	0.66	0.09	0.03	0.10	1.35	0.11
C48	9.07	0.67	0.19	0.06	0.20	1.44	0.14
C50	10.34	0.86	0.11	0.04	0.22	1.79	0.19
C52	8.33	1.08	0.08	0.04	0.24	2.22	0.19
C54	3.83	1.14	0.17	0.04	0.19	2.33	0.09

1) Expanded (k = 2) and rounded uncertainty; uncertainties are always rounded up [18] and in a way that the rounding error corresponds to 3 % to 30 % of the uncertainty.

8 Metrological traceability and commutability

8.1 Metrological traceability

Box 8. Metrological traceability

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

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

This requires that these measurements

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

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

8.1.1 Identity

The relative mass fraction of all triglycerides (C24 to C54) and cholesterol are operationally defined measurands in ERM-BD519 and can only be obtained by following the measurement procedure specified in the documentary standard ISO 17678:2019/IDF 202:2019 [4]. Adherence to this measurement procedure was confirmed by agreement of results of the independent datasets with the assigned values for the CRM BCR-632A [10] that was used as quality control sample. The measurands are therefore operationally defined by measurement procedure ISO 17678:2019/IDF 202:2019 [4].

8.1.2 Quantity value

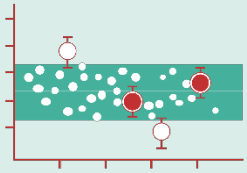
Traceability of the obtained results is based on the traceability of all relevant input factors. Investigation of the method and measurement details of the individual results show that all relevant input parameters of each technically accepted dataset have been properly calibrated. All technically accepted datasets are therefore traceable to the same reference, namely the SI. This traceability to the same reference is also confirmed by the agreement of results within their respective uncertainties. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

Box 9. Commutability

Commutability is a prerequisite for RMs intended to be used for calibration or quality control of different measurement procedures targeting the same measurand. The concept of commutability of a RM is defined by the VIM [19] as:

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



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

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

The certified values of this CRM are operationally defined and can only be obtained when tested in accordance with the documentary standard ISO 17678:2019/IDF 202:2019 [4]. Therefore, commutability of this reference material has not been assessed.

If the CRM is used with a different method, then the user has to assess the commutability of the reference material. For further guidance, see Application Note 8 [20].

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

9.2 Storage conditions

The material should be stored at (-20 ± 5) °C in the dark.

For more information regarding the shelf life of reference materials please consult ERM Application Note 7 [21].

Note that the European Commission cannot be held responsible for changes that may happen to samples after opening or under storage conditions at the customer's premises that differ from those recommended here.

9.3 Use of the material

Before opening an ampoule and taking out a portion for analysis, it must be guaranteed that the content is a liquid and properly mixed to ensure homogeneity. The following procedure is recommended:

1) Immerse the unopened ampoule in a water bath at a temperature not exceeding 50 °C, with occasional agitation until the fat is completely melted.

2) When a clear bright oil is obtained, mix the contents by repeated inversion for at least 1 min.

3) Before significant cooling can occur, score the neck of the ampoule at the thinnest part of its head and remove the head.

4) Immediately transfer the liquid contents of the ampoule in a clean and dry vial, which can be tightly closed. The material should be used on the day of opening. Please note that repeated sampling or use has not been tested and occurs under the responsibility of the user.

For general information on handling of reference materials, please consult ERM Application Note 6 [22].

9.4 Minimum sample size

The minimum sample size representative for all measurands is 10 mg.

9.5 Use of the certified values

The intended use of this material is either for calibration or for the evaluation of method performance according to ISO 17678:2019/IDF 202:2019 [3], i.e. for checking accuracy of measurement results. Measurement results can be made metrologically traceable by calibrating a measurement with a suitable CRM that is metrologically traceable to the same reference. Using a CRM as quality control material in a measurement procedure serves to verify the metrological traceability. Certified values can be used for calibration and trueness controls.

Use as a calibrant

If this matrix material is used as calibrant, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty.

Comparing a measurement result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1 [23]).

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

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine the measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty (u_{Δ}) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %.

— If $\Delta_{meas} \leq U_{\Delta}$ then no significant difference exists between the measurement result and the certified value, at a confidence level of approximately 95 %.

Use in quality control charts

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

10 Conclusions

ERM-BD519 is an anhydrous milk fat material certified for the relative mass fraction of triglycerides (C24-C54) and cholesterol. This material was produced and certified in accordance with ISO 17034:2016 [1] and ISO 33405:2024 [2]. ERM-BD519 was produced within the scope of ISO 17034 accreditation.

According to Commission Implementing Regulation (EU) 2018/150 [5], the purity of milk fat should be determined using ISO 17678 [4]. ERM-BD519 supports the implementation of ISO 17678:2019/IDF 202:2019 [4] by providing laboratories with the necessary tool to demonstrate their proficiency in applying this measurement procedure and ensuring the proper functioning of their instruments.

The following values were assigned:

Table 14. Values assigned to ERM-BD519.

Relative mass fraction		
Property [1)]	Certified value [2)] [g/100 g]	Uncertainty [3)] [g/100 g]
C24	0.078	0.009
Cholesterol	0.303	0.022
C26	0.338	0.026
C28	0.76	0.03
C30	1.45	0.04
C32	3.03	0.07
C34	6.60	0.14
C36	11.25	0.29
C38	12.46	0.22
C40	9.91	0.16
C42	7.40	0.09
C44	7.10	0.09
C46	7.74	0.11
C48	9.07	0.14
C50	10.34	0.19
C52	8.33	0.19
C54	3.83	0.09

[1)] As obtained by gas chromatography according to ISO 17678:2019/IDF 202:2019.

[2)] Certified values are values that fulfil the highest standards of accuracy. The given values represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI).

[3)] The uncertainty of the certified value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of 95 %, estimated in accordance with ISO 17034:2016 and ISO 33405:2024.

The material is intended either for calibration or for the evaluation of method performance according to ISO 17678:2019/IDF 202:2019 [4]. As any reference material, it can be used for establishing quality control charts or in validation studies.

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List of abbreviations

AMF	Anhydrous milk fat
ANOVA	Analysis of variance
b	Slope of the linear regression $y = a + bx$
BCR®	Trademark owned by the European Commission; used by the JRC for reference materials
C24	Triglyceride with a total of 24 carbon atoms in the fatty acid chains (sum of three fatty acids)
C26	Triglyceride with a total of 26 carbon atoms in the fatty acid chains (sum of three fatty acids)
C28	Triglyceride with a total of 28 carbon atoms in the fatty acid chains (sum of three fatty acids)
C30	Triglyceride with a total of 30 carbon atoms in the fatty acid chains (sum of three fatty acids)
C32	Triglyceride with a total of 32 carbon atoms in the fatty acid chains (sum of three fatty acids)
C34	Triglyceride with a total of 34 carbon atoms in the fatty acid chains (sum of three fatty acids)
C36	Triglyceride with a total of 36 carbon atoms in the fatty acid chains (sum of three fatty acids)
C38	Triglyceride with a total of 38 carbon atoms in the fatty acid chains (sum of three fatty acids)
C40	Triglyceride with a total of 40 carbon atoms in the fatty acid chains (sum of three fatty acids)
C42	Triglyceride with a total of 42 carbon atoms in the fatty acid chains (sum of three fatty acids)
C44	Triglyceride with a total of 44 carbon atoms in the fatty acid chains (sum of three fatty acids)
C46	Triglyceride with a total of 46 carbon atoms in the fatty acid chains (sum of three fatty acids)
C48	Triglyceride with a total of 48 carbon atoms in the fatty acid chains (sum of three fatty acids)
C50	Triglyceride with a total of 50 carbon atoms in the fatty acid chains (sum of three fatty acids)
C52	Triglyceride with a total of 52 carbon atoms in the fatty acid chains (sum of three fatty acids)
C54	Triglyceride with a total of 54 carbon atoms in the fatty acid chains (sum of three fatty acids)
CCC	Glyceryl trioctanoate, belonging to C24
CRM	Certified reference material
DDD	Glyceryl tricaprinate, belonging to C30
ERM®	Trademark owned by the European Commission; used by the JRC for reference materials

GC-FID	Gas chromatography-flame ionisation detection
GUM	Guide to the Expression of Uncertainty in Measurement
IDF	International Dairy Federation
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
LC-MS	Liquid chromatography-mass spectrometry
LLL	Glyceryl trilaurate, belonging to C36
MMM	Glyceryl trimyristate, belonging to C42
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicate analysis per unit
n.a.	Not applicable
n.c.	Not calculated
NV	Non-volatile/inorganic impurities
OS	Residual organic solvent/volatile organic impurities
p	Number of technically valid datasets
PPP	Glyceryl tripalmitate, belonging to C48
QC	Quality control
r	Repeatability limit
R	Reproducibility limit
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material
RMP	Reference material producer
RF	Response factor
RS	Related-structure impurities
RSD	Relative standard deviation
RSE	Relative standard error ($=RSD/\sqrt{n}$)
s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate; this parameter is linked to the homogeneity of the material
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SI	International System of Units
SMS	1,3-distearate-2-myristate-glycerol, belonging to C50
SPS	1,3-distearate-2-palmitate-glycerol, belonging to C52
SSS	Glyceryl tristearate, belonging to C54
s_{wb}	Within-unit standard deviation; an additional index "rel" is added when appropriate; this parameter is linked to the homogeneity of the material

s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
$\bar{t}$	Mean of all *ti*
TBHQ	t-butylhydroquinone
t_i	Time point for each replicate
TG	Triglyceride
t_{sl}	Proposed shelf life
t_{tt}	Proposed transport time
u	Standard uncertainty
U	Expanded uncertainty
u_{a1}	Standard uncertainty modelled as rectangular distribution of the slope (b)
u_{a2}	Standard uncertainty of the short-term stability
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
$u_{bb\ cal}$	Standard uncertainty related to a possible between-unit inhomogeneity in the calibrant; an additional index "rel" is added as appropriate
u_{cal}	Standard uncertainty of common calibrant; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_{Δ}	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_P	Standard uncertainty of primary component
u_{NV}	Standard uncertainty of non-volatile/inorganic impurities
u_{OS}	Standard uncertainty of residual organic solvent/volatile organic impurities
u_{RS}	Standard uncertainty of related-structure impurities
u_{wi}	Standard uncertainty of substance i in common calibrant, an additional index "rel" is added as appropriate
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
VC	Variation coefficient

VIM	International Vocabulary of Metrology – Basic and General Concepts and Associated Terms
w_i	Mass fraction of substance i expressed as g triglyceride/100 g of the total triglyceride content of common calibrant
w_P	Mass fraction of primary component
w_{NV}	Mass fraction of non-volatile/inorganic impurities
w_{OS}	Mass fraction of residual organic solvent/volatile organic impurities
w_{RS}	Mass fraction of related-structure impurities
$\bar{x}_{outlier}$	Largest outlying unit mean of the homogeneity study
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}
$\bar{y}$	Mean of all results of the homogeneity study

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Annexes

Annex 1. Results of the homogeneity measurements

Data points represent data as reported by the laboratory, unless indicated as “trend-corrected”. Results are expressed as relative mass fractions [g/100 g], i.e. the mass fraction of each triglyceride (C24, C26, C28, C30, C32, C34, C36, C38, C40, C42, C44, C46, C48, C50, C52, C54) and cholesterol is expressed as a percentage of the total triglycerides.

Figure 1.1. Analytical trend-corrected individual measurement replicates for C24 in the order of measurement.

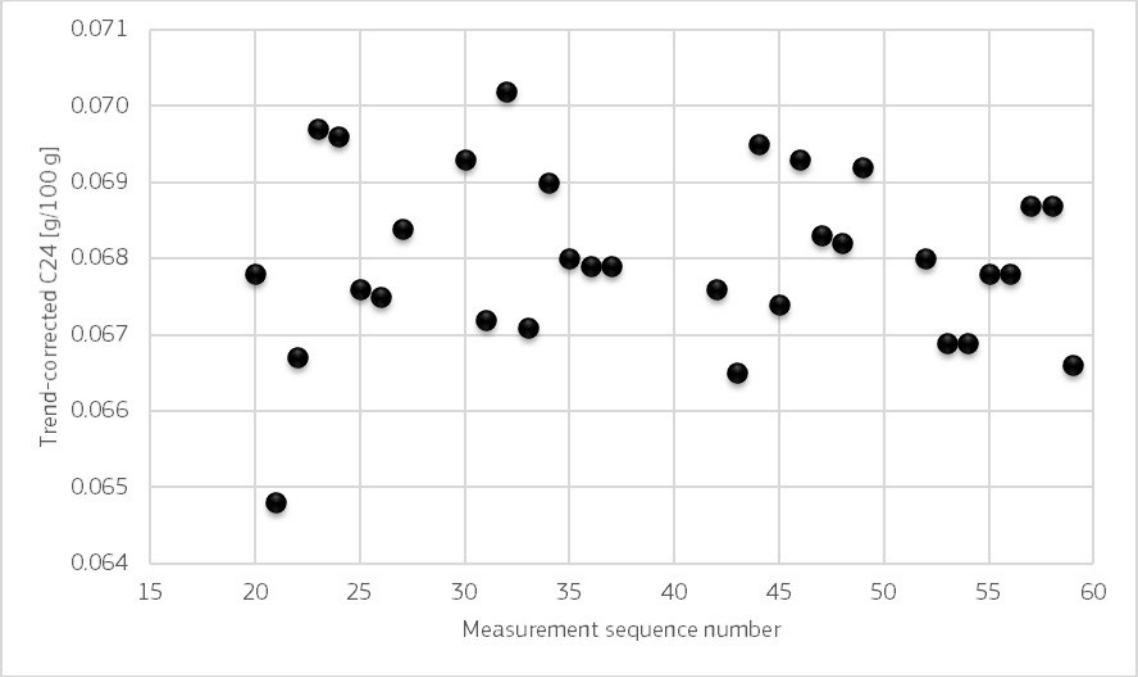


Figure 1.2. Analytical trend-corrected unit means for C24, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

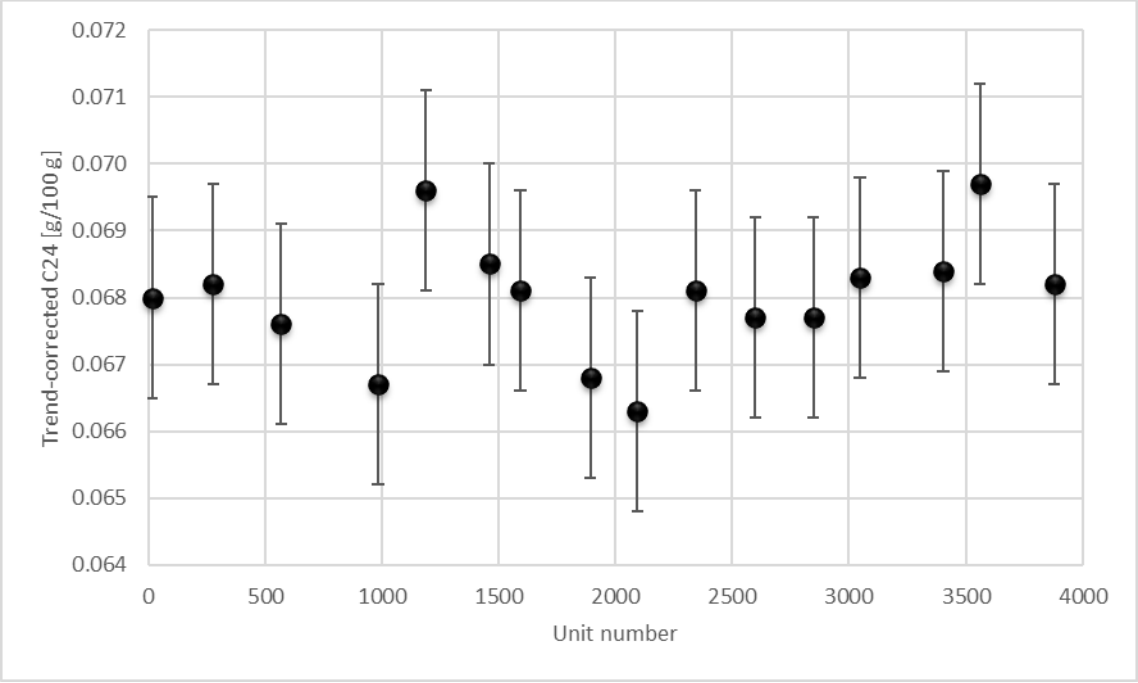


Figure 1.3. Analytical trend-corrected individual measurement replicates for cholesterol in the order of measurement.

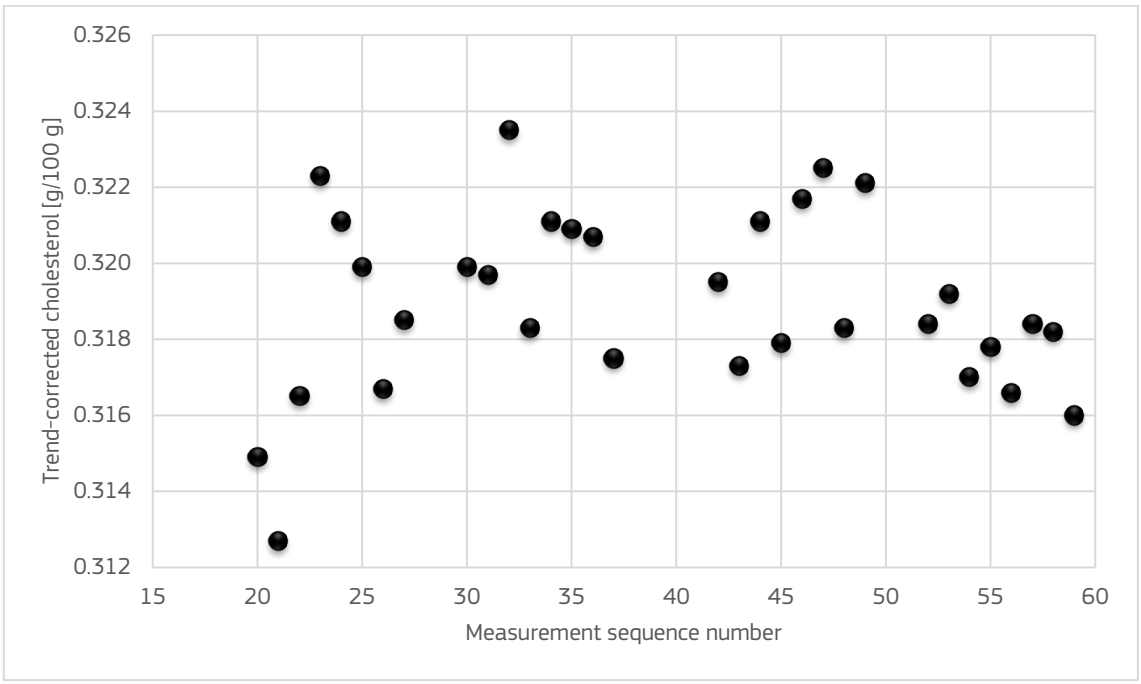


Figure 1.4. Analytical trend-corrected unit means for cholesterol, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

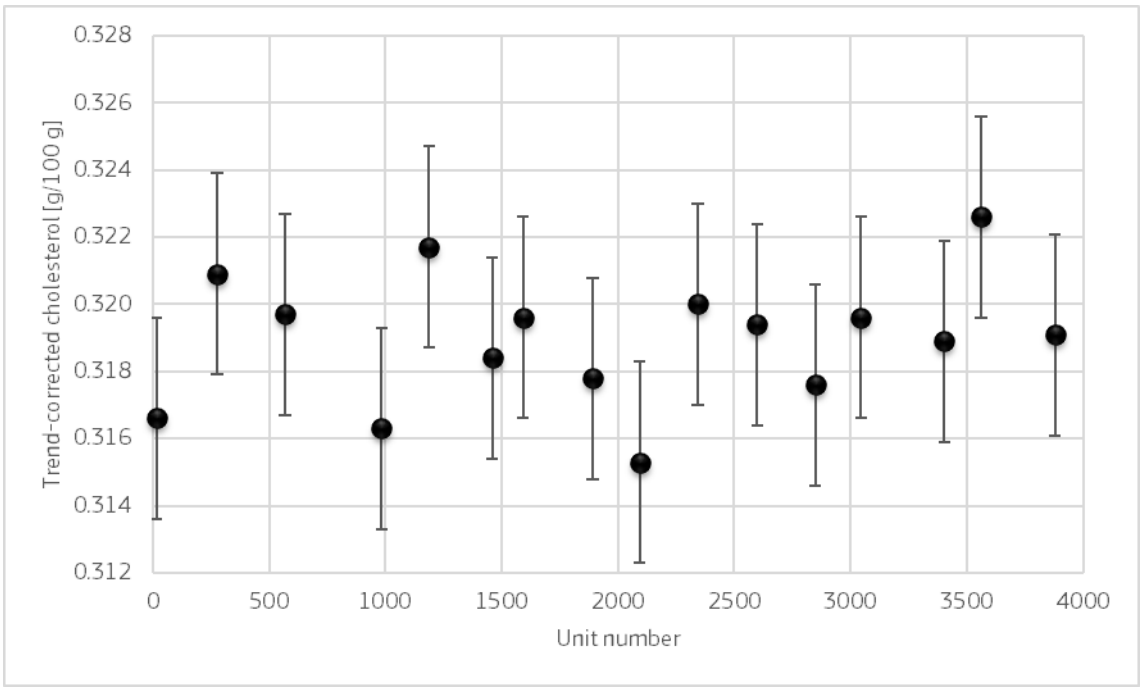


Figure 1.5. Individual measurement replicates for C26 in the order of measurement.

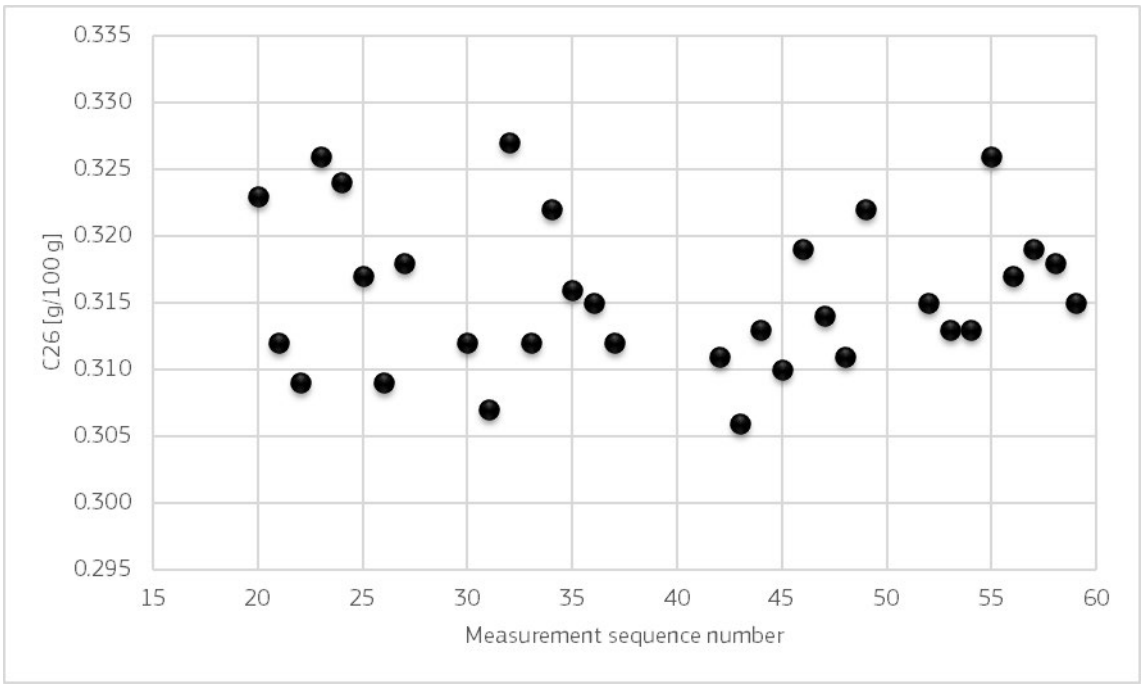


Figure 1.6. Unit means for C26, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

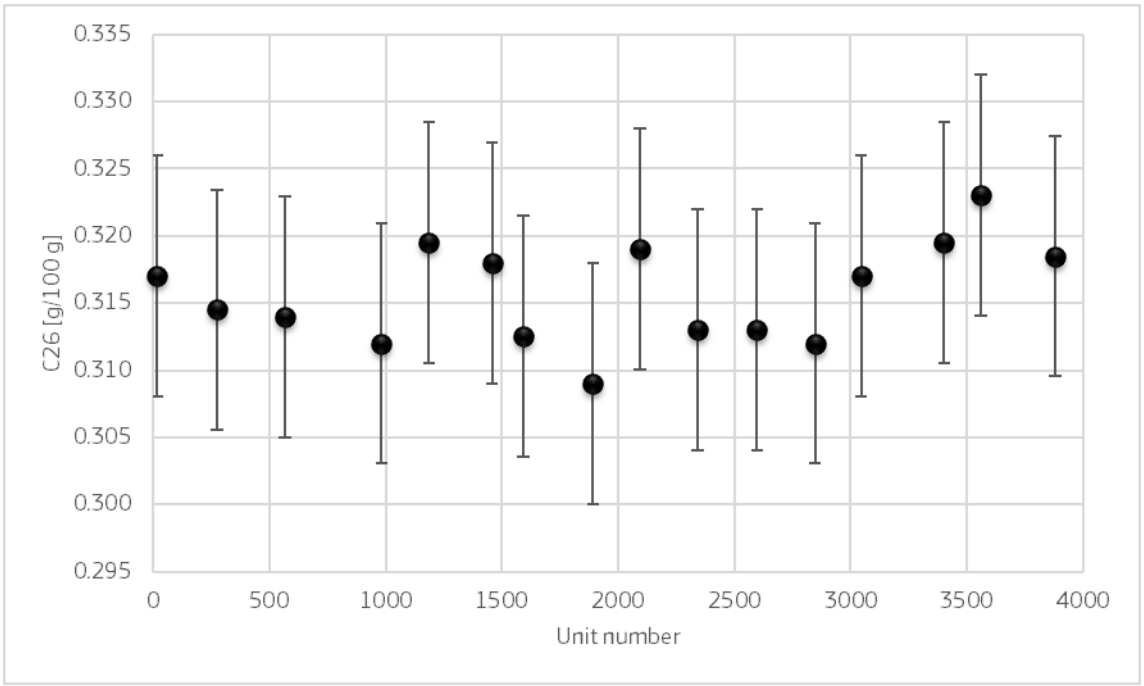


Figure 1.7. Individual measurement replicates for C28 in the order of measurement.

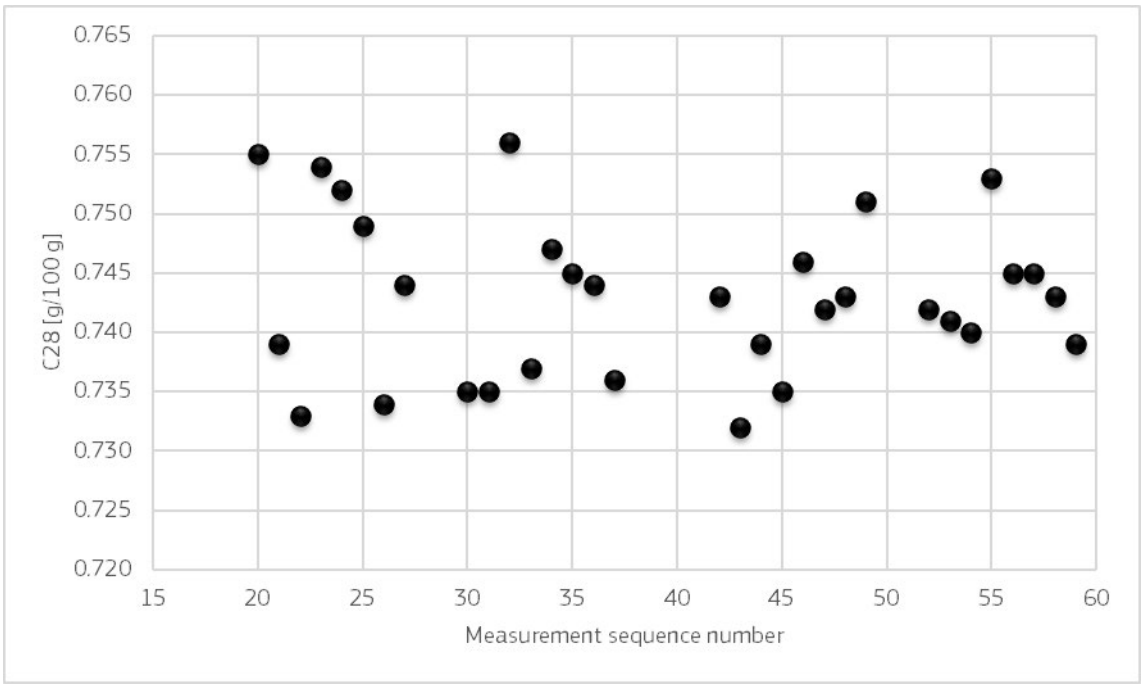


Figure 1.8. Unit means for C28, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

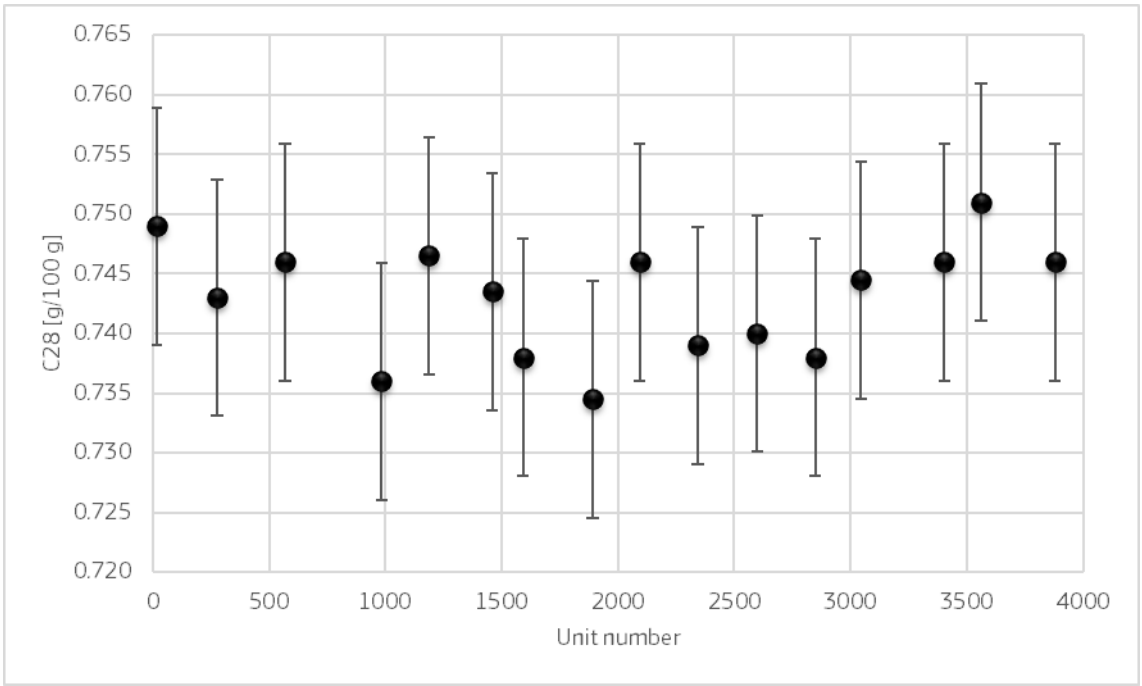


Figure 1.9. Individual measurement replicates for C30 in the order of measurement.

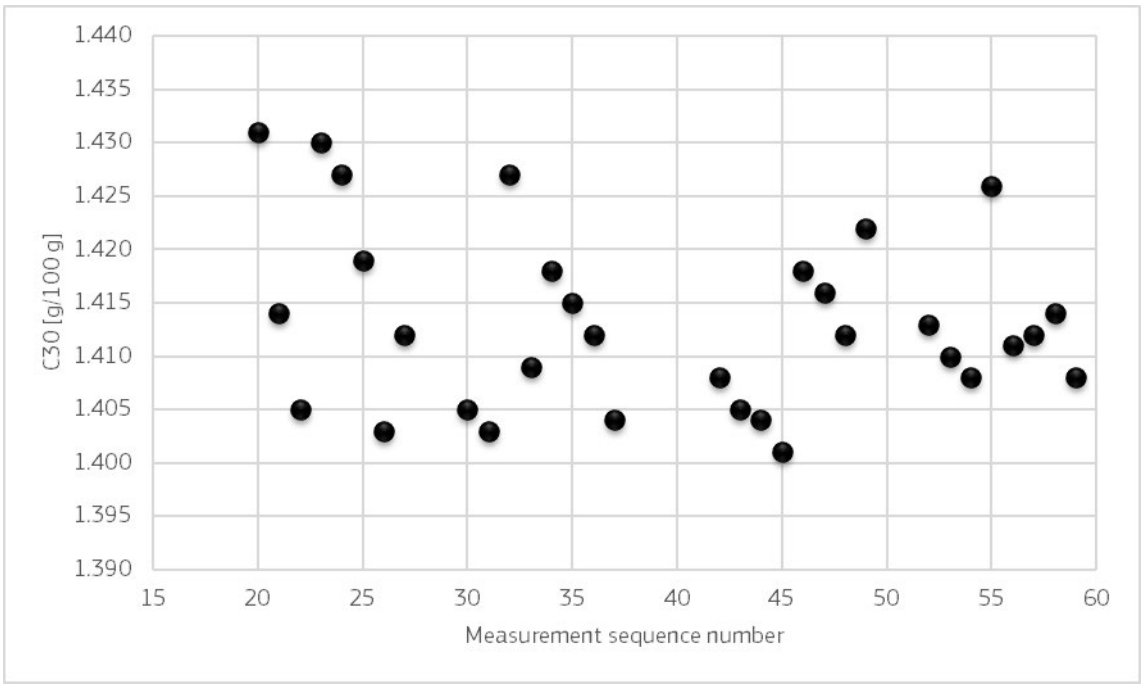


Figure 1.10. Unit means for C30, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

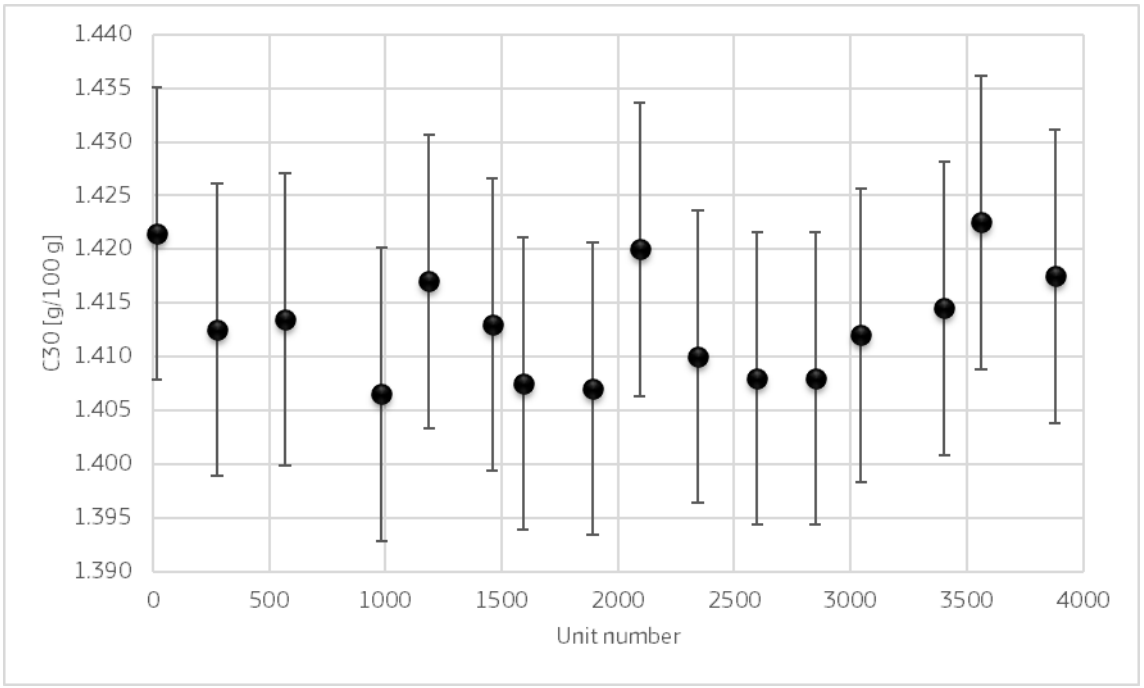


Figure 1.11. Individual measurement replicates for C32 in the order of measurement.

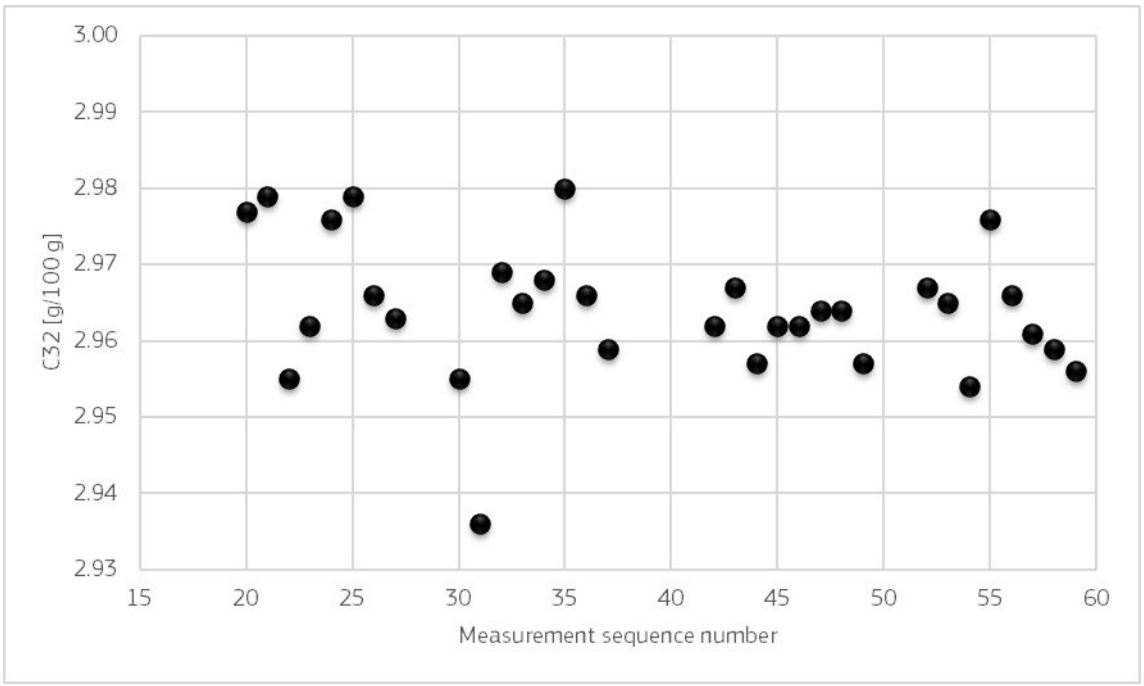


Figure 1.12. Unit means for C32, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

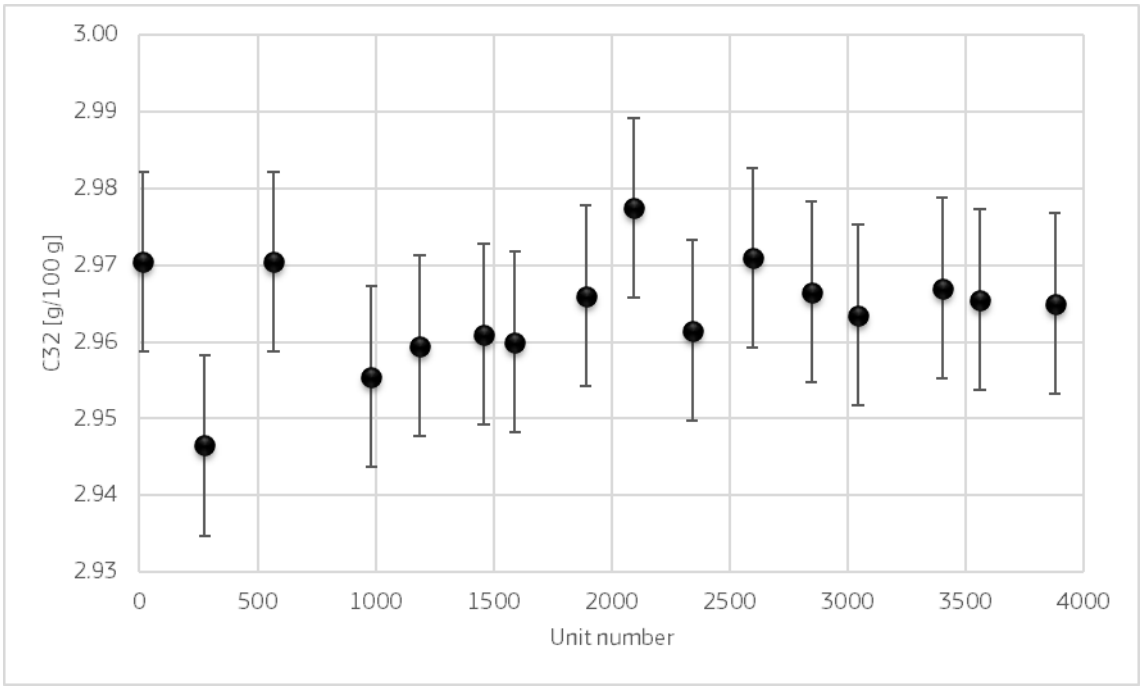


Figure 1.13. Analytical trend-corrected individual measurement replicates for C34 in the order of measurement.

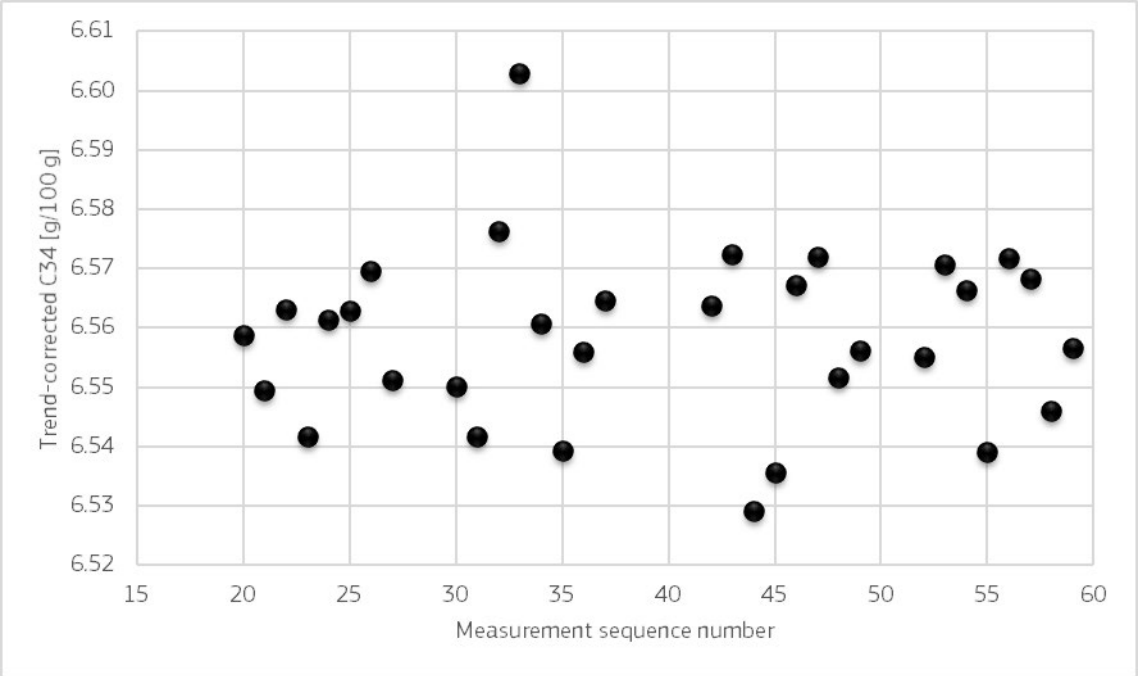


Figure 1.14. Analytical trend-corrected unit means for C34, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

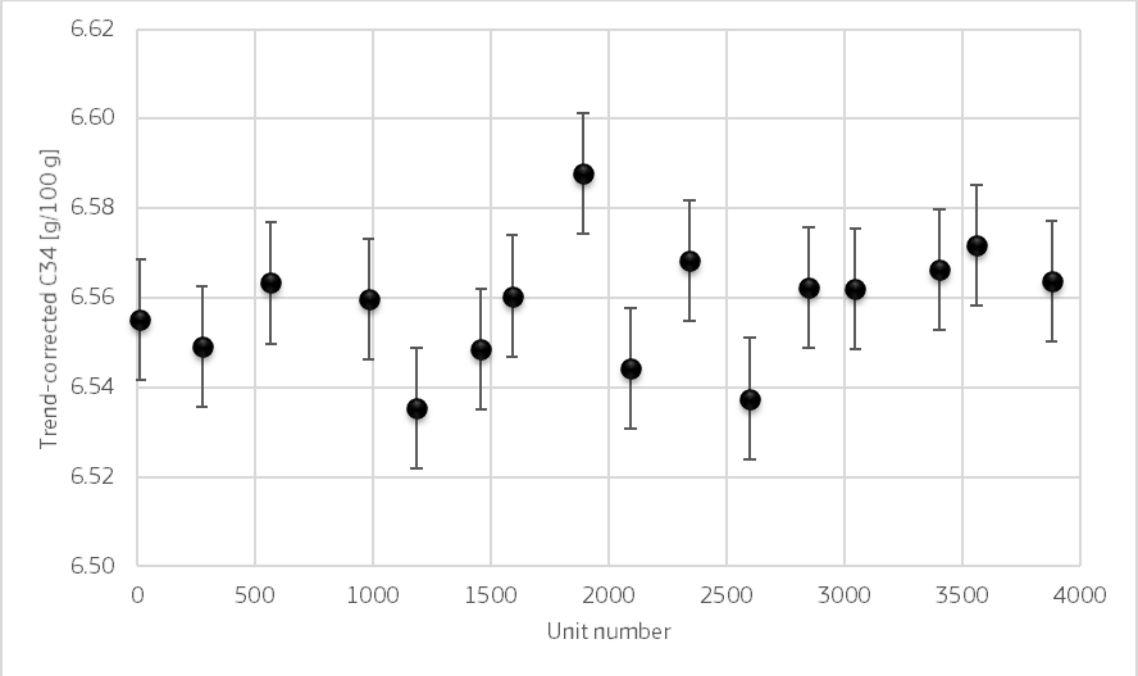


Figure 1.15. Individual measurement replicates for C36 in the order of measurement.

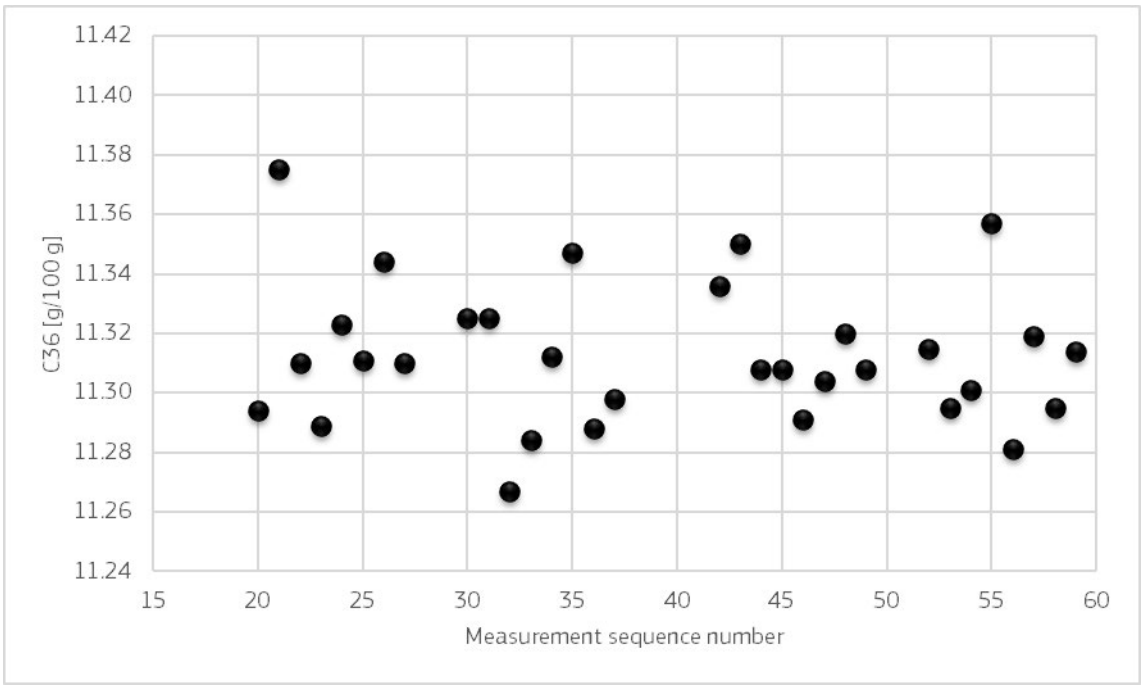


Figure 1.16. Unit means for C36, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

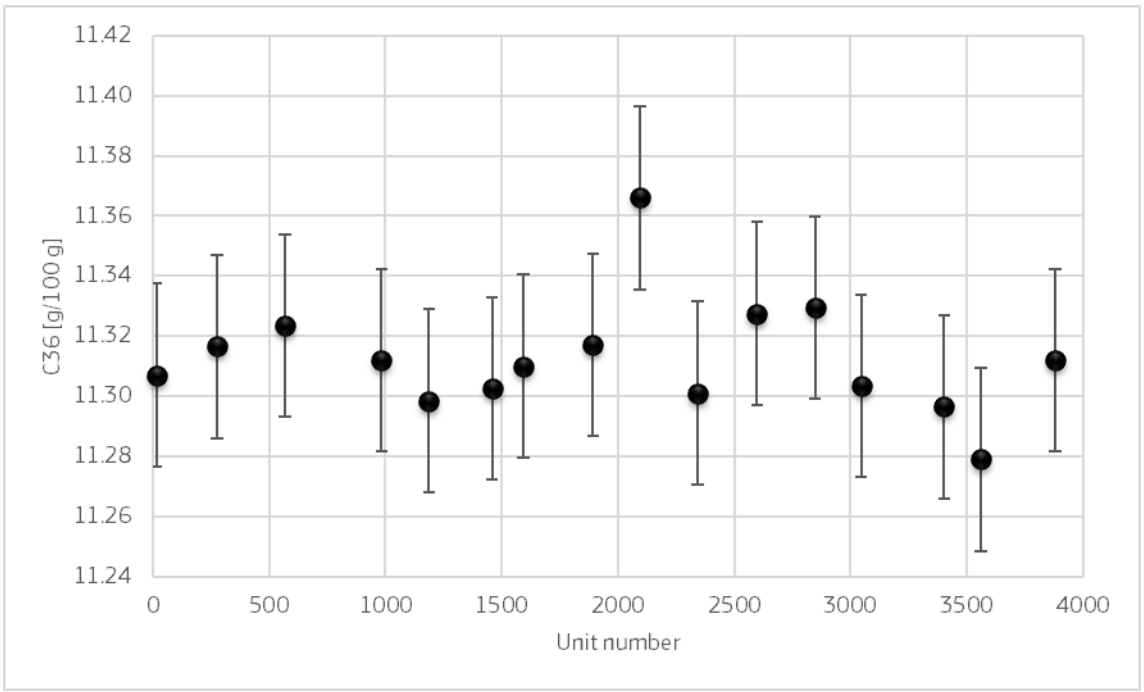


Figure 1.17. Individual measurement replicates for C38 in the order of measurement.

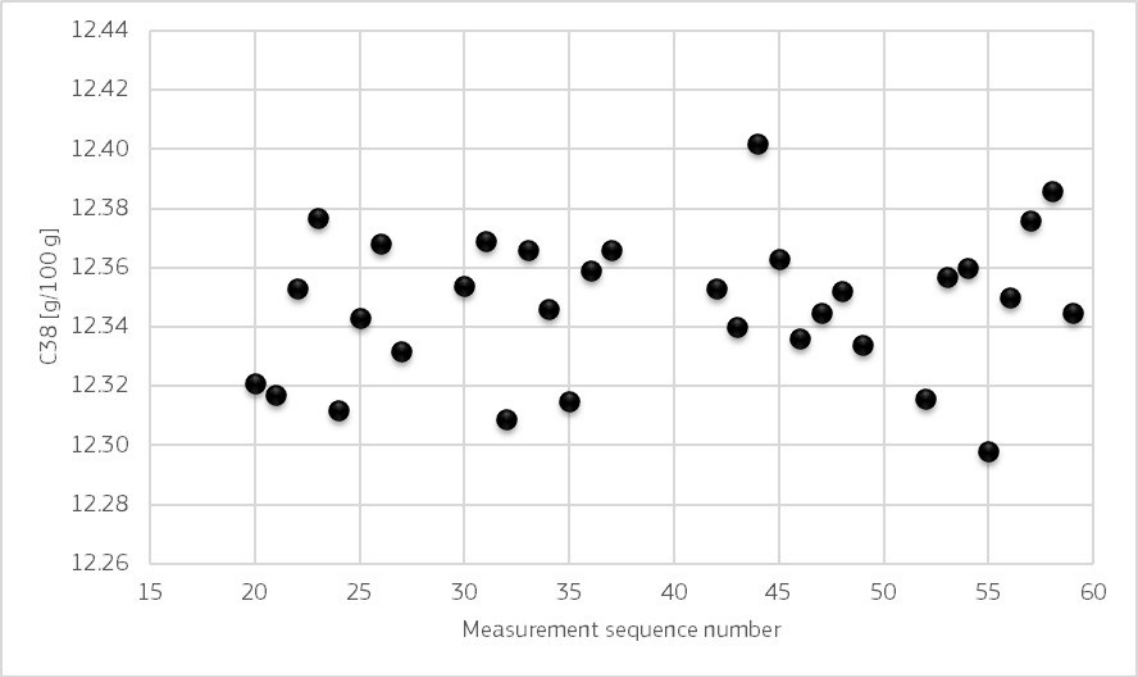


Figure 1.18. Unit means for C38, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

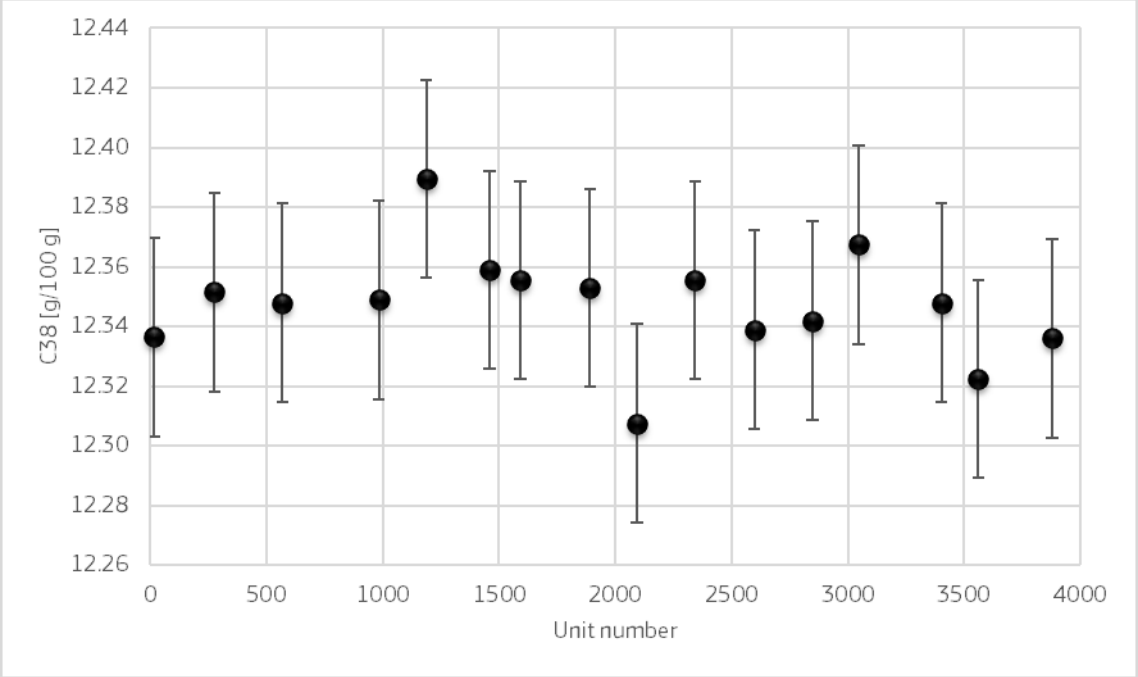


Figure 1.19. Individual measurement replicates for C40 in the order of measurement.

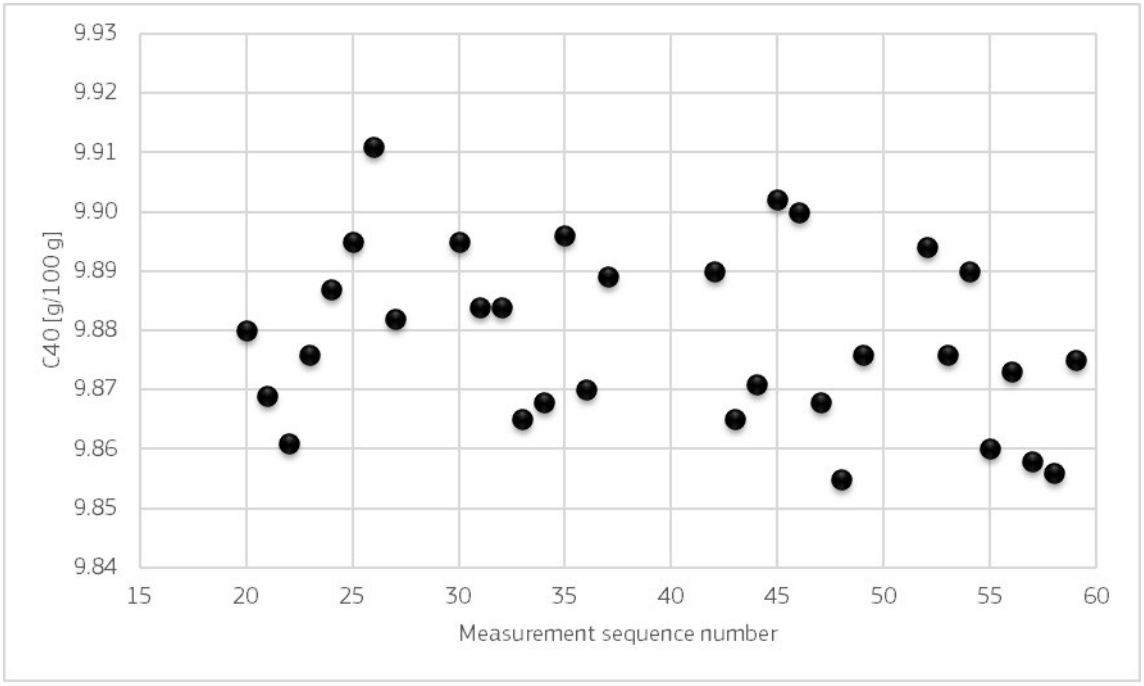


Figure 1.20. Unit means for C40, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

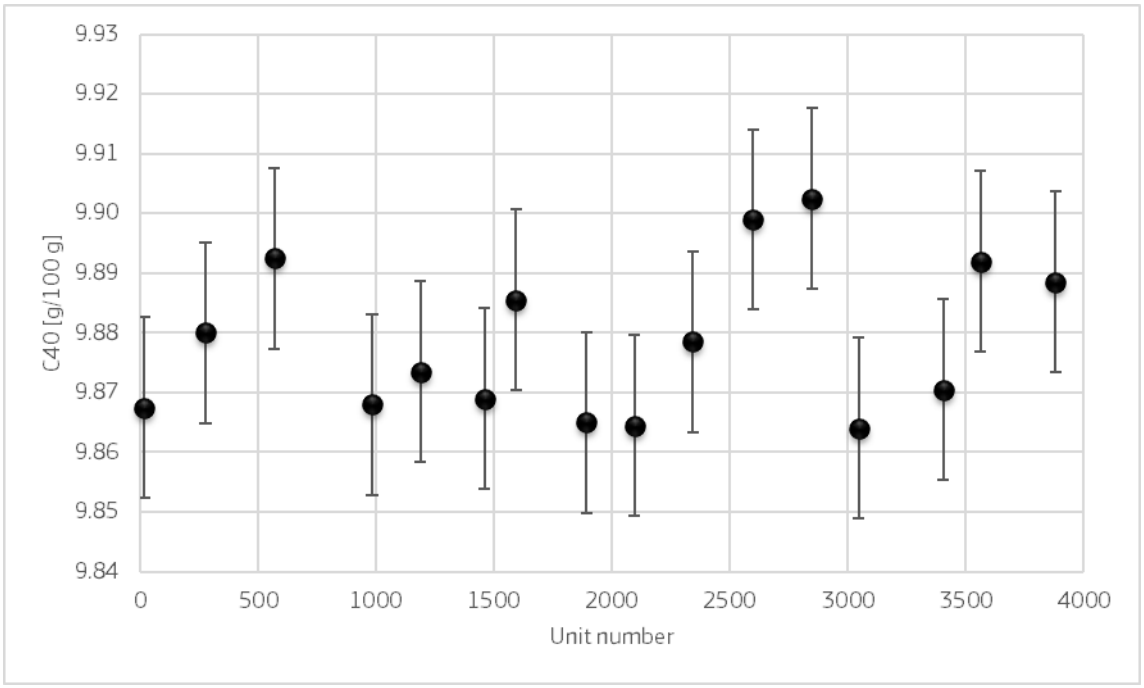


Figure 1.21. Analytical trend-corrected individual measurement replicates for C42 in the order of measurement.

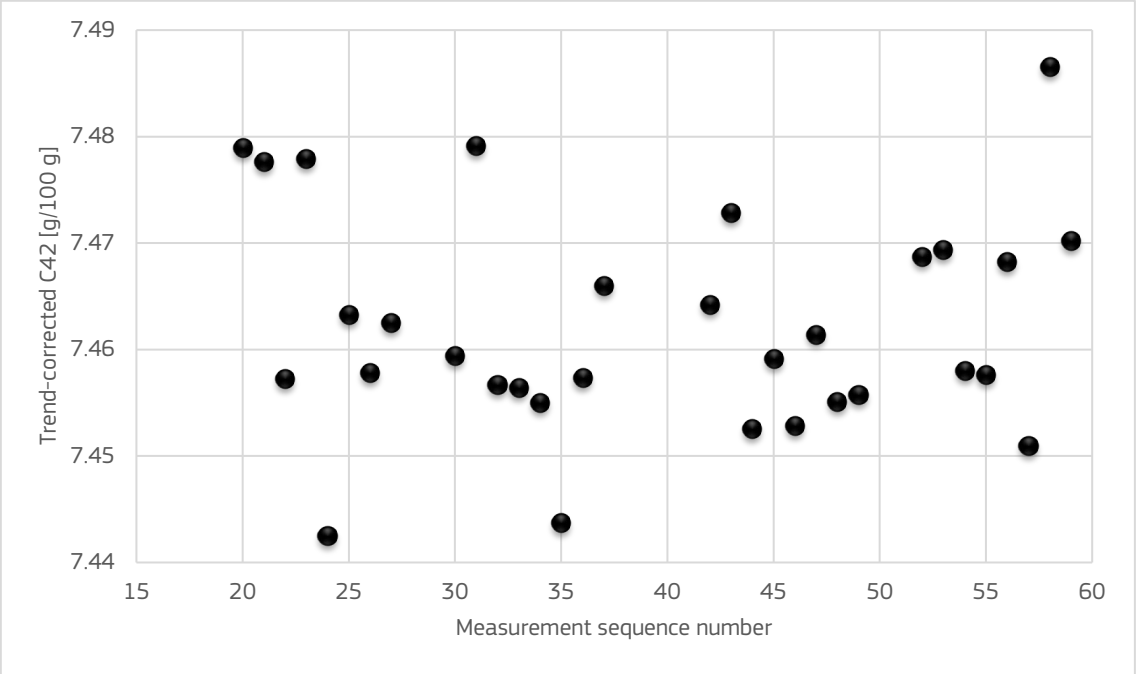


Figure 1.22. Analytical trend-corrected unit means for C42, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

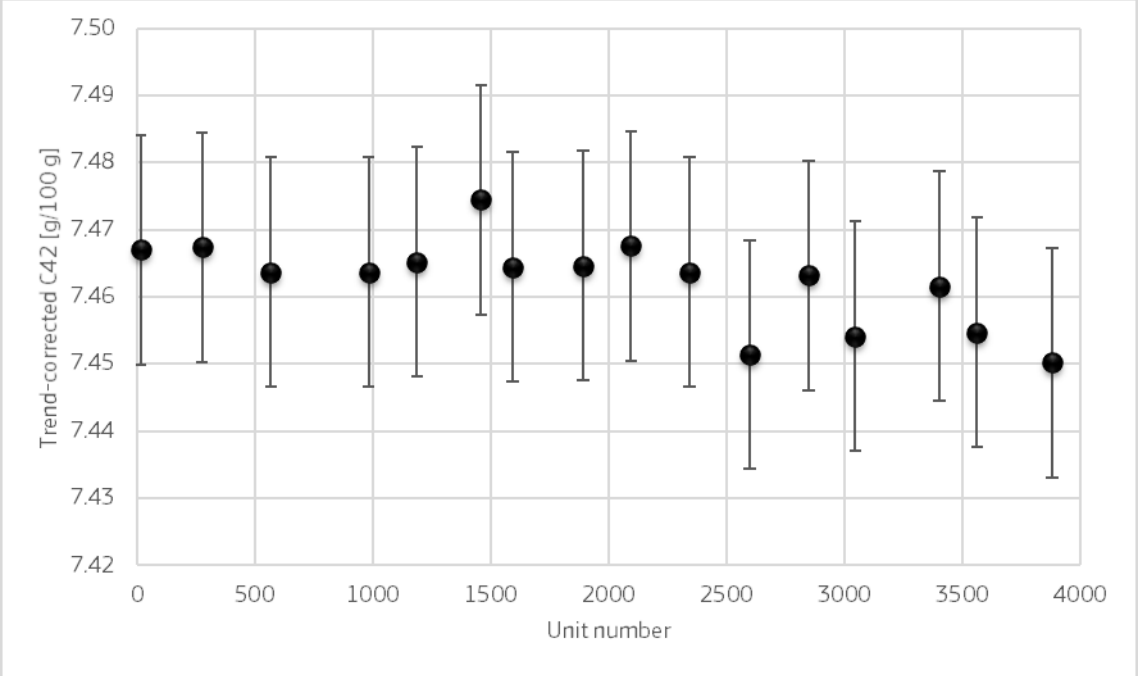


Figure 1.23. Analytical trend-corrected individual measurement replicates for C44 in the order of measurement.

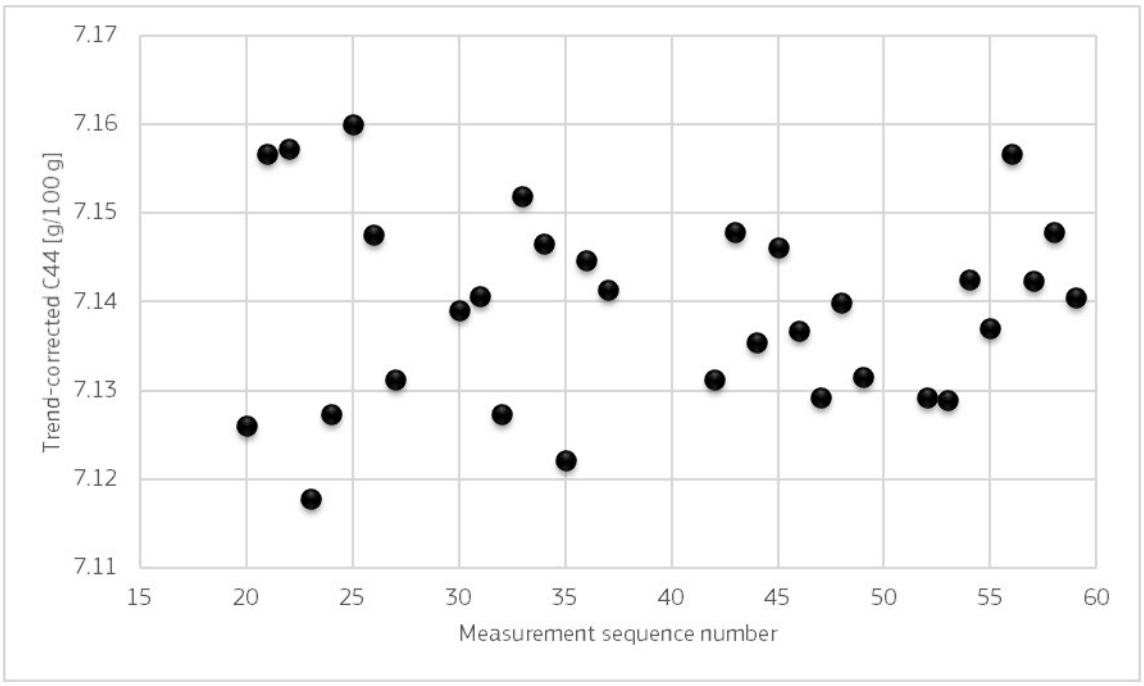


Figure 1.24. Analytical trend-corrected unit means for C44, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

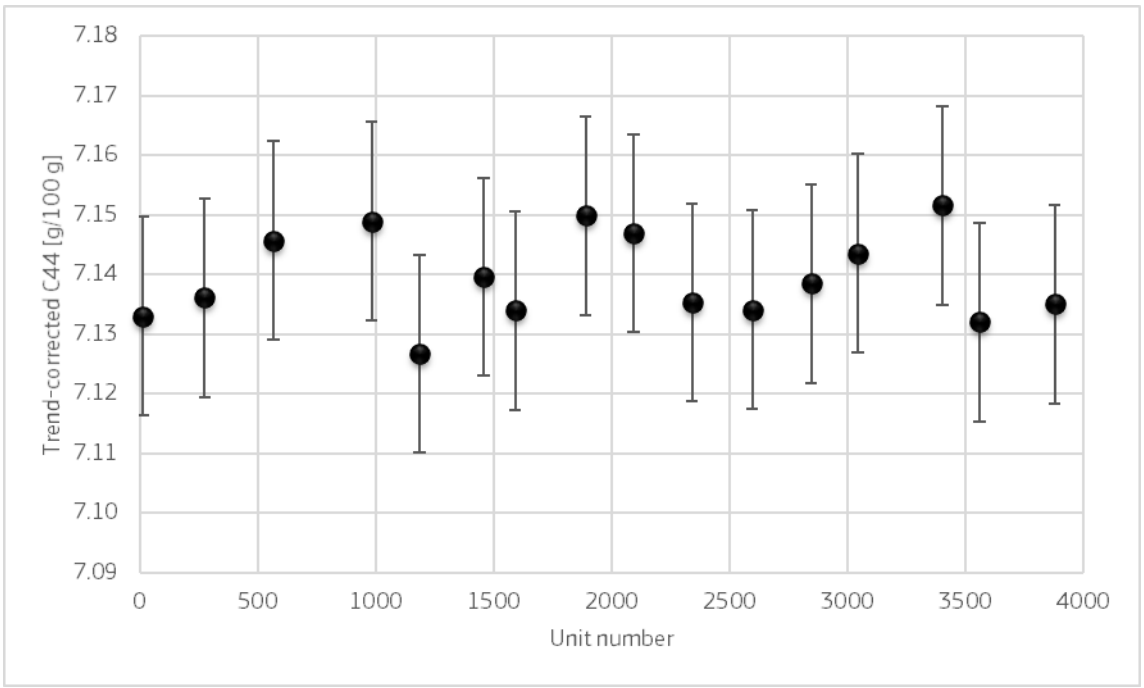


Figure 1.25. Analytical trend-corrected individual measurement replicates for C46 in the order of measurement.

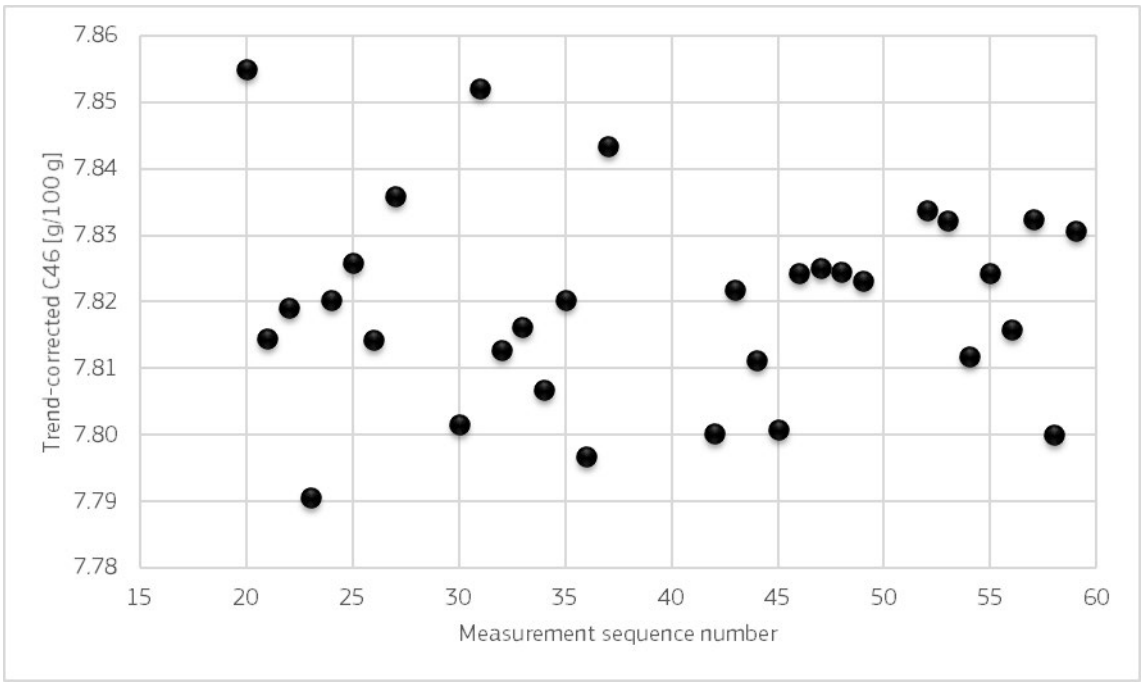


Figure 1.26. Analytical trend-corrected unit means for C46, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

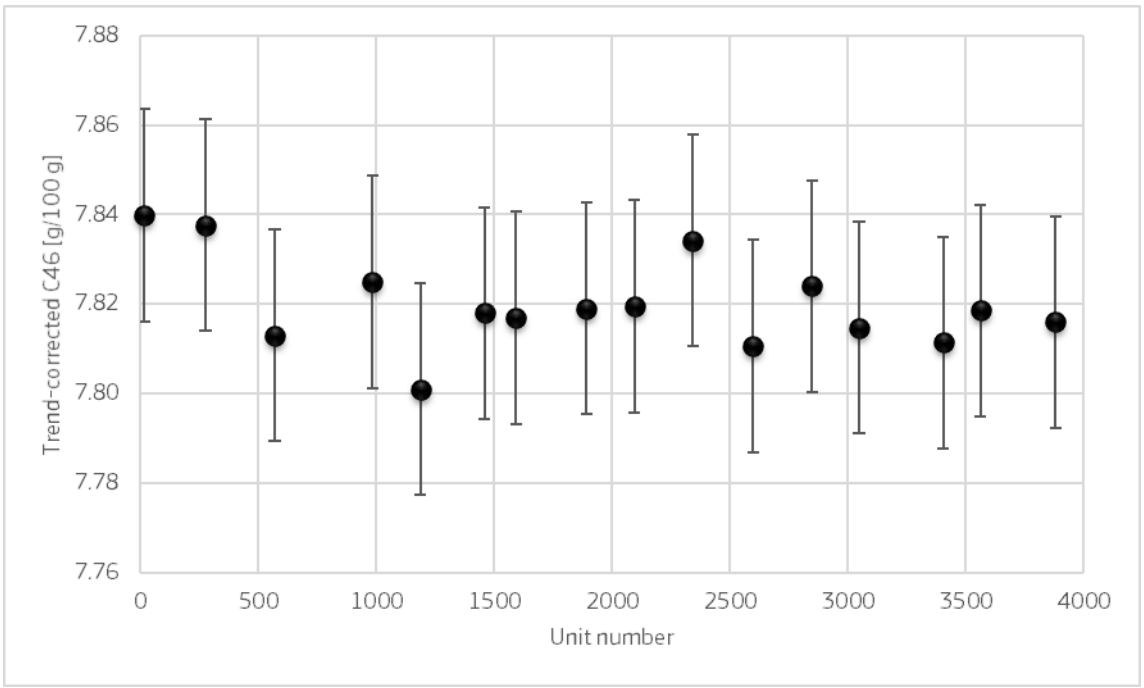


Figure 1.27. Individual measurement replicates for C48 in the order of measurement.

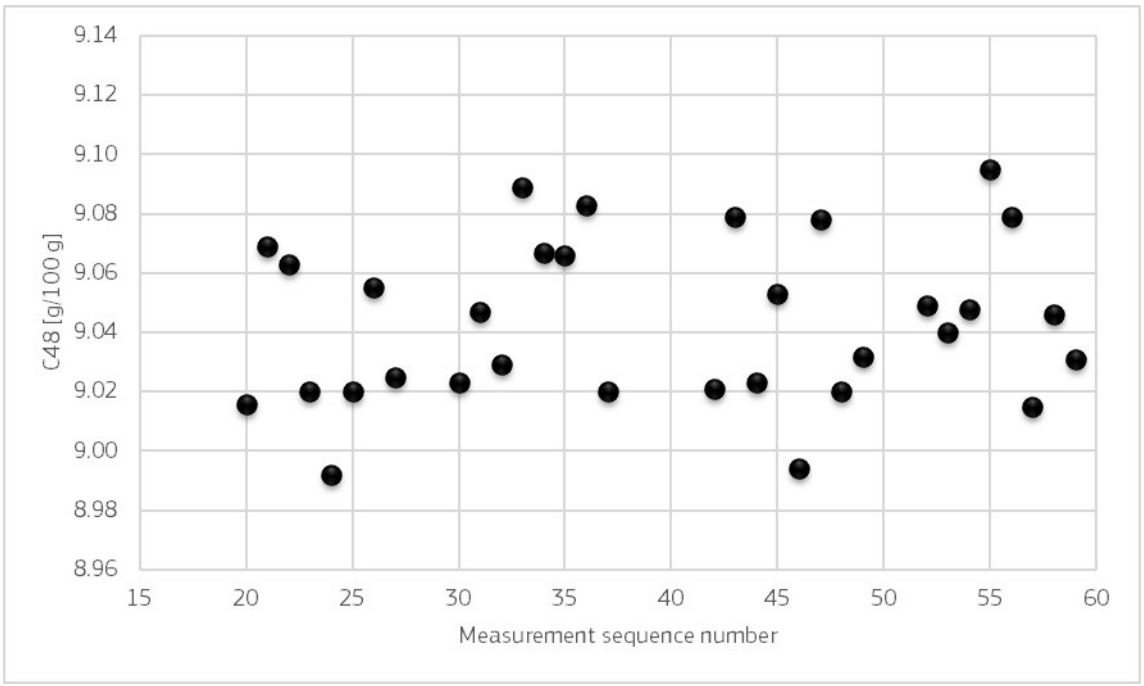


Figure 1.28. Unit means for C48, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

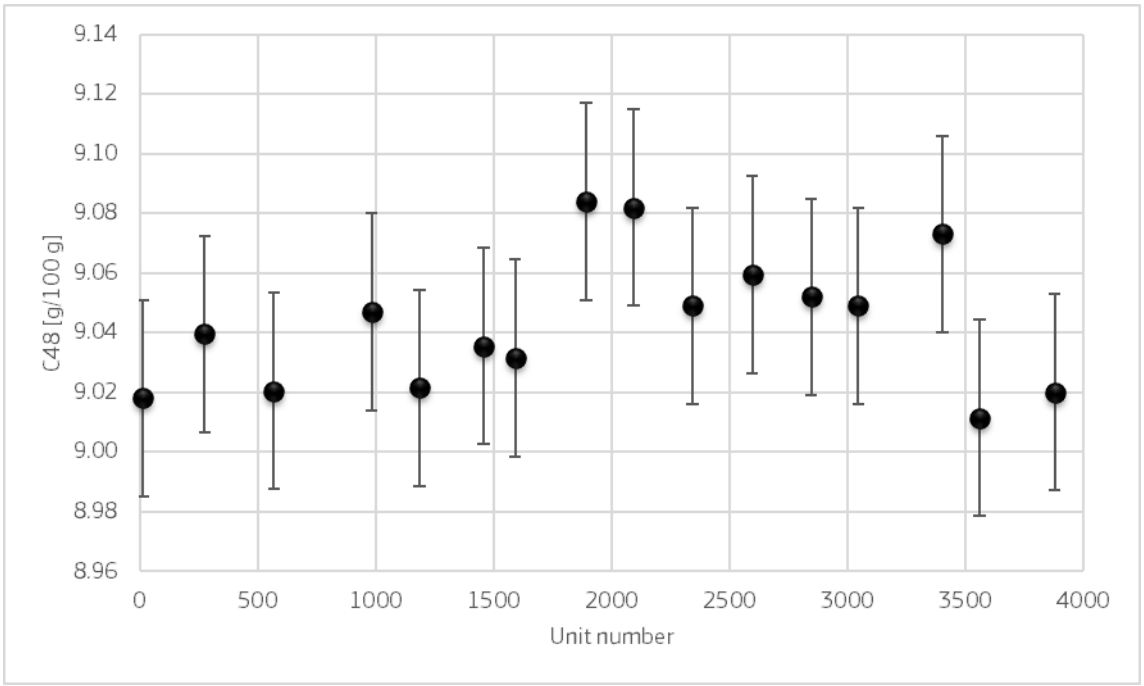


Figure 1.29. Analytical trend-corrected individual measurement replicates for C50 in the order of measurement.

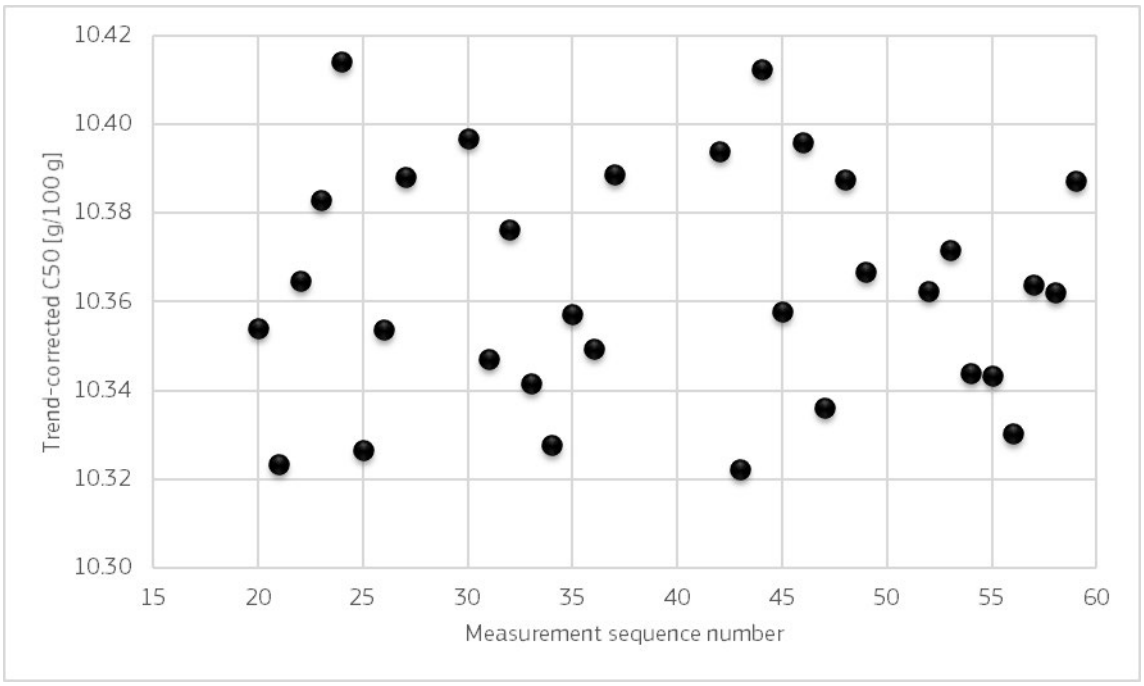


Figure 1.30. Analytical trend-corrected unit means for C50, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

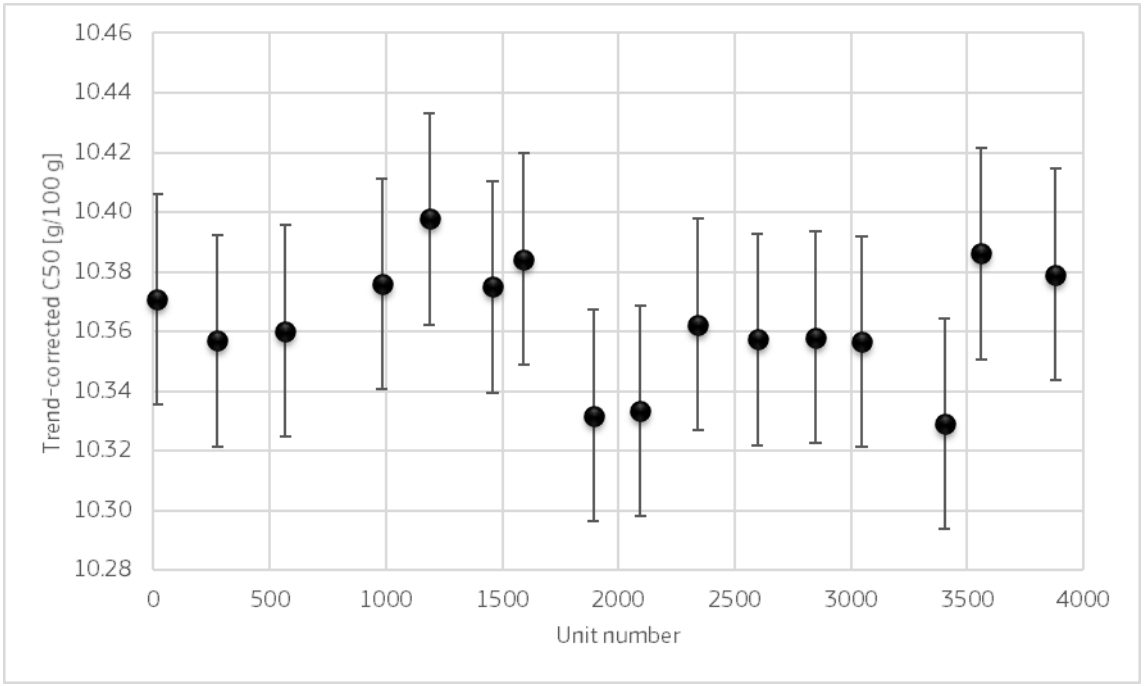


Figure 1.31. Analytical trend-corrected individual measurement replicates for C52 in the order of measurement.

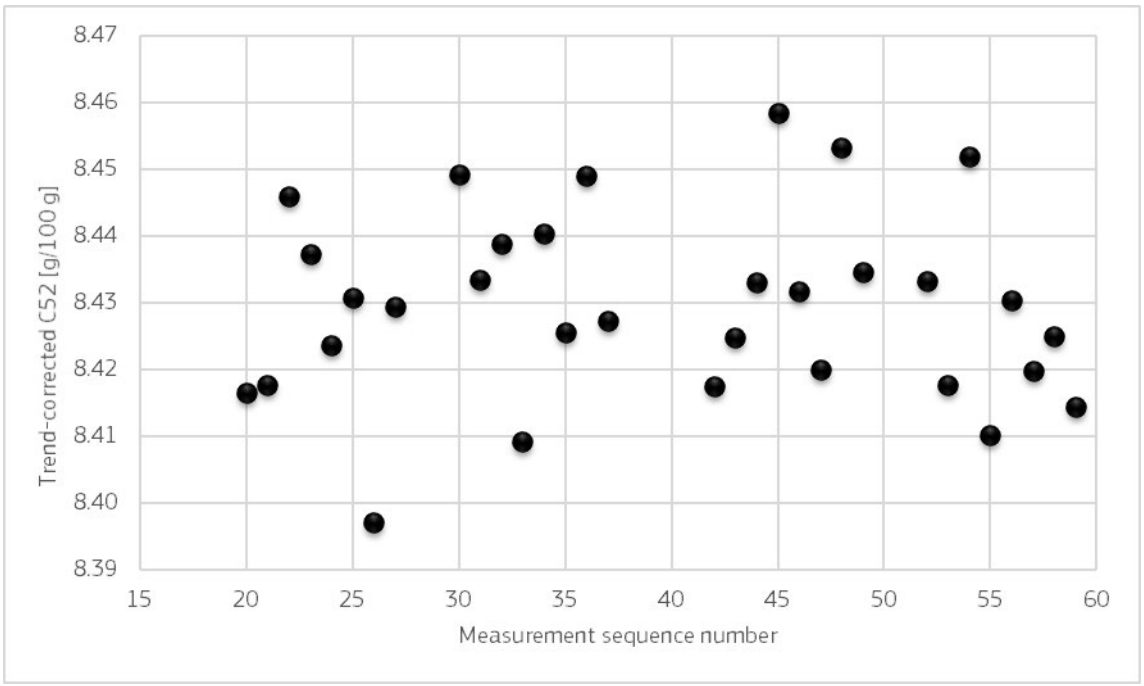


Figure 1.32. Analytical trend-corrected unit means for C52, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.

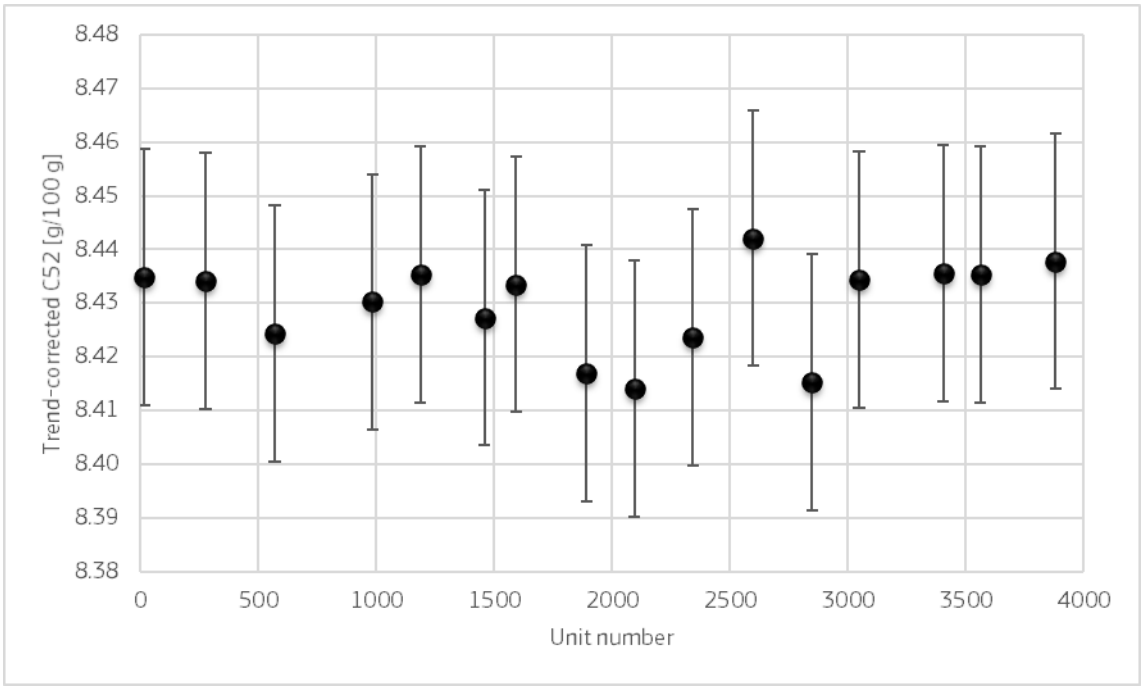


Figure 1.33. Individual measurement replicates for C54 in the order of measurement.

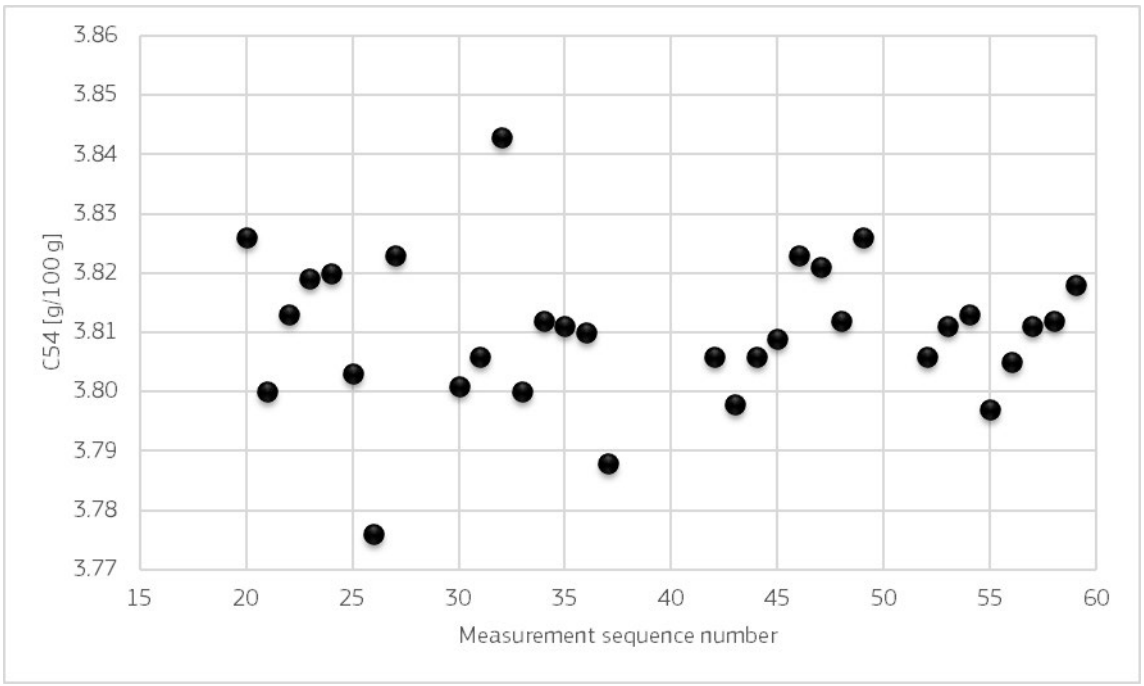
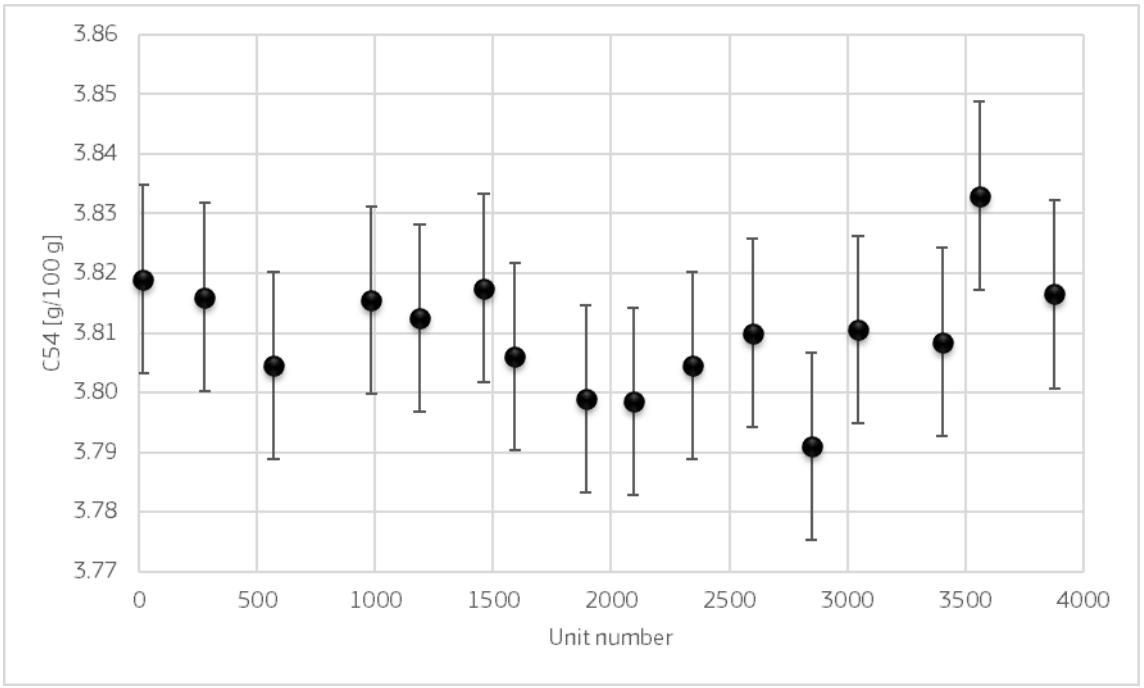


Figure 1.34. Unit means for C54, against unit number. Error bars are a 95 % confidence interval derived from s_{wb} from ANOVA for all units of the homogeneity study.



Annex 2. Results of the short-term stability measurements

In each graph the means measured at each time-point are given. The error bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA. Results are expressed as relative mass fractions [g/100 g], i.e. the mass fraction of each triglyceride (C24, C26, C28, C30, C32, C34, C36, C38, C40, C42, C44, C46, C48, C50, C52, C54) and cholesterol is expressed as a percentage of the total triglycerides.

Figure 2.1. Transport stability of C24 in ERM-BD519 assessed at 60 °C over four weeks.

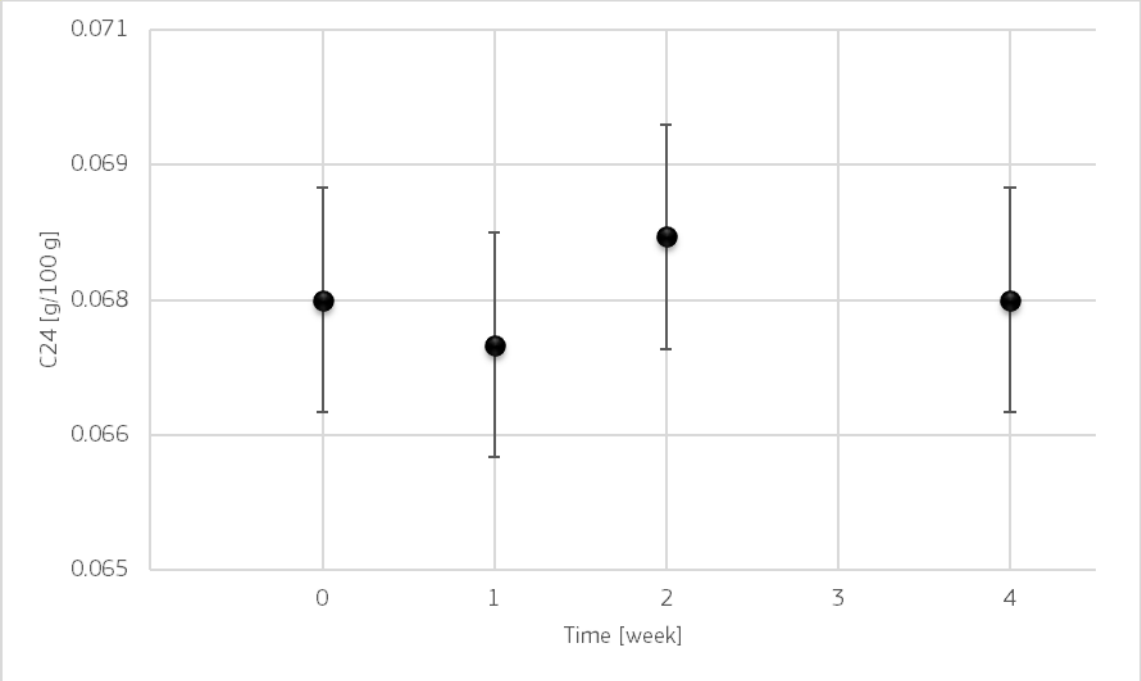


Figure 2.2. Transport stability of cholesterol in ERM-BD519 assessed at 60 °C over four weeks.

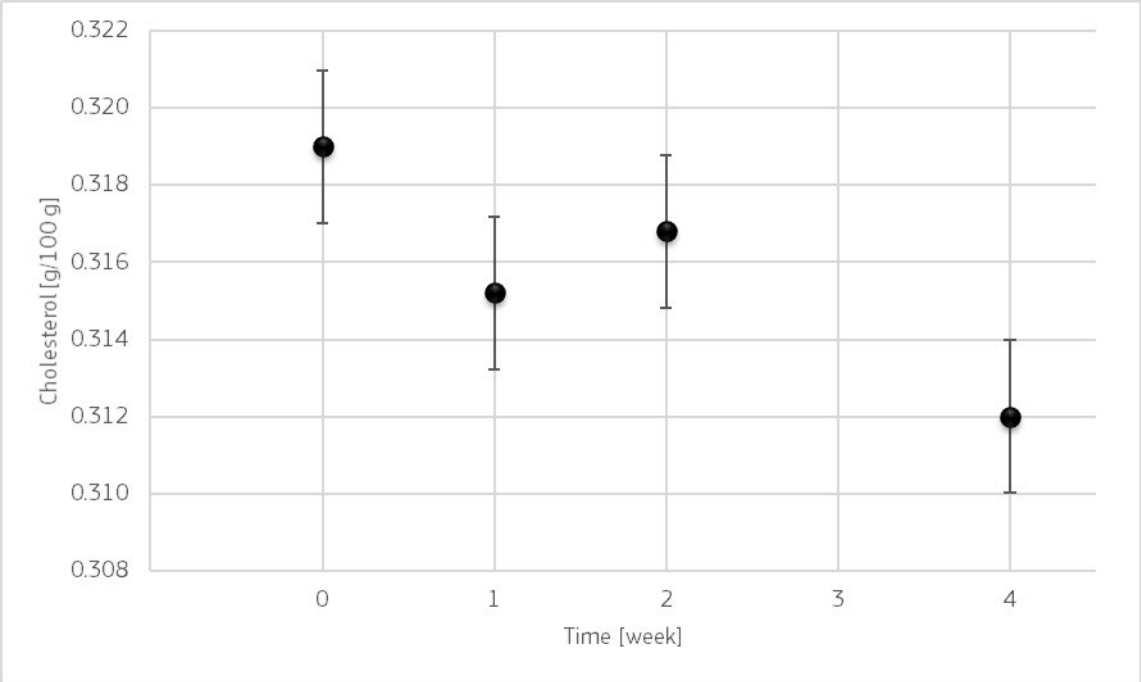


Figure 2.3. Transport stability of C26 in ERM-BD519 assessed at 60 °C over four weeks.

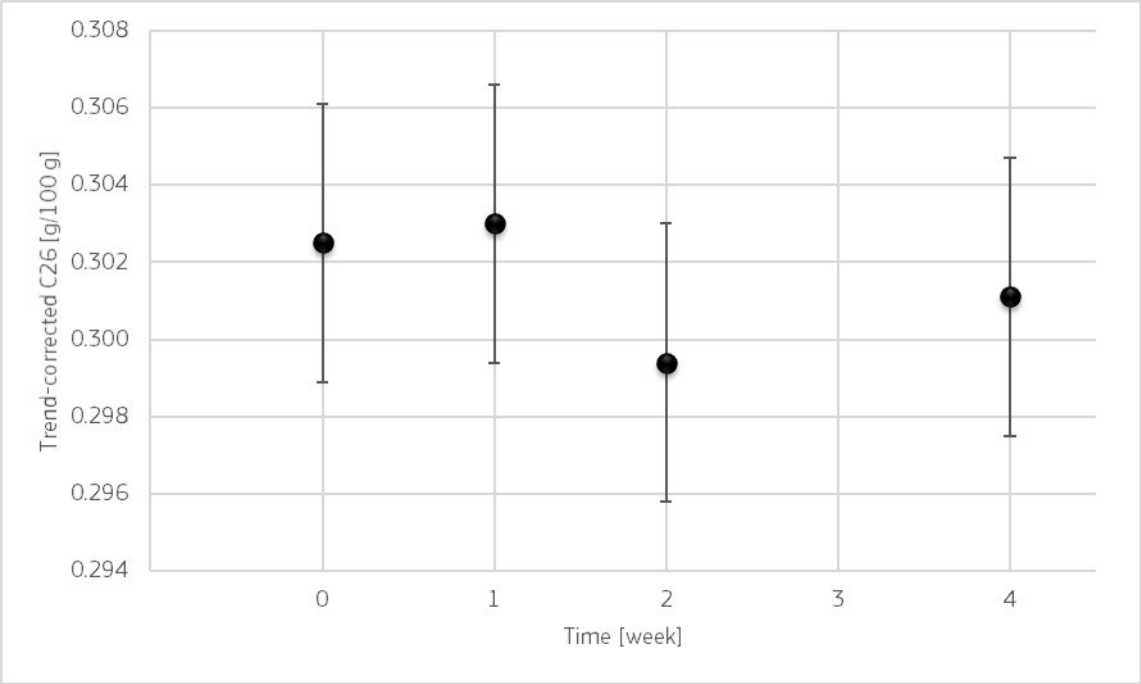


Figure 2.4. Transport stability of C28 in ERM-BD519 assessed at 60 °C over four weeks.

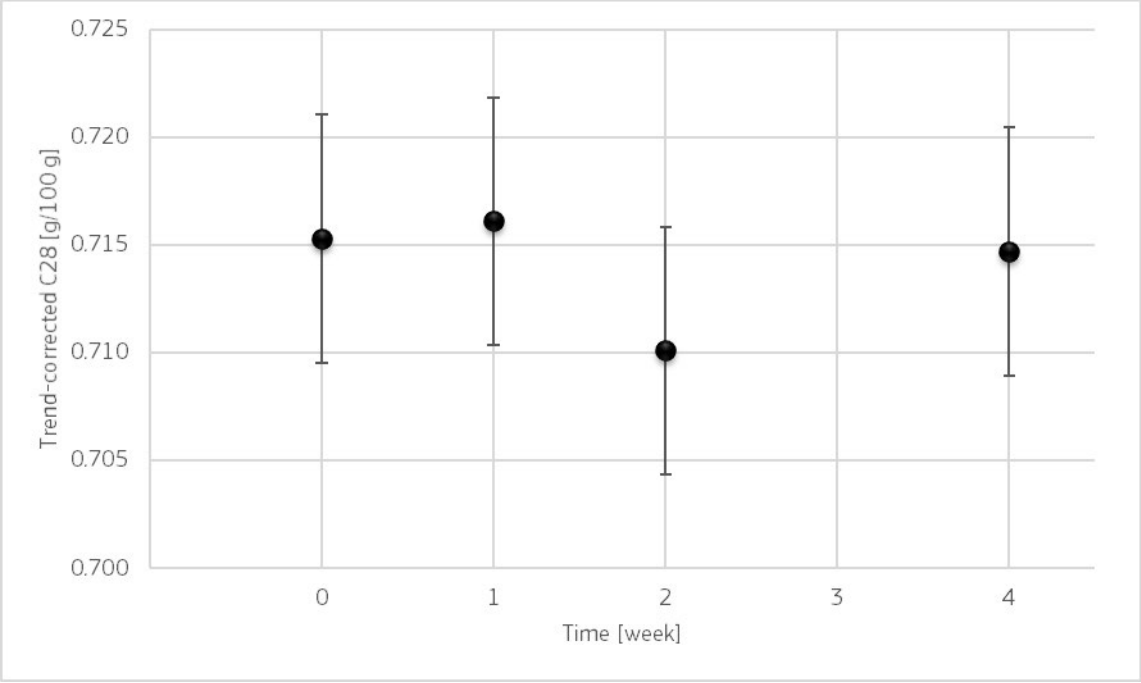


Figure 2.5. Transport stability of C30 in ERM-BD519 assessed at 60 °C over four weeks.

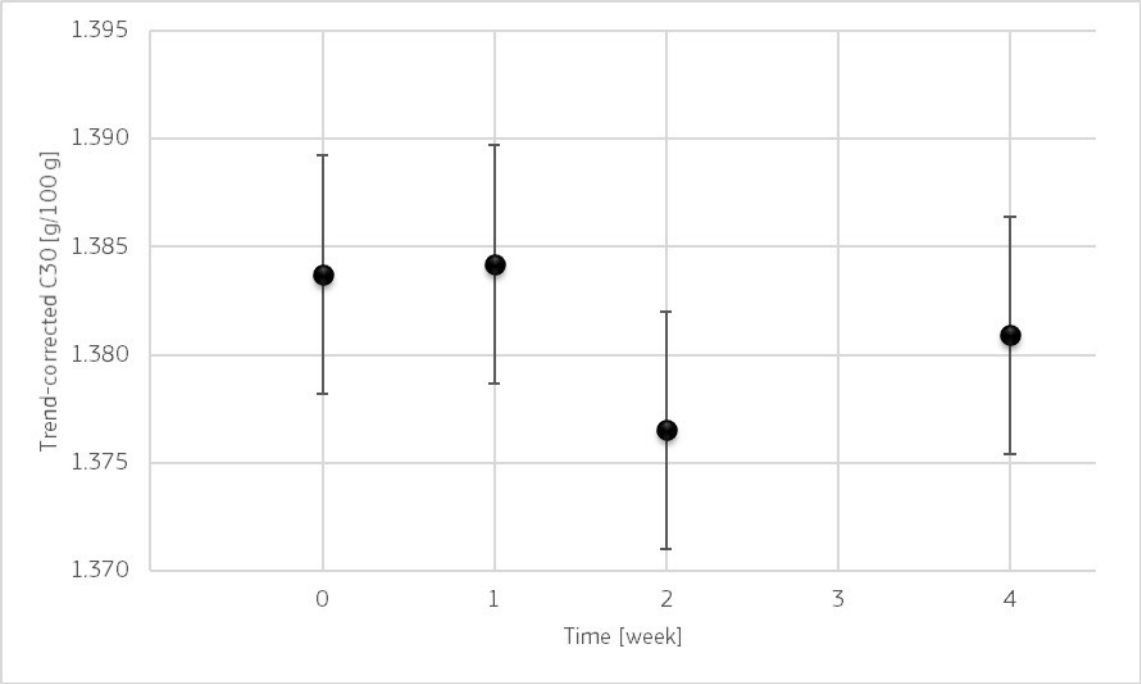


Figure 2.6. Transport stability of C32 in ERM-BD519 assessed at 60 °C over four weeks.

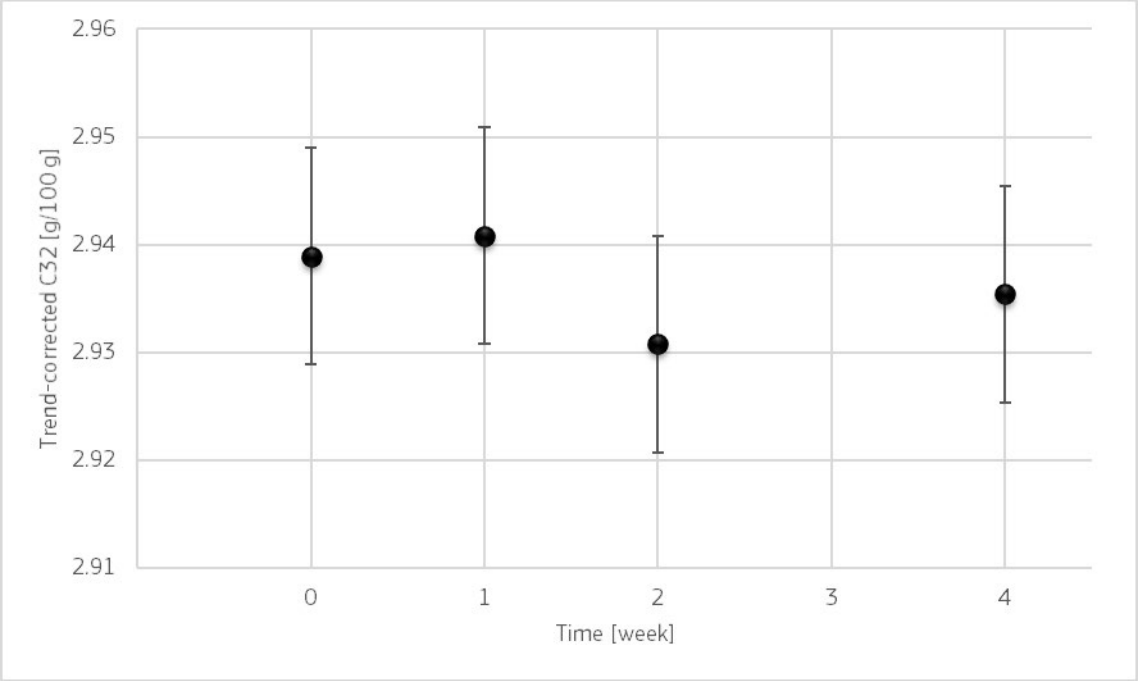


Figure 2.7. Transport stability of C34 in ERM-BD519 assessed at 60 °C over four weeks.

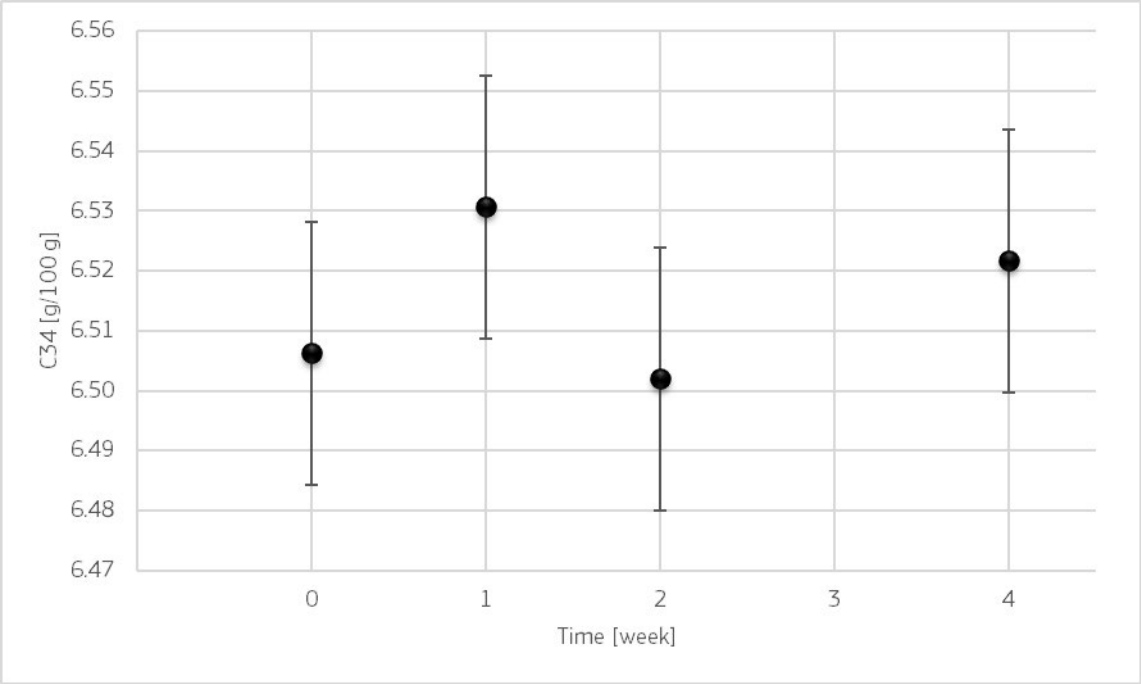


Figure 2.8. Transport stability of C36 in ERM-BD519 assessed at 60 °C over four weeks.

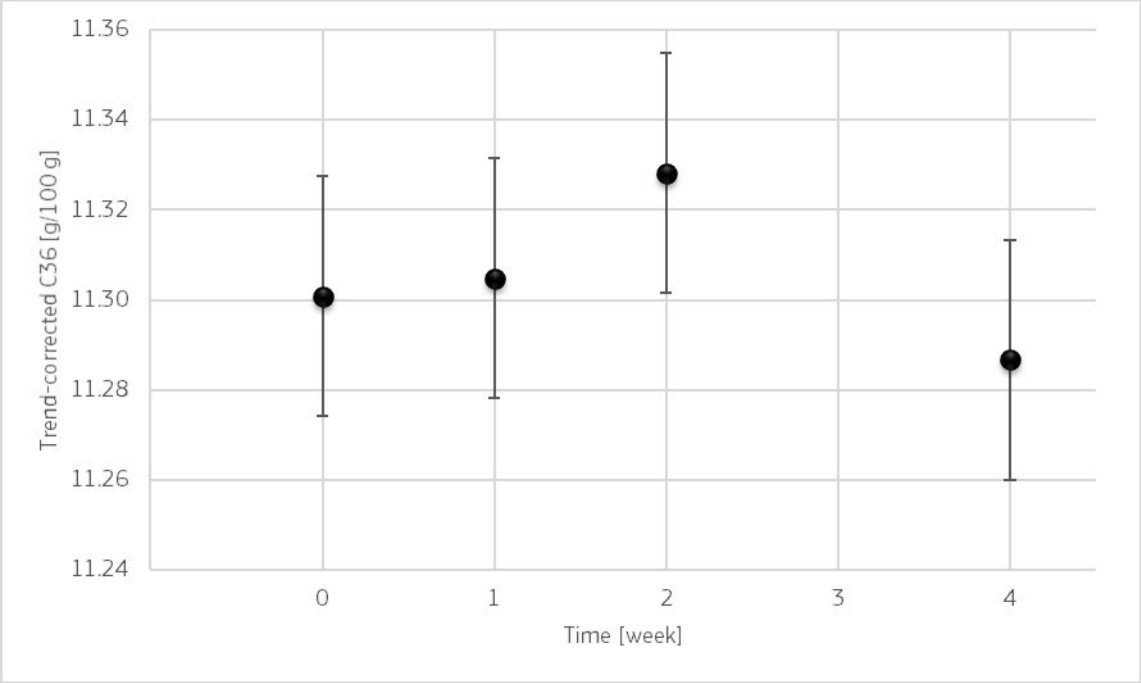


Figure 2.9. Transport stability of C38 in ERM-BD519 assessed at 60 °C over four weeks.

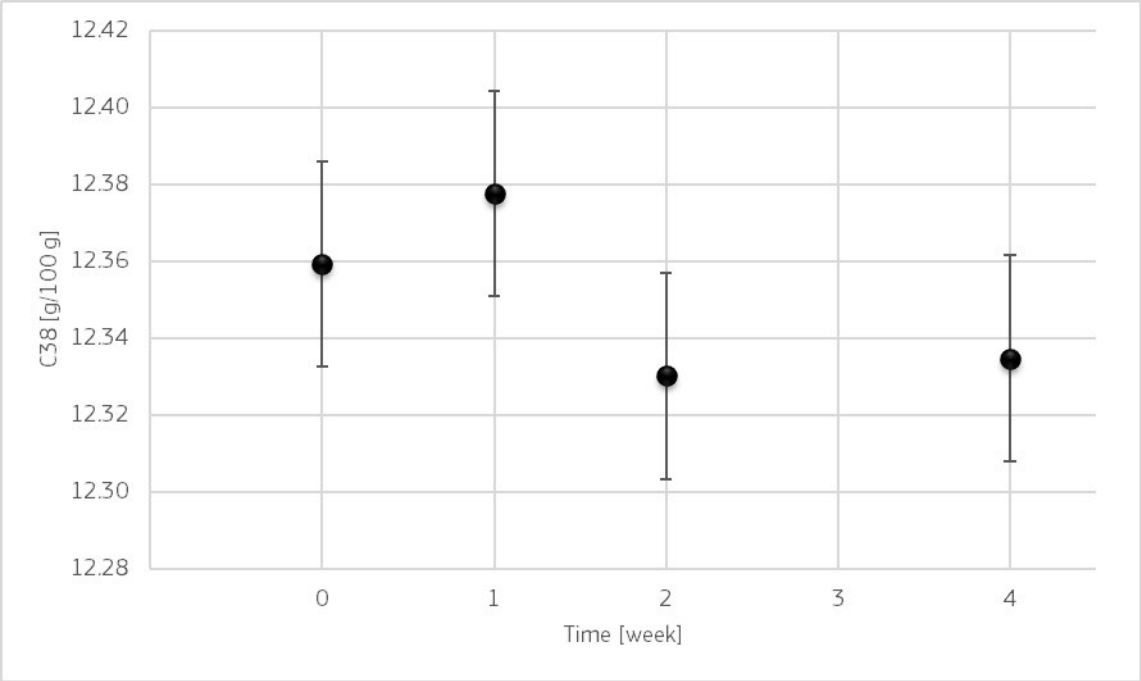


Figure 2.10. Transport stability of C40 in ERM-BD519 assessed at 60 °C over four weeks.

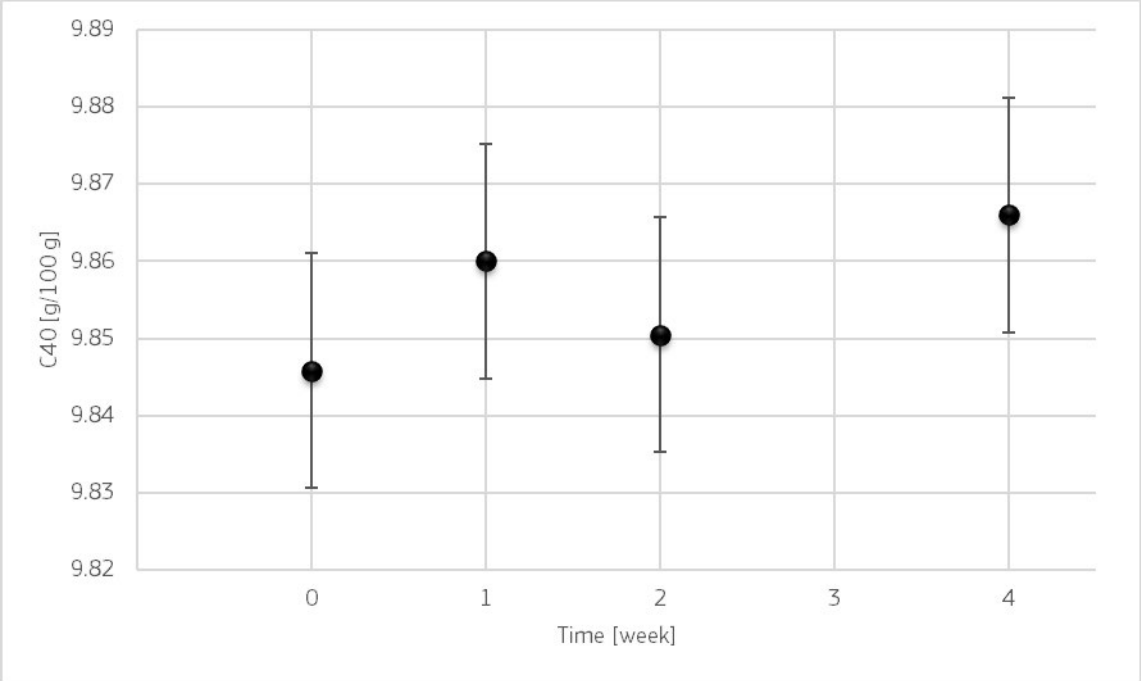


Figure 2.11. Transport stability of C42 in ERM-BD519 assessed at 60 °C over four weeks.

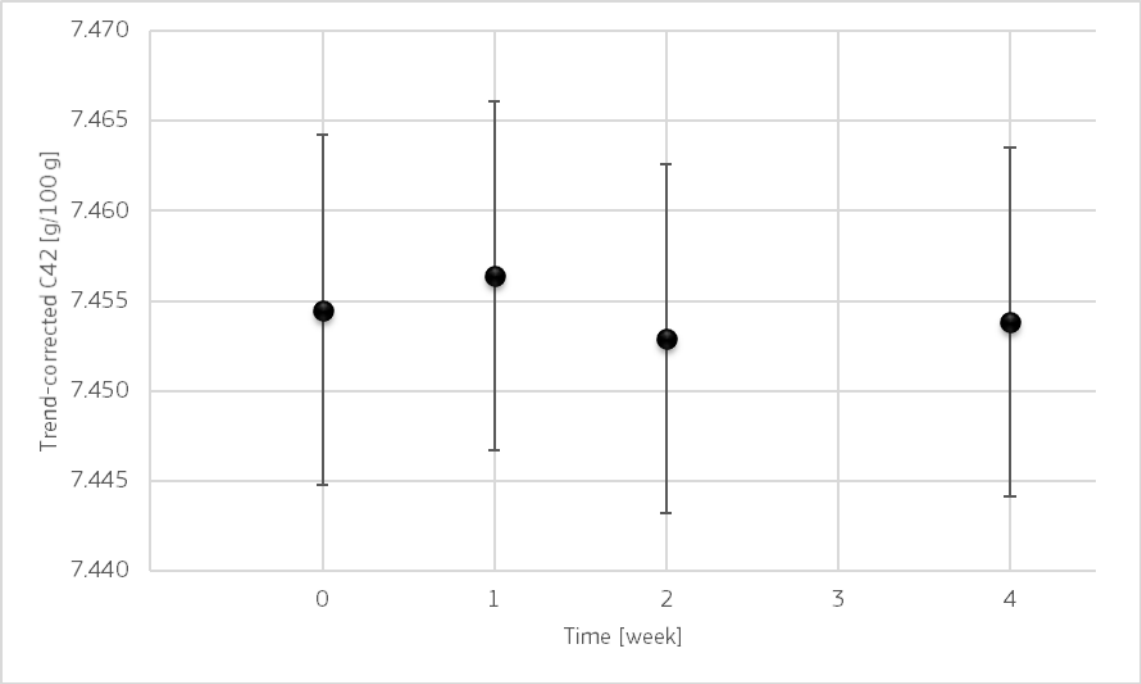


Figure 2.12. Transport stability of C44 in ERM-BD519 assessed at 60 °C over four weeks.

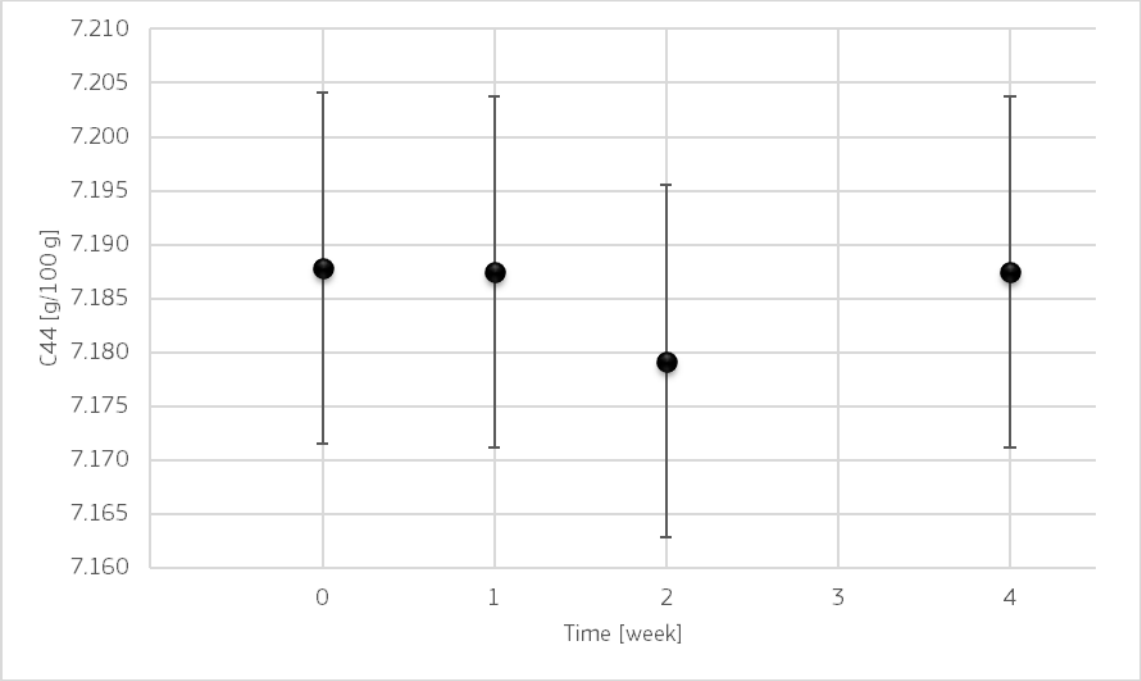


Figure 2.13. Transport stability of C46 in ERM-BD519 assessed at 60 °C over four weeks.

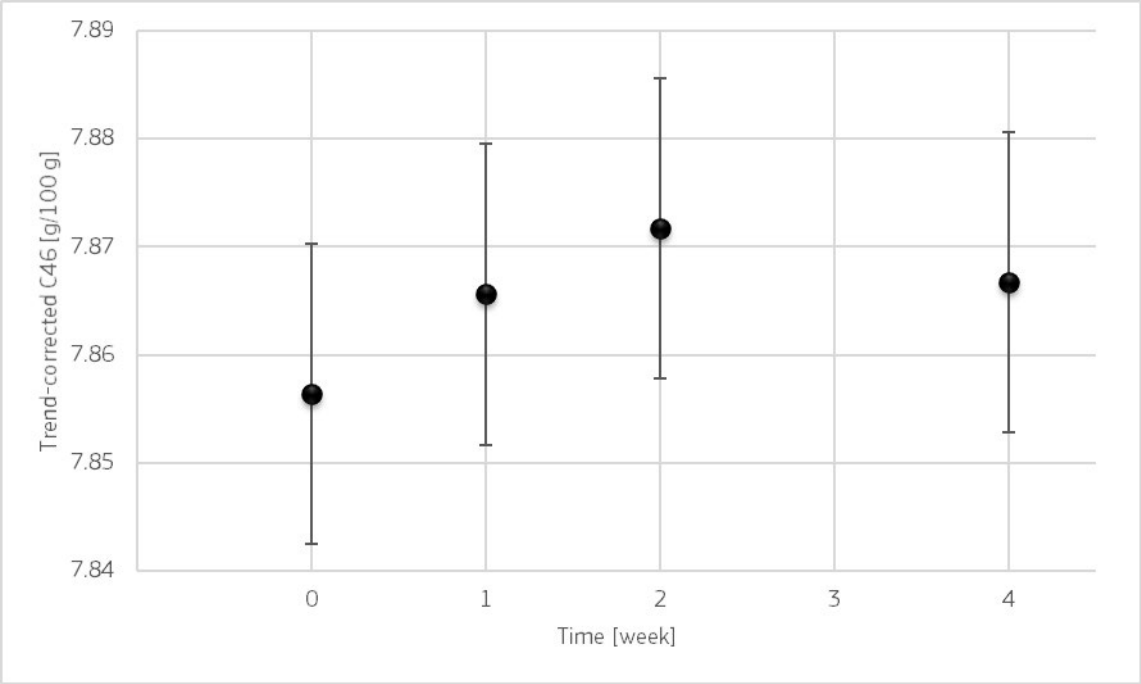


Figure 2.14. Transport stability of C48 in ERM-BD519 assessed at 60 °C over four weeks.

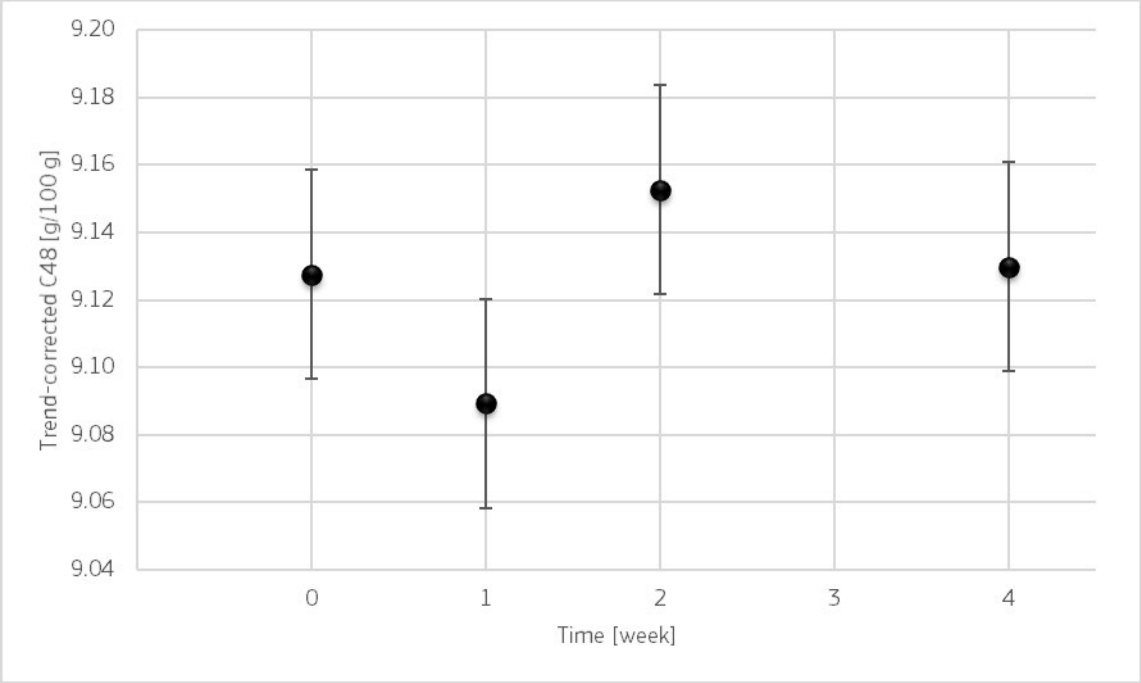


Figure 2.15. Transport stability of C50 in ERM-BD519 assessed at 60 °C over four weeks.

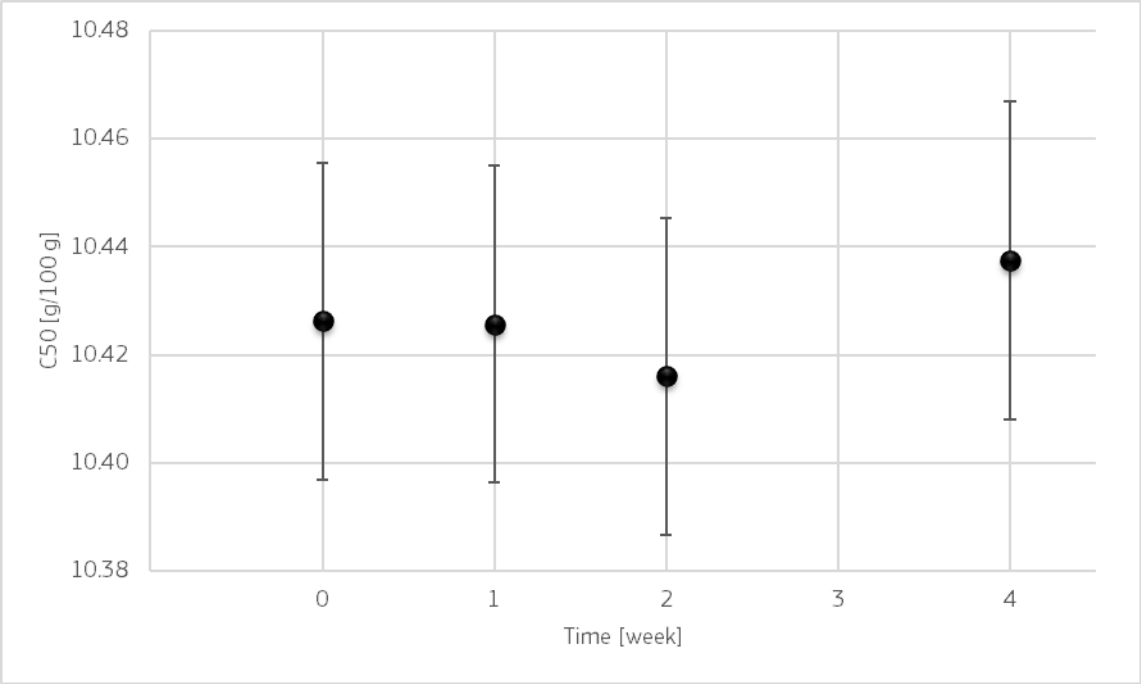


Figure 2.16. Transport stability of C52 in ERM-BD519 assessed at 60 °C over four weeks.

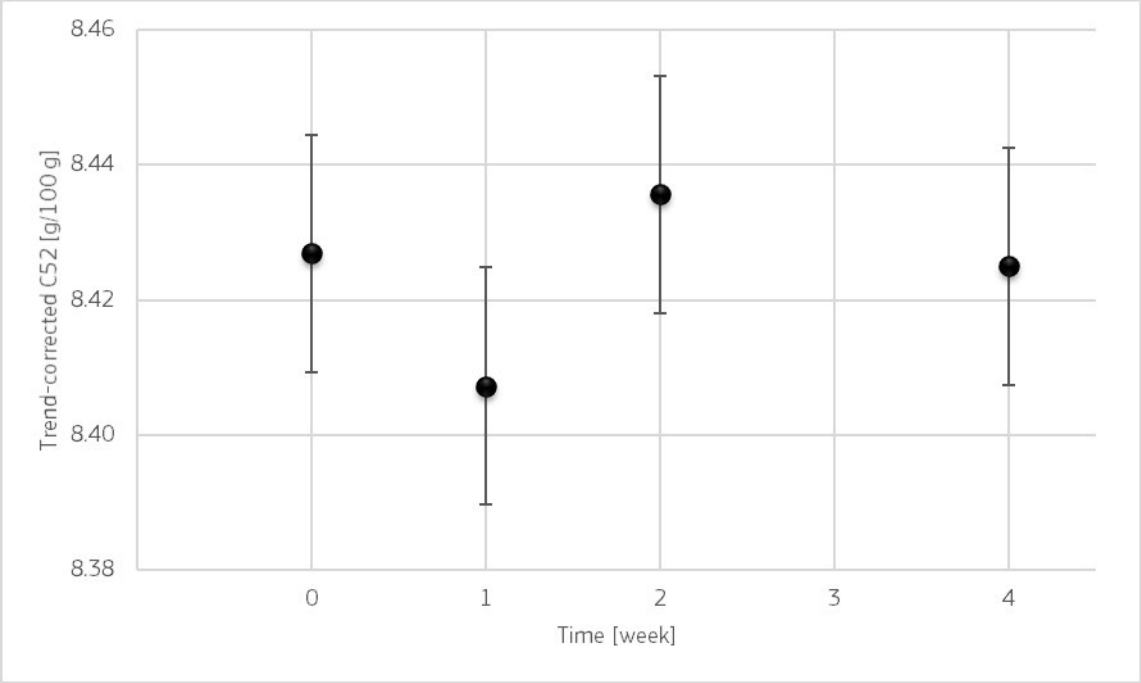
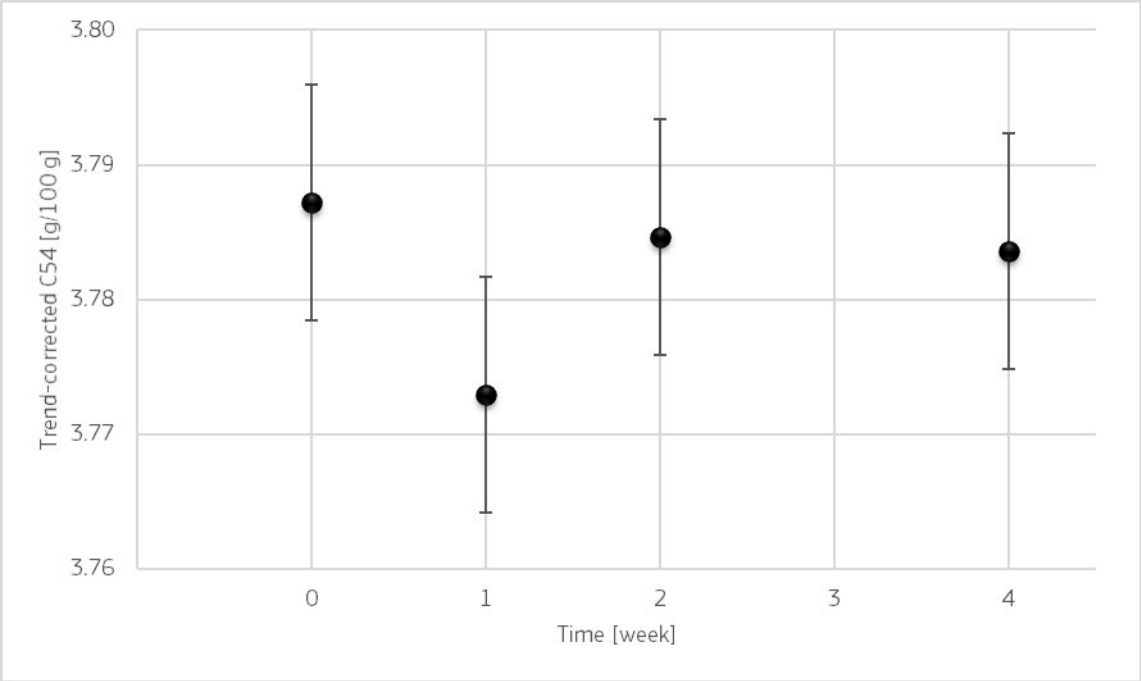


Figure 2.17. Transport stability of C54 in ERM-BD519 assessed at 60 °C over four weeks.



Annex 3. Results of the long-term stability measurements

In each graph the normalised means (results at storage temperature normalised to average result at reference temperature) measured at each time-point are given. Error bars represent the 95 % confidence interval of the mean, based on the within-group standard deviation as obtained by single-factor ANOVA.

Figure 3.1. Storage stability of C24 in BCR-632A assessed at -20 °C over 144 months.

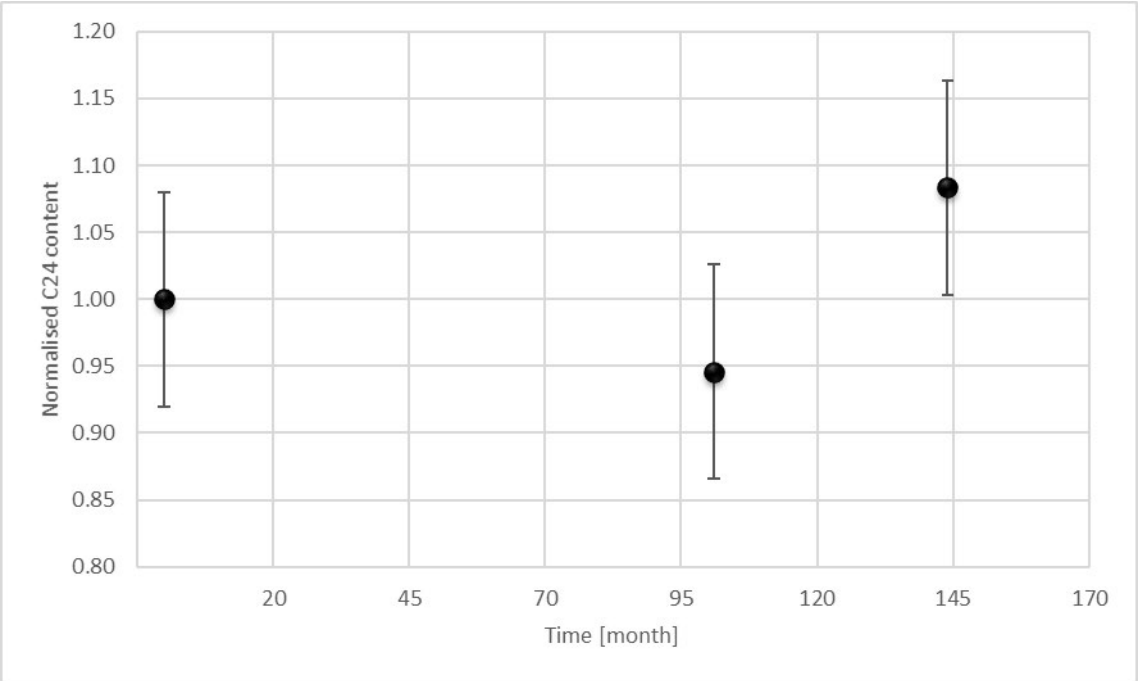


Figure 3.2. Storage stability of cholesterol in BCR-632A assessed at -20 °C over 181 months.

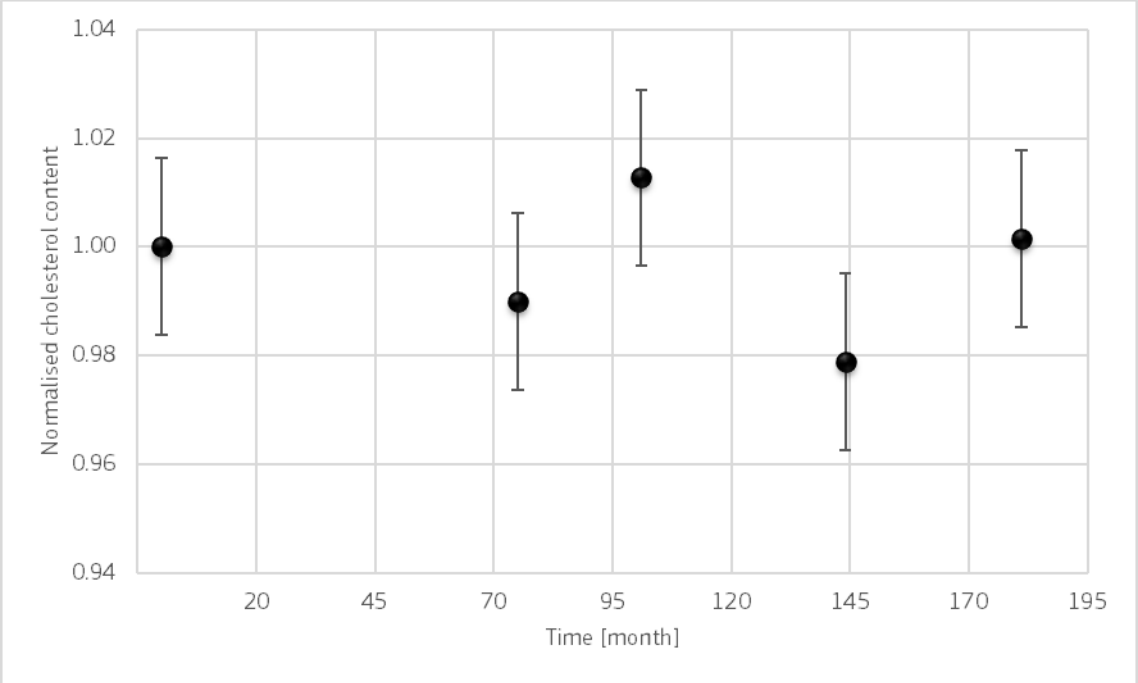


Figure 3.3. Storage stability of C26 in BCR-632A assessed at -20 °C over 181 months.

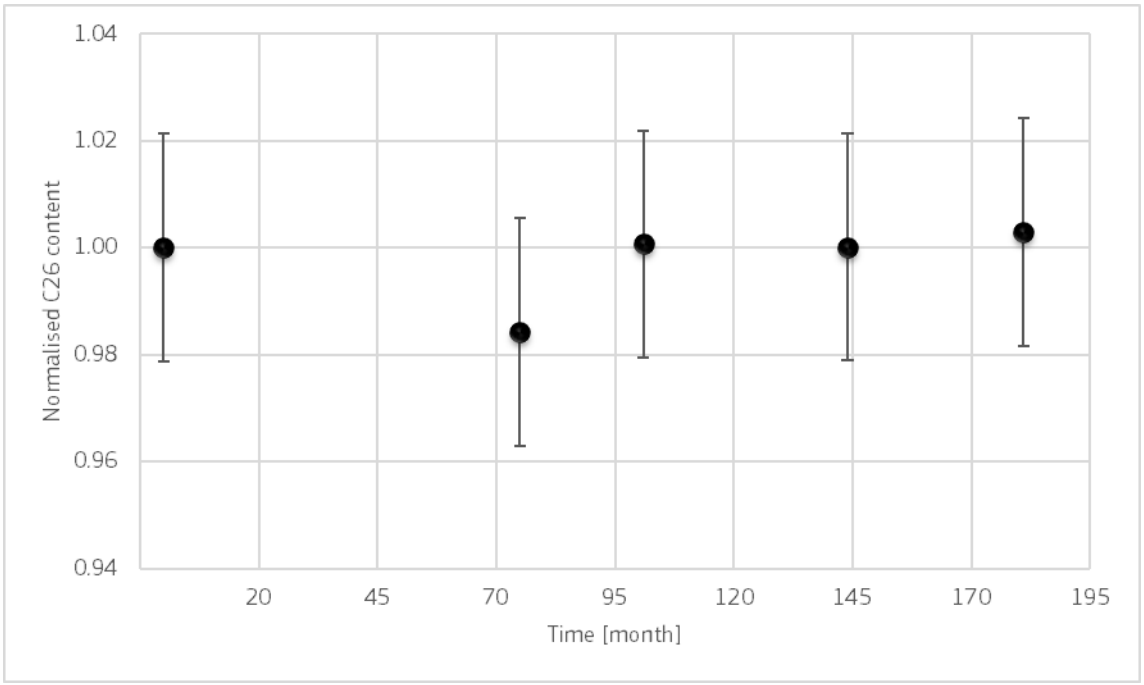


Figure 3.4. Storage stability of C28 in BCR-632A assessed at -20 °C over 181 months.

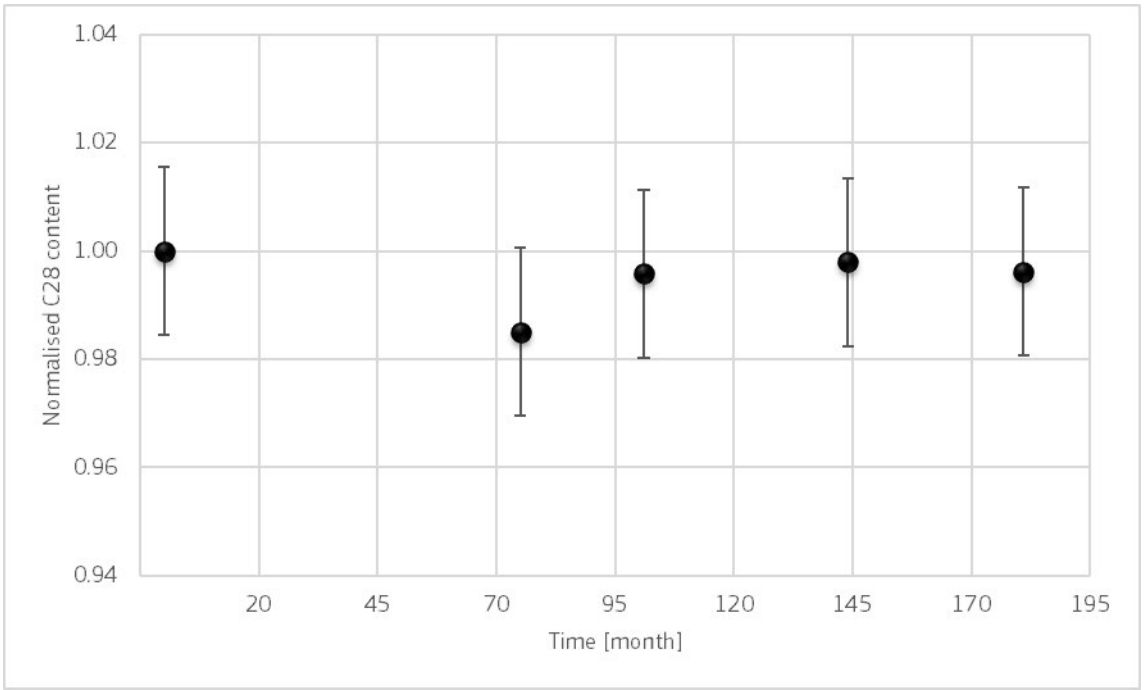


Figure 3.5. Storage stability of C30 in BCR-632A assessed at -20 °C over 181 months.

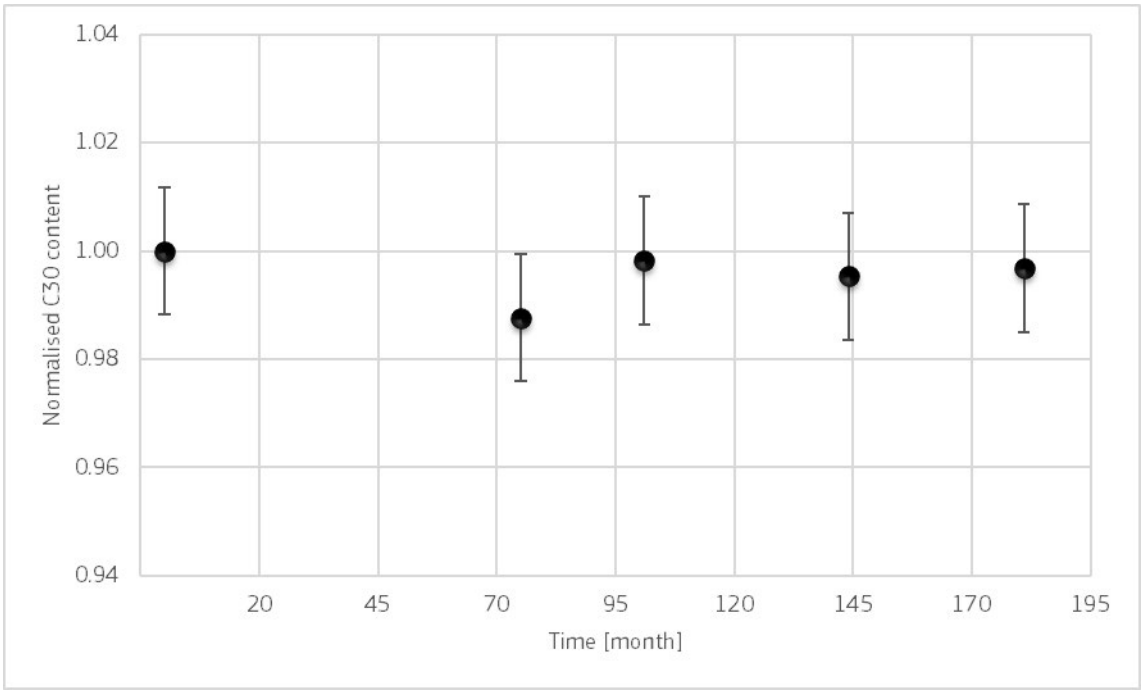


Figure 3.6. Storage stability of C32 in BCR-632A assessed at -20 °C over 181 months.

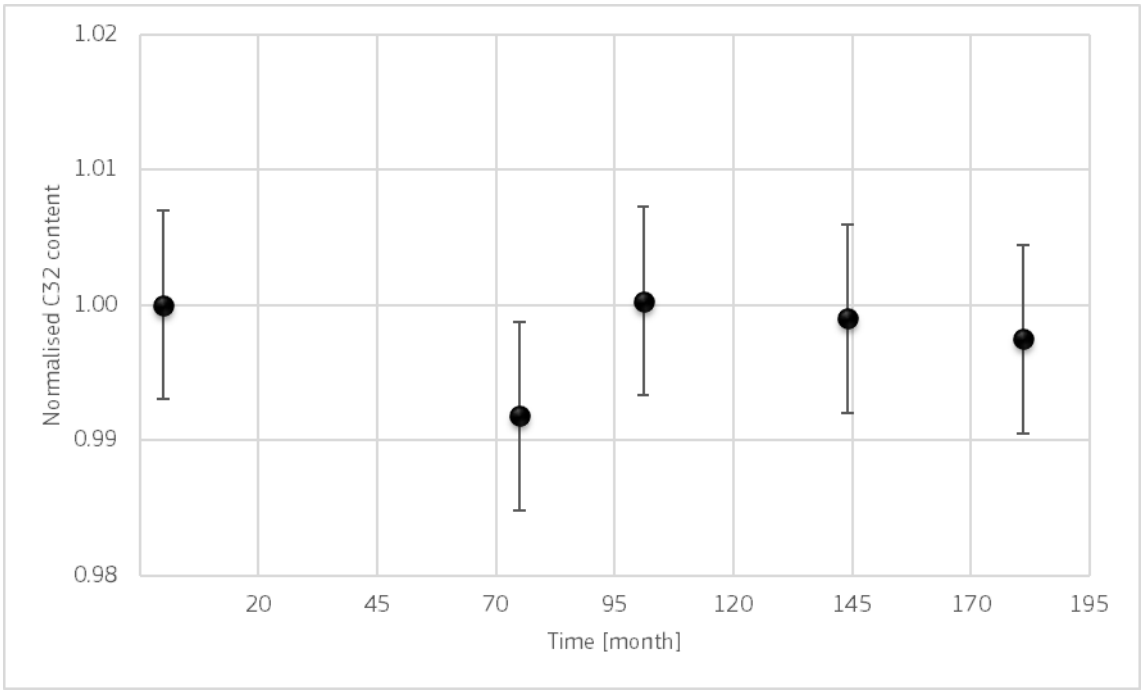


Figure 3.7. Storage stability of C34 in BCR-632A assessed at -20 °C over 181 months.

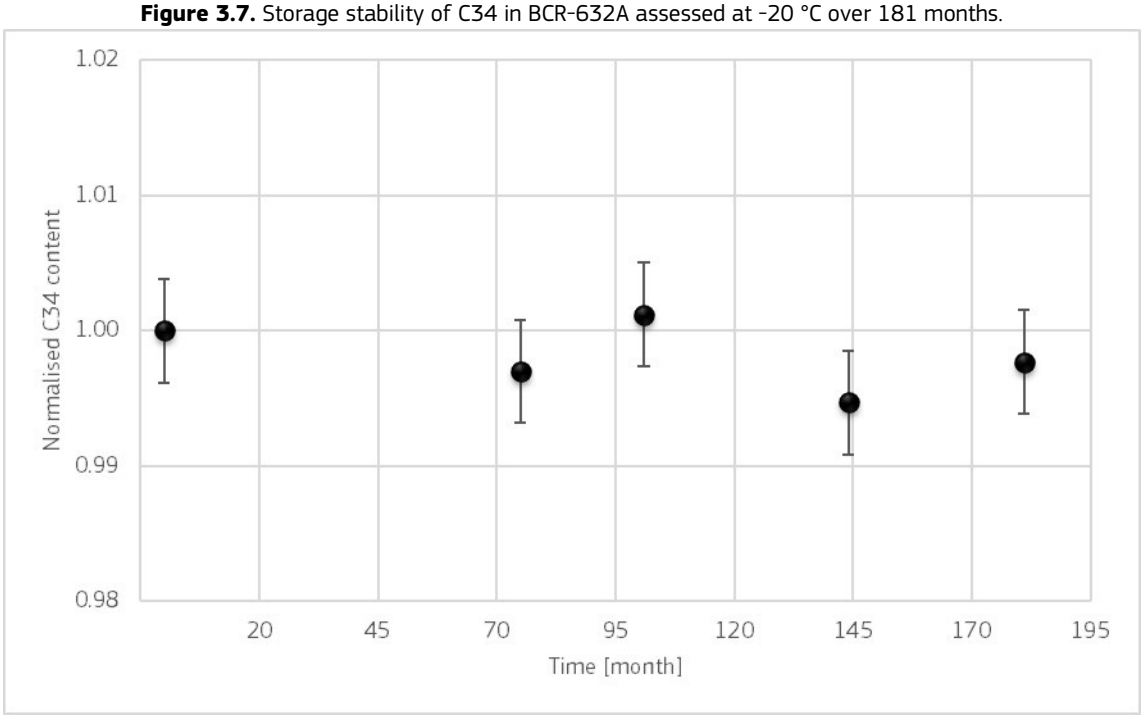


Figure 3.8. Storage stability of C36 in BCR-632A assessed at -20 °C over 181 months.

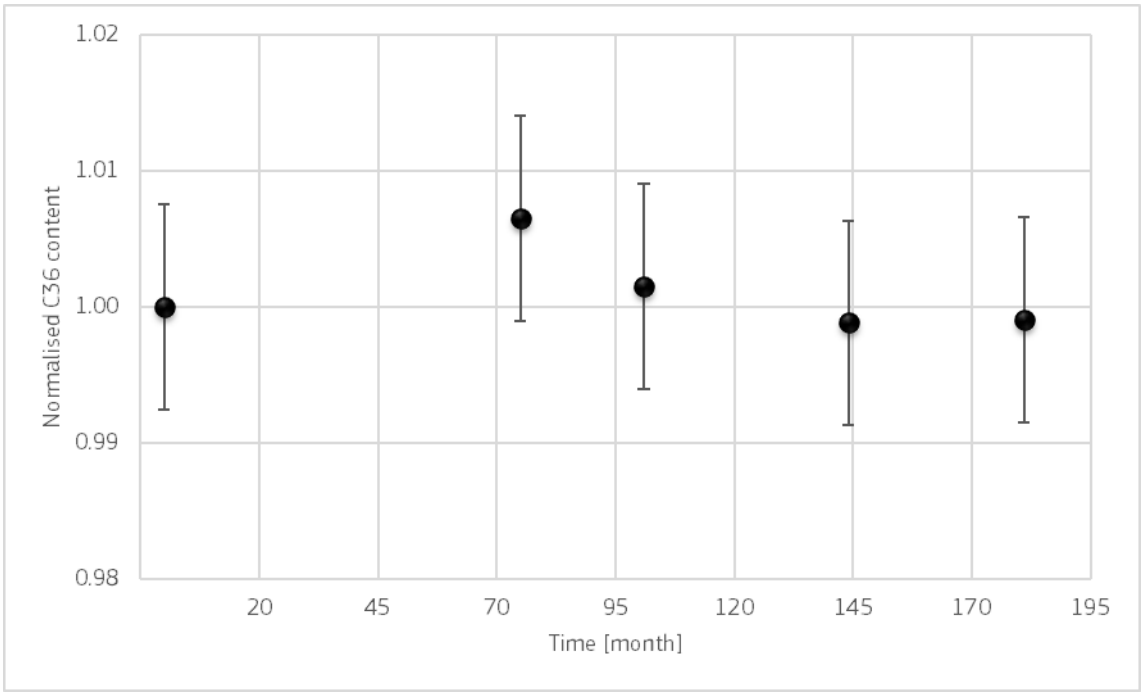


Figure 3.9. Storage stability of C38 in BCR-632A assessed at -20 °C over 181 months.

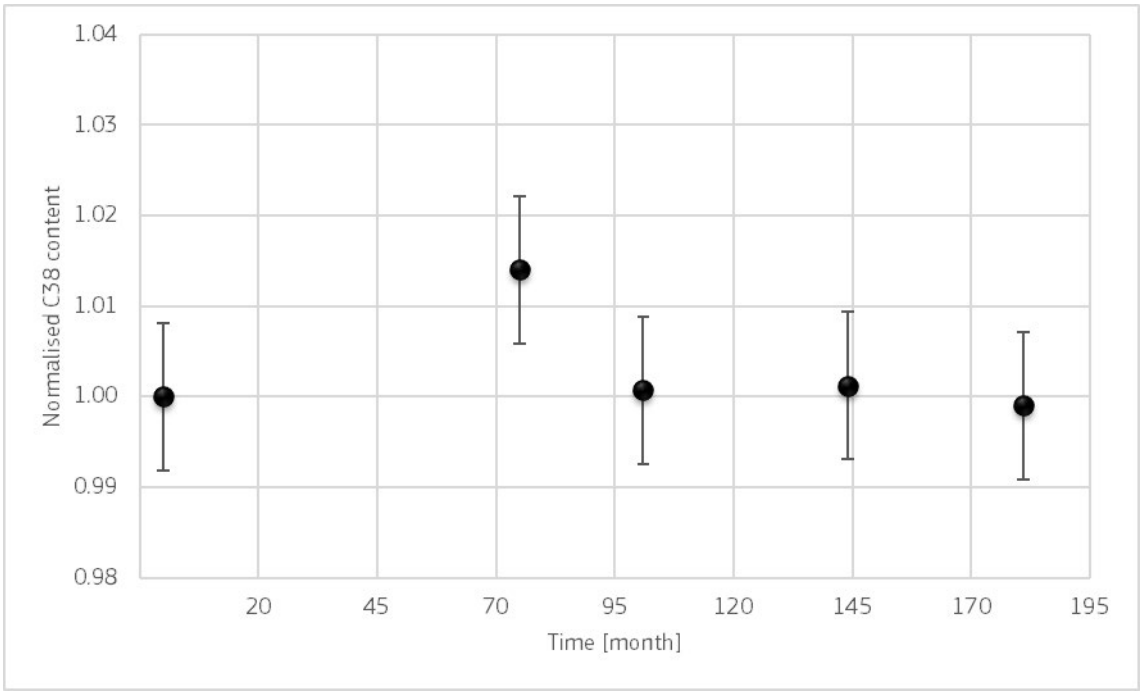


Figure 3.10. Storage stability of C40 in BCR-632A assessed at -20 °C over 181 months.

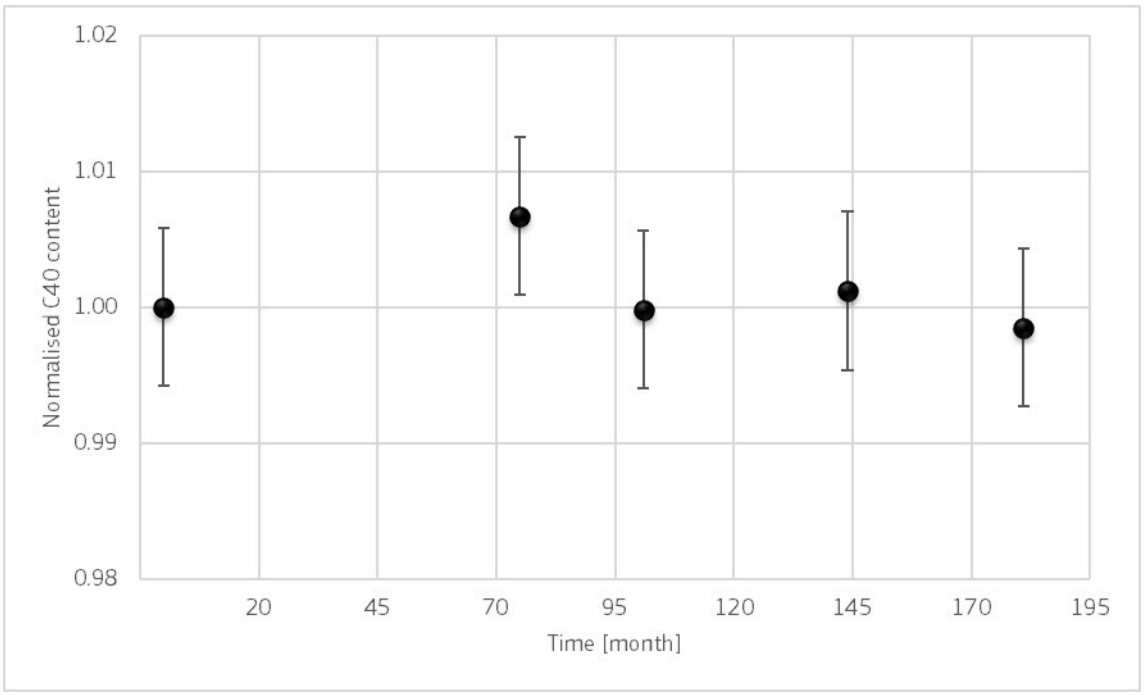


Figure 3.11. Storage stability of C42 in BCR-632A assessed at -20 °C over 181 months.

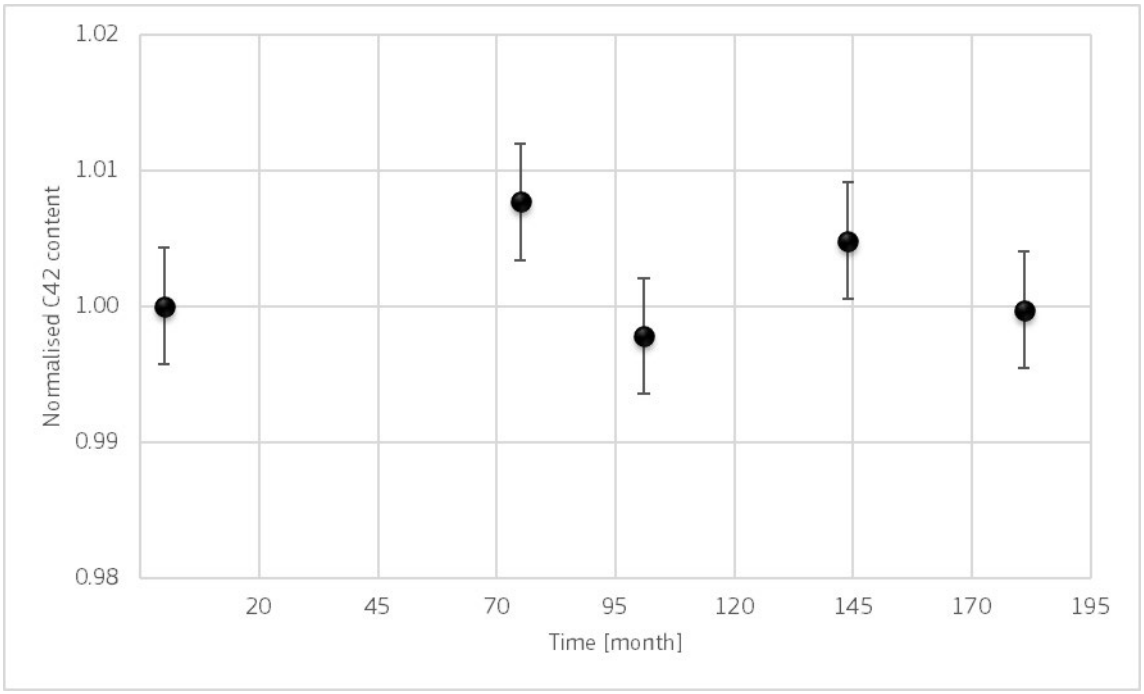


Figure 3.12. Storage stability of C44 in BCR-632A assessed at -20 °C over 181 months.

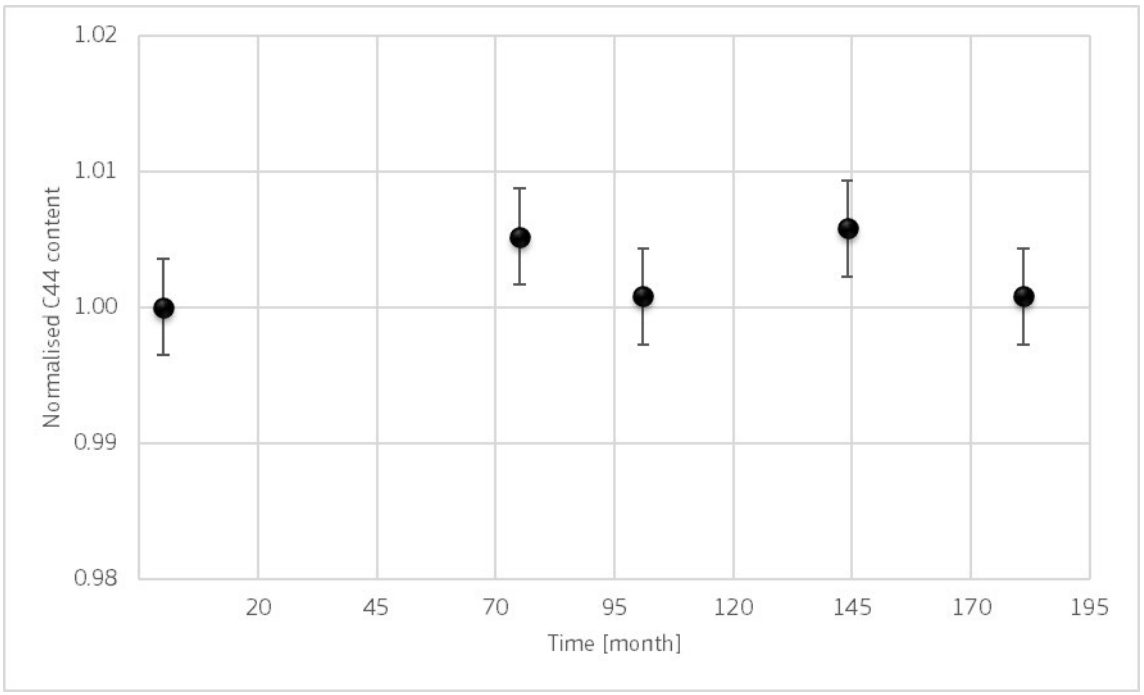


Figure 3.13. Storage stability of C46 in BCR-632A assessed at -20 °C over 181 months.

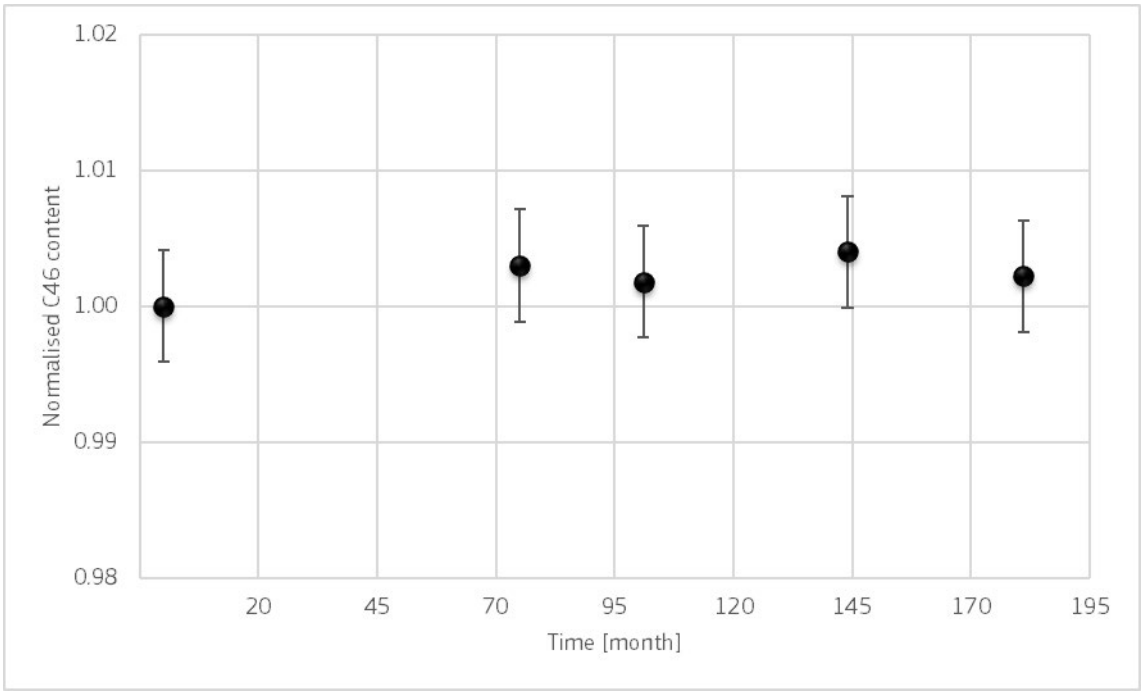


Figure 3.14. Storage stability of C48 in BCR-632A assessed at -20 °C over 181 months.

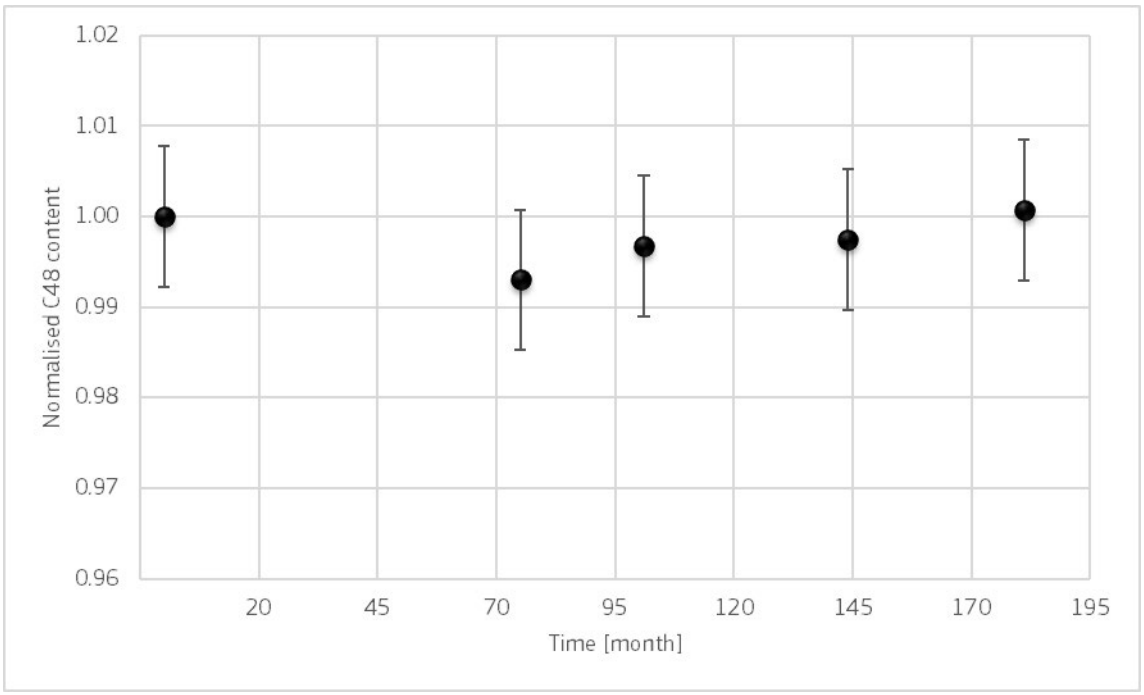


Figure 3.15. Storage stability of C50 in BCR-632A assessed at -20 °C over 181 months.

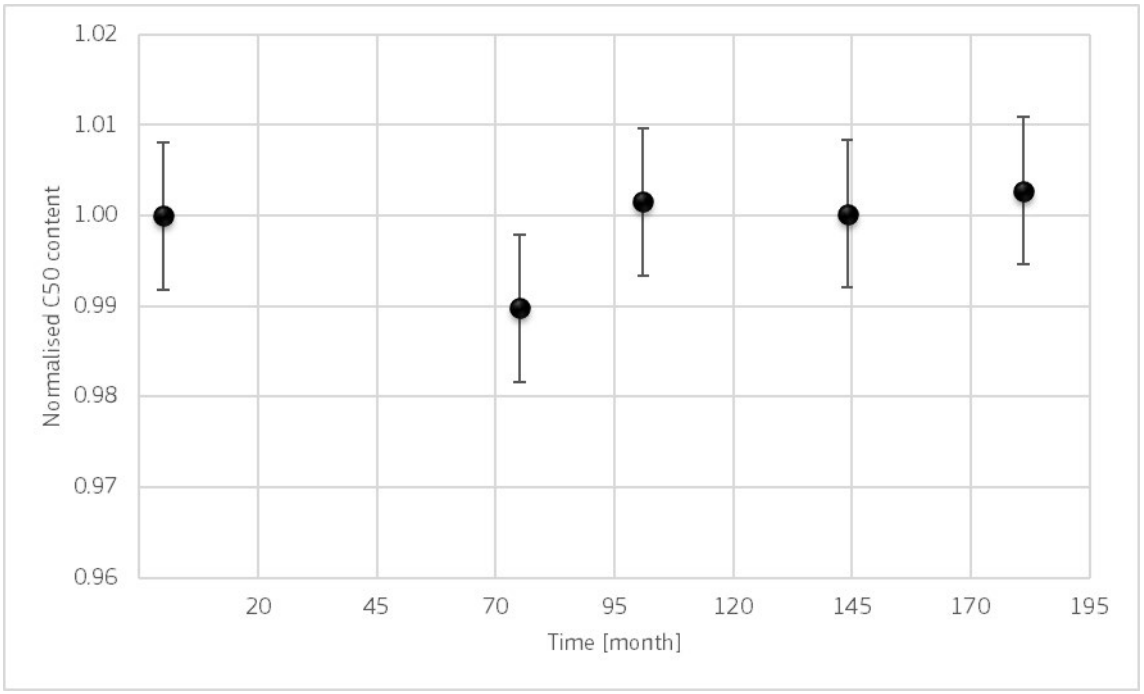


Figure 3.16. Storage stability of C52 in BCR-632A assessed at -20 °C over 181 months.

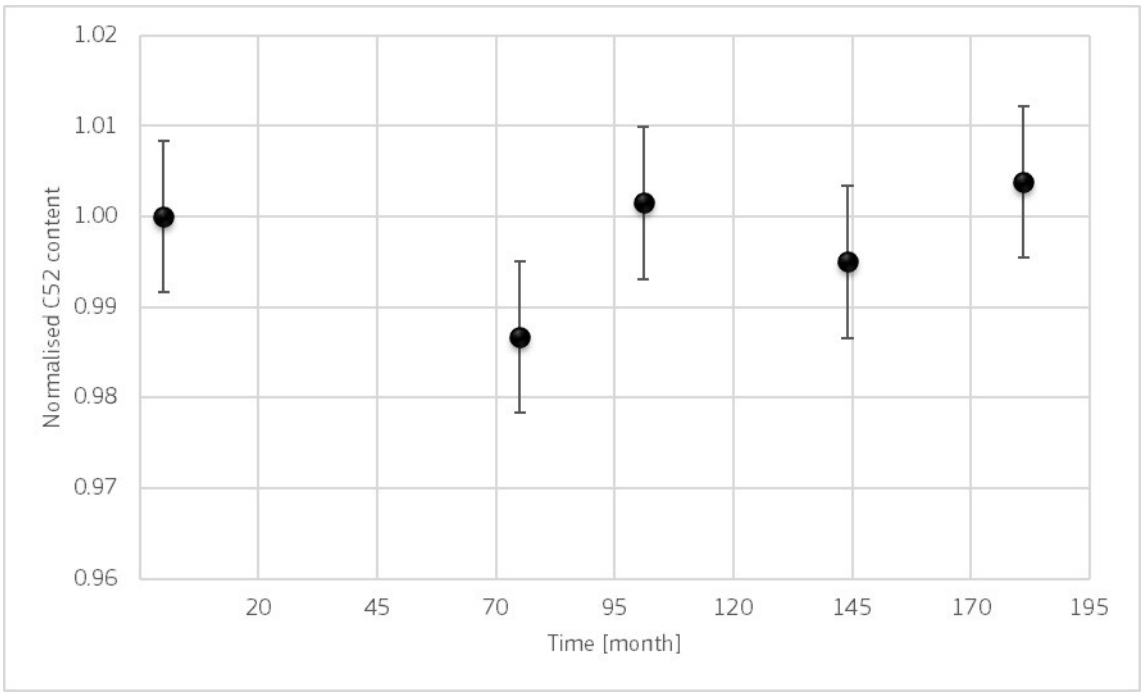
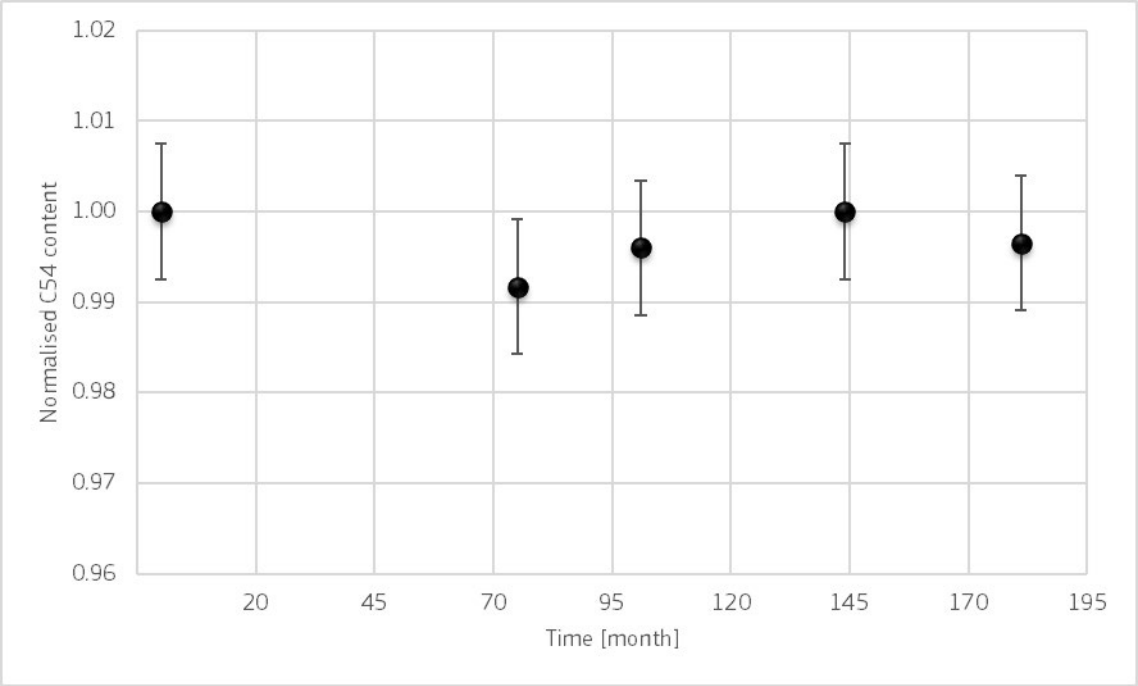


Figure 3.17. Storage stability of C54 in BCR-632A assessed at -20 °C over 181 months.



Annex 4. Results of preparation of common calibrant

Table 4.1. The absolute masses (m_i) and relative mass fractions (w_i) of substances and related uncertainties (u_{mi} and u_{wi}) in the four calibration solutions.

Solution	Compound	m_i [mg]	u_{mi} [mg]	w_i [g/100 g]	u_{wi} [g/100 g]
1	C24	2.56	0.0153	0.1273	0.0008
1	Cholesterol	9.61	0.0153	0.4780	0.0008
1	C30	48.67	0.046	2.4198	0.0022
1	C36	389.39	0.046	19.3600	0.0021
1	C42	255.47	0.046	12.7016	0.0021
1	C48	320.91	0.046	15.9552	0.0021
1	C50	404.40	0.046	20.1062	0.0022
1	C52	381.35	0.046	18.9602	0.0021
1	C54	198.95	0.046	9.8915	0.0021
2	C24	2.53	0.0153	0.1256	0.0008
2	Cholesterol	9.73	0.0153	0.4831	0.0008
2	C30	47.49	0.046	2.3591	0.0022
2	C36	389.29	0.046	19.3387	0.0021
2	C42	253.75	0.046	12.6055	0.0021
2	C48	321.54	0.046	15.9731	0.0021
2	C50	403.94	0.046	20.0664	0.0022
2	C52	385.20	0.046	19.1355	0.0021
2	C54	199.55	0.046	9.9130	0.0021
3	C24	2.57	0.0153	0.1282	0.0008
3	Cholesterol	8.54	0.0153	0.4255	0.0008
3	C30	46.35	0.046	2.3090	0.0022
3	C36	390.16	0.046	19.4362	0.0022
3	C42	253.95	0.046	12.6508	0.0021
3	C48	320.21	0.046	15.9516	0.0021
3	C50	404.82	0.046	20.1665	0.0022
3	C52	380.35	0.046	18.9475	0.0021
3	C54	200.43	0.046	9.9846	0.0021
4	C24	2.53	0.0153	0.1266	0.0008
4	Cholesterol	8.38	0.0153	0.4192	0.0008
4	C30	47.55	0.046	2.3797	0.0023
4	C36	388.13	0.046	19.4243	0.0022
4	C42	252.93	0.046	12.6581	0.0021
4	C48	318.53	0.046	15.9411	0.0021
4	C50	405.29	0.046	20.2831	0.0022
4	C52	379.48	0.046	18.9914	0.0022
4	C54	195.35	0.046	9.7765	0.0022

Figure 4.1. Response factors of C24 determined in four calibration solutions.

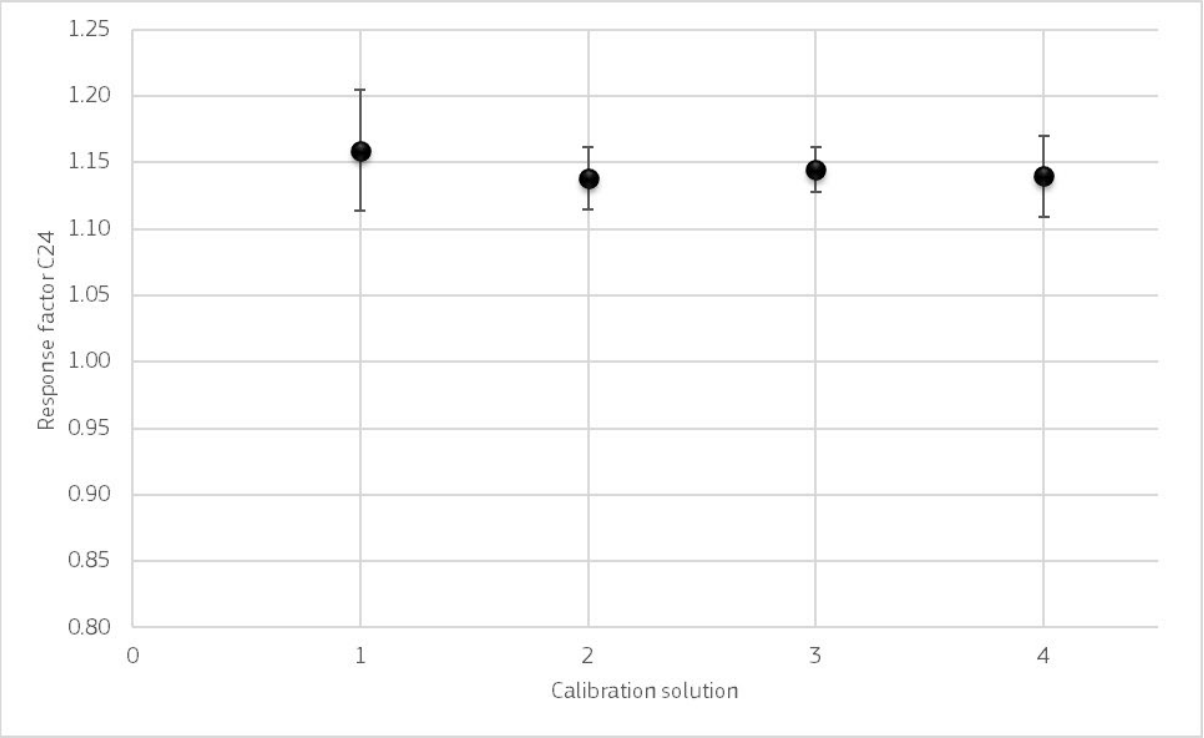


Figure 4.2. Response factors of cholesterol determined in four calibration solutions.

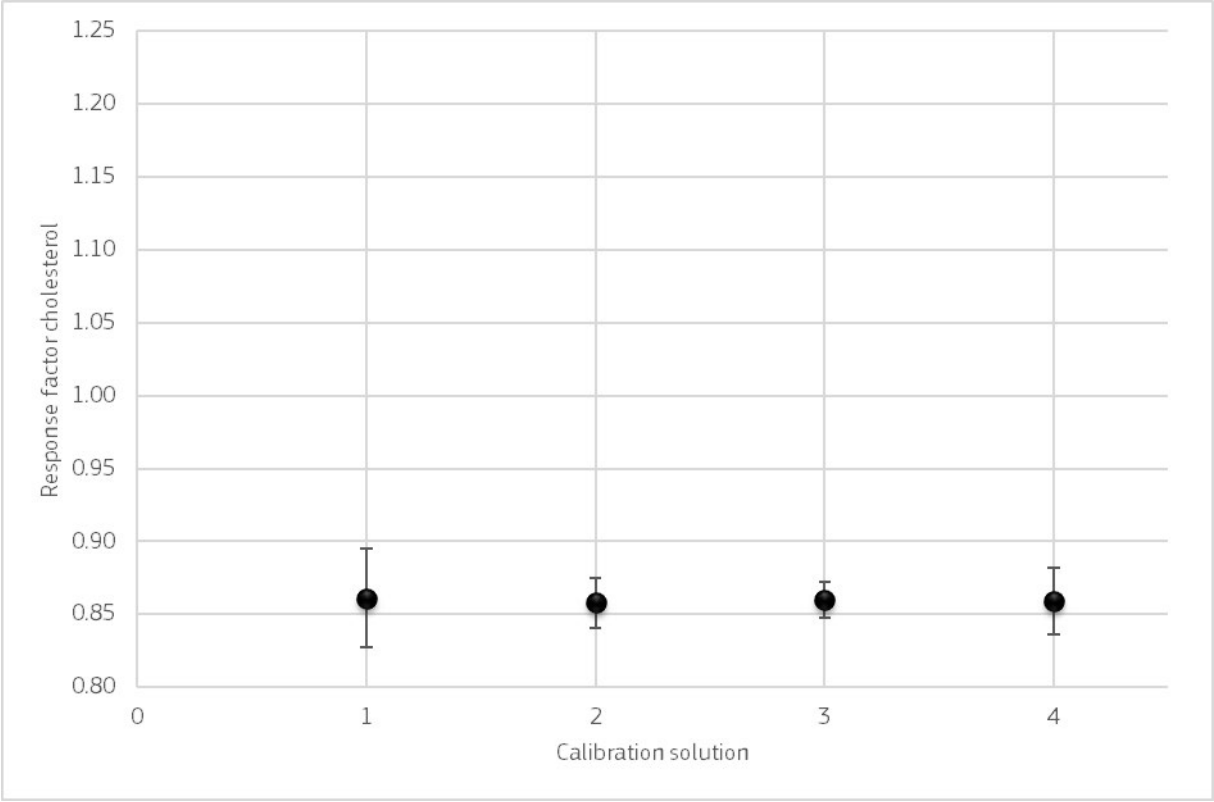


Figure 4.3. Response factors of C30 determined in four calibration solutions.

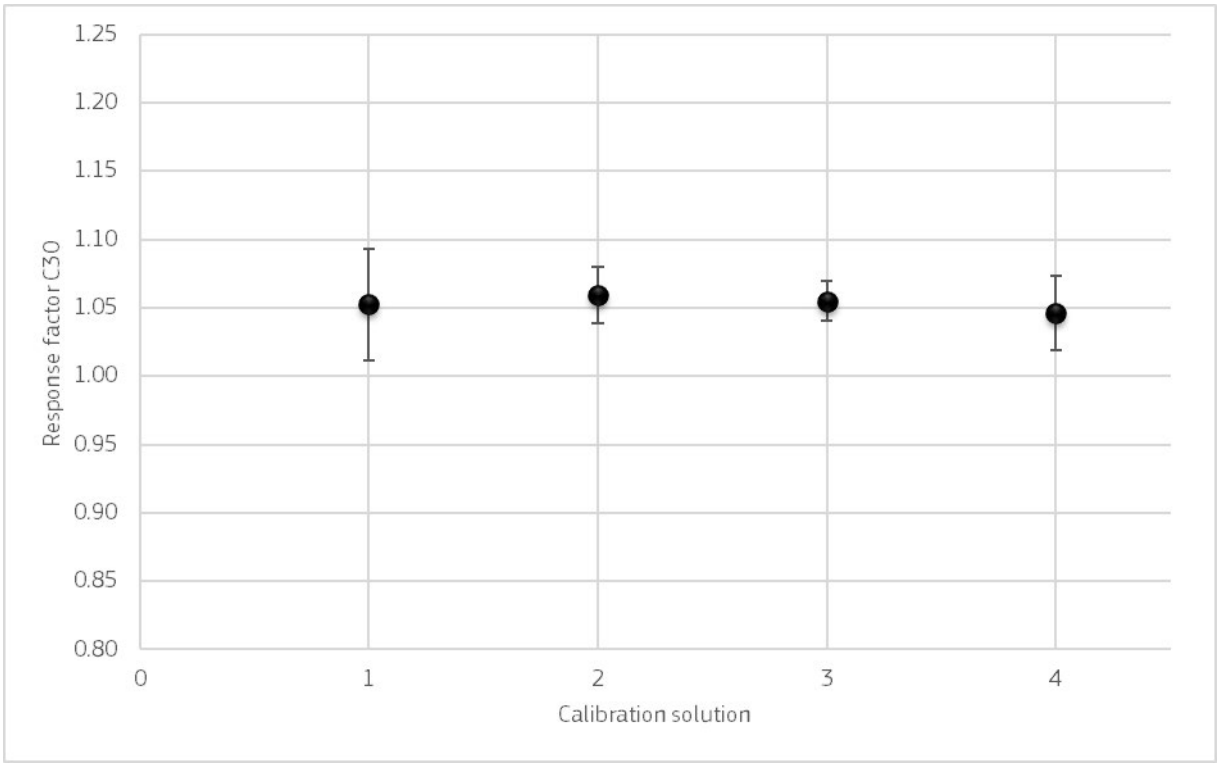


Figure 4.4. Response factors of C36 determined in four calibration solutions.

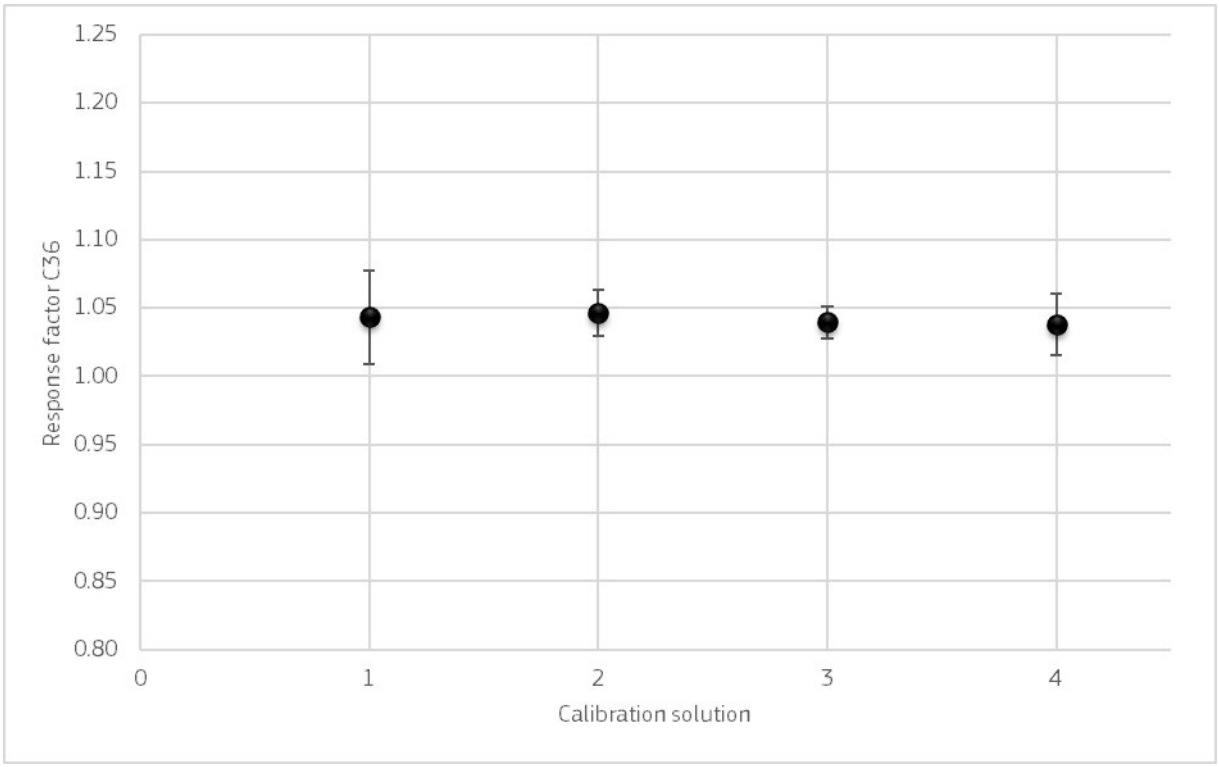


Figure 4.5. Response factors of C42 determined in four calibration solutions.

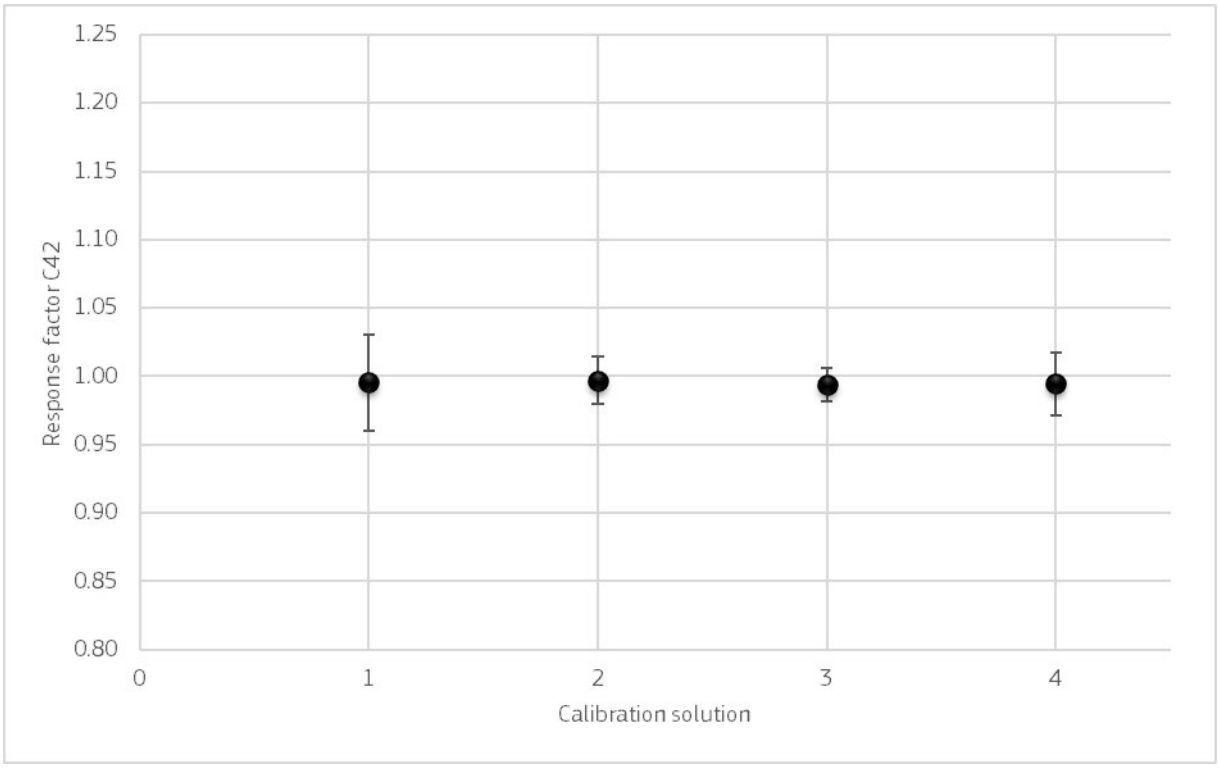


Figure 4.6. Response factors of C48 determined in four calibration solutions.

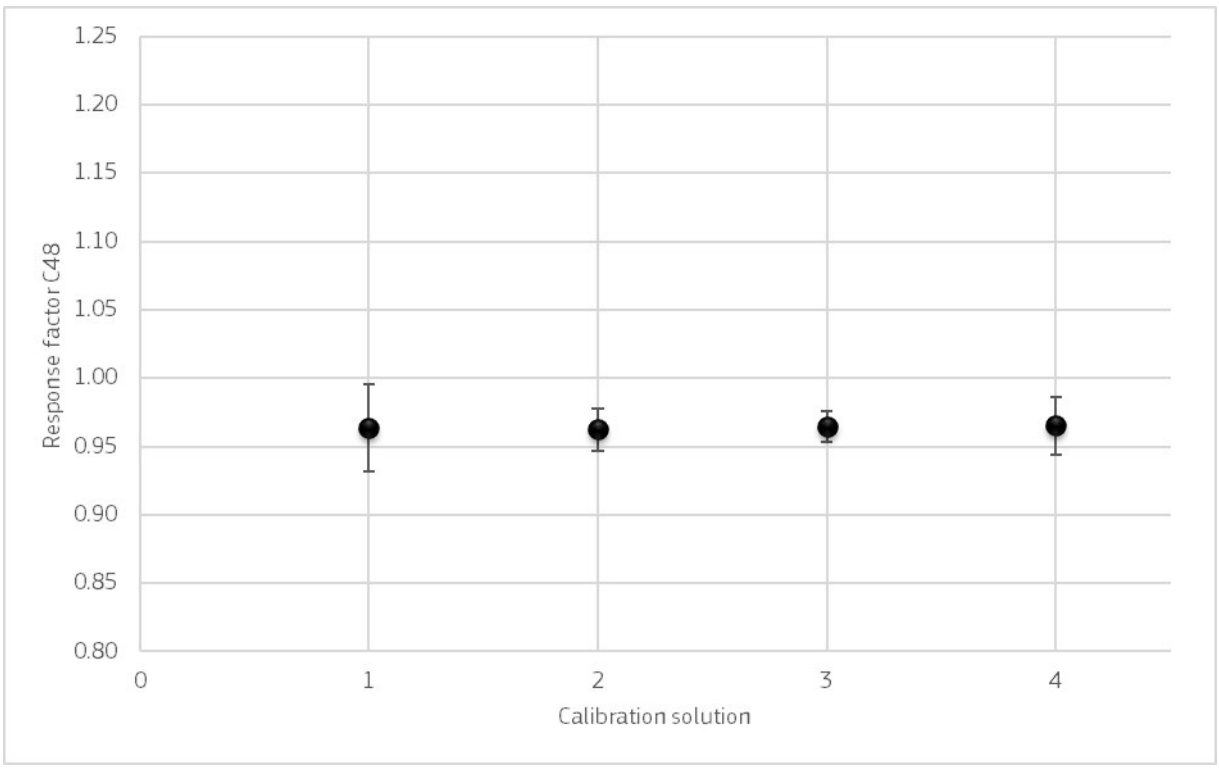


Figure 4.7. Response factors of C50 determined in four calibration solutions.

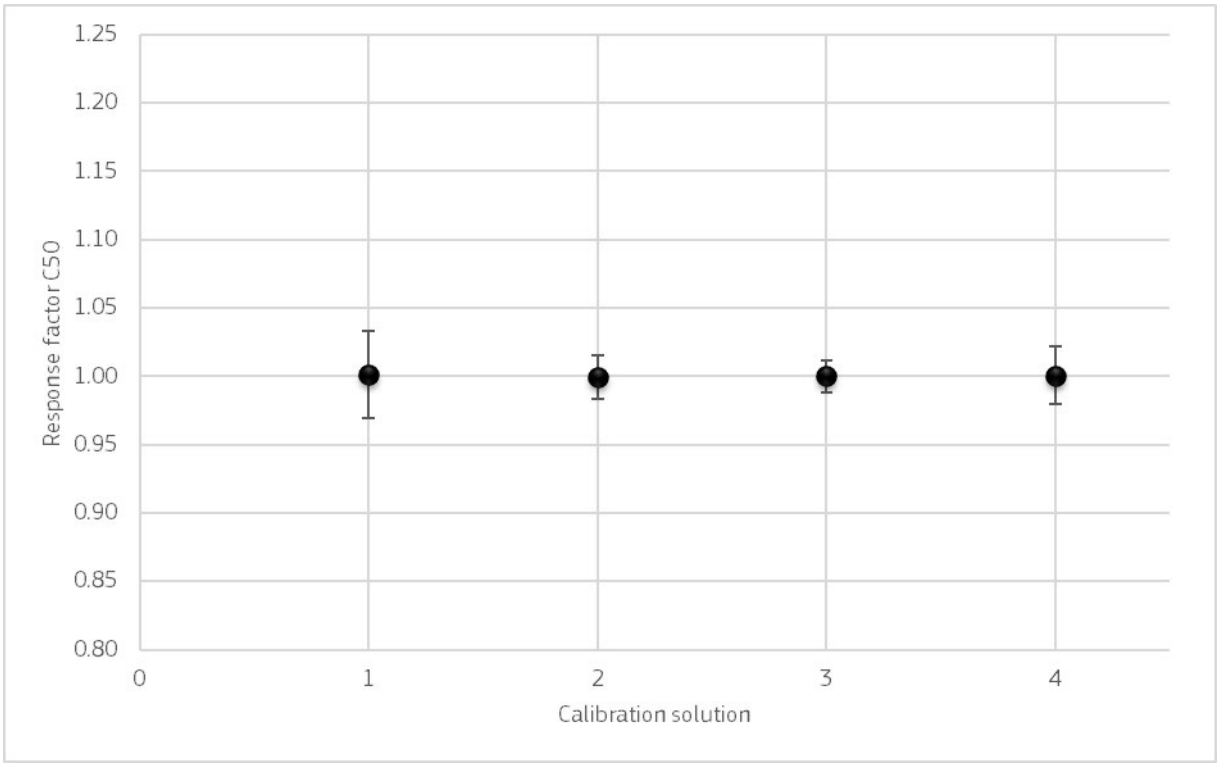


Figure 4.8. Response factors of C52 determined in four calibration solutions.

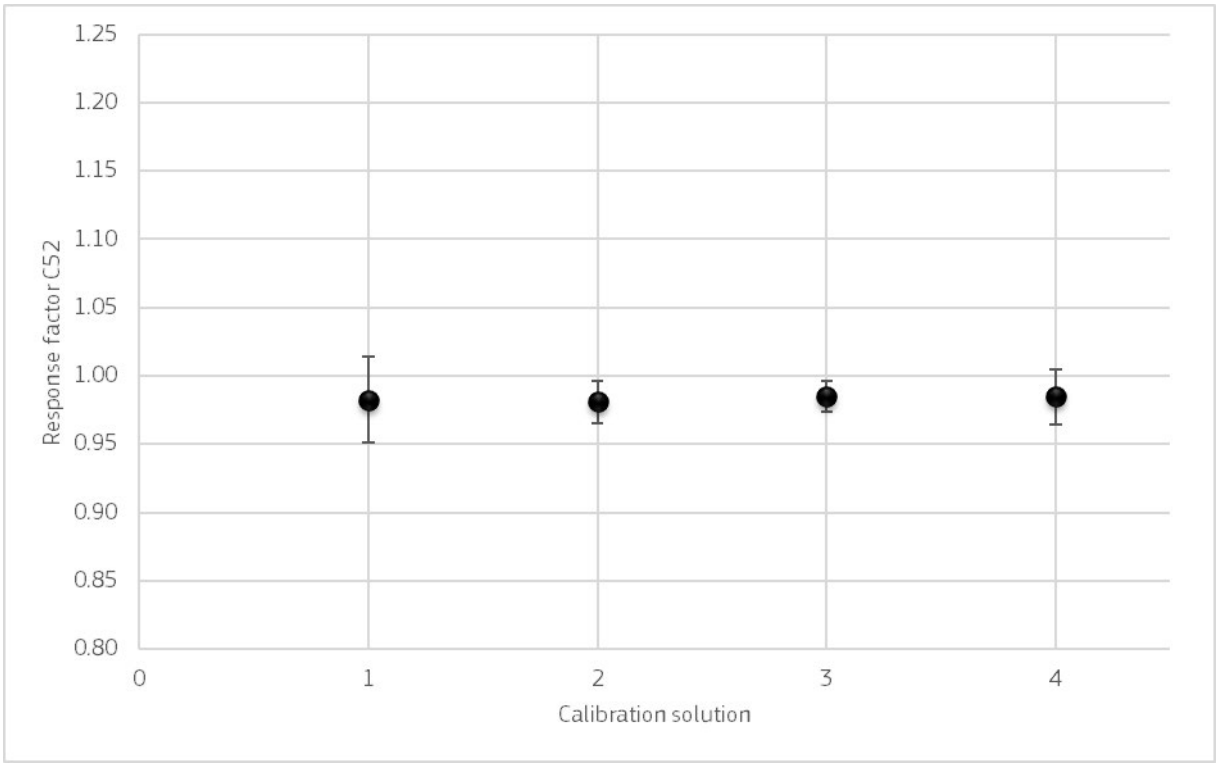


Figure 4.9. Response factors of C54 determined in four calibration solutions.

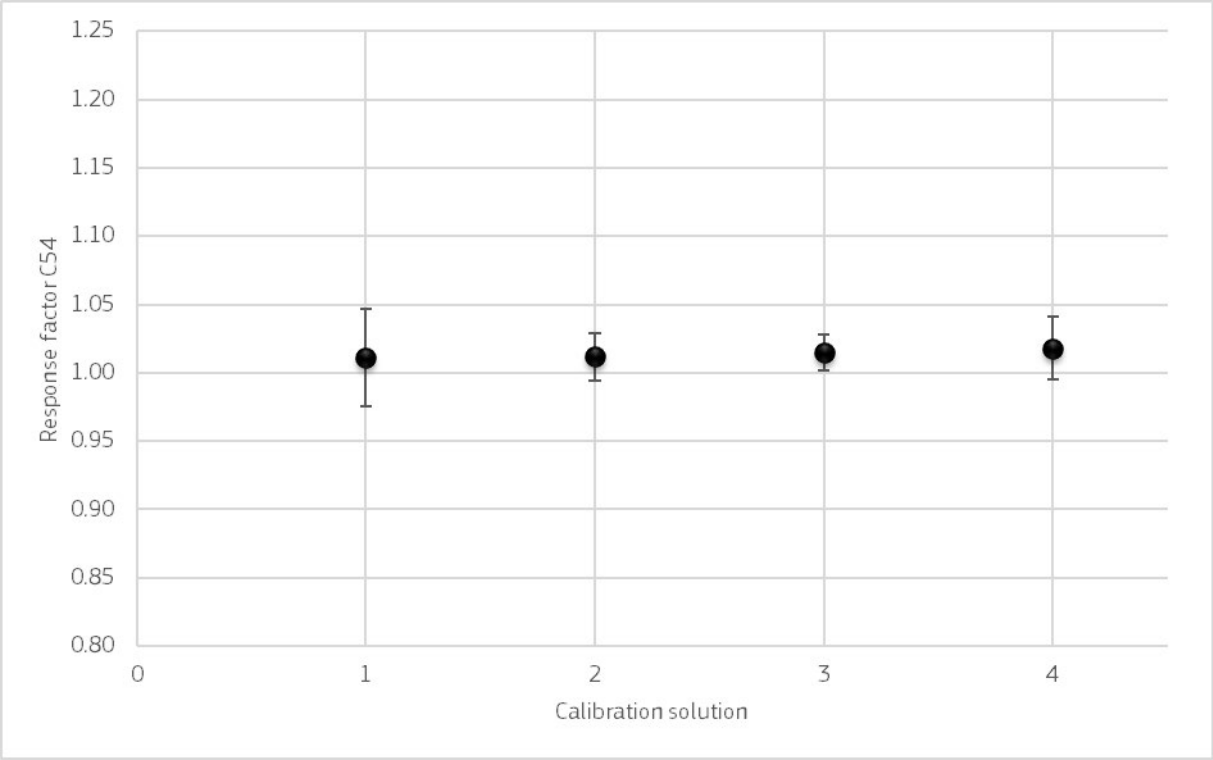


Table 4.2. Masses of substances determined by LC-MS (columns) and in the common calibrant (rows).

	CCC [mg]	Chol [mg]	DDD [mg]	LLL [mg]	MMM [mg]	PPP [mg]	SMS [mg]	SPS [mg]	SSS [mg]	m_i [mg]
C24	2.4638		0.0040							2.4677
Chol		9.5392								9.5392
C30			47.0507	0.0041	0.0118					47.0666
C36			0.0010	377.4190	0.0072					377.4272
C42			0.0206		247.7490		0.0215			247.7912
C48			0.0064			321.5400	3.8663			325.4126
C50						0.3269	393.8720	0.5791		394.7780
C52							3.6589	384.0590	0.1540	387.8718
C54									199.0110	199.0110

Note: The final values assigned for each substance in the common calibrant are obtained by adding up the values across the rows in Table 4.2. For example, the substance CCC contains 2.4638 mg of CCC, but an additional contribution of CCC comes from the substance DDD (0.004 mg), which contains CCC as an impurity. To obtain the final mass of C24 in the calibrant the two amounts are added, resulting in 2.4677 mg C24.

Table 4.3. Uncertainties of substances based on the LC-MS (columns) and in the common calibrant (rows).

	CCC [mg]	Chol [mg]	DDD [mg]	LLL [mg]	MMM [mg]	PPP [mg]	SMS [mg]	SPS [mg]	SSS [mg]	u_{mi} [mg]
C24	0.0164		0.0007							0.0164
Chol		0.0316								0.0316
C30			0.1322	0.0010	0.0009					0.1322
C36			0.0003	4.7424	0.0012					4.7424
C42			0.0037		0.6952		0.0062			0.6953
C48			0.0004			0.9832	0.2276			1.0092
C50						0.3272	1.1060	0.0577		1.1549
C52							0.2486	1.0017	0.0314	1.0326
C54									0.5209	0.5209

Table 4.4. Results of the statistical evaluation of the homogeneity study for the common calibrant.

Substance	Trends [1]		Outliers [2]		Distribution	
	Measurement sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
C24	no	no	no	none	normal	normal
Cholesterol	no	no	no	yes	normal	normal
C30	no	no	no	none	normal	normal
C36	no	no	no	none	normal	normal
C42	no	no	no	none	normal	normal
C48	no	yes	no	none	normal	normal
C50	no	no	yes	none	normal	normal
C52	no	no	no	none	normal	normal
C54	no	no	no	none	normal	normal

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

Table 4.5. Results of the homogeneity study for the common calibrant.

Substance	s_{wb} [mg]	s_{bb} [mg]	u^*_{bb} [mg]	u_{rec} [mg]	u_{bb} [mg]
C24	0.0047	n.c.	0.0025	n.a.	0.0025
Cholesterol	0.0056	0.0155	0.0030	0.0134	0.0155
C30	0.0813	0.0313	0.0437	n.a.	0.0437
C36	0.6499	n.c.	0.3491	n.a.	0.3491
C42	0.5632	n.c.	0.3026	n.a.	0.3026
C48	0.2210	0.2496	0.1188	0.2343	0.2496
C50	0.3198	n.c.	0.1717	n.a.	0.1717
C52	0.4837	0.2475	0.2599	n.a.	0.2599
C54	0.3787	n.c.	0.2034	n.a.	0.2034

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

[2] n.a.: not applicable.

Annex 5. Summary of method used in the characterisation study

Table 5.1. Scope of ISO 17678:2019/IDF 202:2019 [4]

Standard	**ISO 17678:2019/IDF 202:2019**
Title	Milk and milk products - Determination of milk fat purity by gas chromatographic analysis of triglycerides
Scope	ISO 17678:2019/IDF 202:2019 specifies a reference method for the determination of milk fat purity using gas chromatographic analysis of triglycerides. The method utilizes the differences in triglyceride fingerprint of milk fat from the individual triglyceride fingerprints of other fats and oils to determine samples which are outside the range normally observed for milk fat. This is achieved by using the defined triglyceride formulae based on the normalized weighted sum of individual triglyceride peaks which are sensitive to the integrity of the milk [6, 7]. The integrity of the milk fat can be determined by comparing the result of these formulae with those previously observed for a range of pure milk fat samples [12]. Both vegetable fats and animal fats such as beef tallow and lard can be detected. The method is applicable to bulk milk, or products made thereof, irrespective of the variation in common feeding practices, breed or lactation conditions. In particular, the method is applicable to fat extracted from milk products purporting to contain pure milk fat with unchanged composition, such as butter, cream, milk and milk powder. Because a false-positive result can occur, the method does not apply to milk fat related to these circumstances: a) obtained from bovine milk other than cow's milk; b) obtained from single cows; c) obtained from cows whose diet contained a particularly high proportion of vegetable oils such as rapeseed, cotton or palm oil, etc.; d) obtained from cows suffering from serious underfeeding (strong energy deficit); e) obtained from colostrum; f) subjected to technological treatment such as removal of cholesterol or fractionation; g) obtained from skim milk, buttermilk or whey; h) obtained from cheeses showing increased lipolysis; i) extracted using the Gerber, Weibull–Berntrop or Schmid–Bondzynski–Ratzlaff methods, or that has been isolated using detergents (e.g. the Bureau of Dairy Industries method).

Table 5.2. Method details that are not strictly specified in ISO 17678:2019/IDF 202:2019 (as reported by the laboratories)

Lab no.	Column type and dimension	Carrier gas and flow rate/pressure	Temperature programme	Injection technique	Detector type	Calibrant
1	CP-SimDist Ultimetal 5 m x 0.53 mm x 0.17 µm	Hydrogen; 3mL/min	80 °C for 0.5 min; with 50 °C/min to 190 °C; with 6 °C/min to 350 °C; 350 °C for 5 min	On column; 80°C	FID; 370 °C	See chapter 6.3
2	HP-1 Simdisk 15 m x 0.53 mm x 0.15 µm	Nitrogen (ECD); 3 mL/min (flow control)	80 °C for 0.5 min; with 50°C/min to 190 °C; with 6°C/min to 370°C; 370°C for 10 min	On column	FID; 370 °C	See chapter 6.3
3	HT5 (AQ) 6 m x 0.53 mm x 0.10 µm	Helium; 37 mL/min / 6 psi	80 °C for 1 min; with 5 °C/min to 200 °C; with 8 °C/min to 360 °C; 360 °C for 10 min	Split; 80 °C	FID; 380 °C	See chapter 6.3
4	ZB-5HT 15 m x 0.25 mm x 0.10 µm	Hydrogen; 250 kPa	80 °C for 0.5 min; with 10 °C/min to 350 °C; 350 °C for 5 min	On column	FID; 370 °C	See chapter 6.3
5	ZB-5HT Inferno 5 m x 0.32 mm x 0.10 µm	Hydrogen; 8 mL/min / 41.5 kPa (constant flow)	200 °C for 0 min; with 25 °C/min to 360 °C; 360 °C for 1 min	Split/splitless Split ratio 1:20, isotherm, 300 °C,	FID; 380 °C	See chapter 6.3
6	CP-SimDist UltiMetal 5 m x 0.53 mm x 0.17 µm	Hydrogen; 8 mL/min	160 °C for 0.2 min; with 40 °C/min to 360°C; 360 °C for 2 min	On column (oven track)	FID; 370 °C	See chapter 6.3
7	DB-5HT 10 m x 0.32 mm x 0.10 µm	Hydrogen; 3 mL/min / 30 kPa	80 °C for 0.5 min; with 50 °C/min to 190 °C; with 6 °C/min to 350 °C; 350 °C for 5 min	On column (oven track)	FID, 370 °C	See chapter 6.3
8	DB-5HT 5 m x 0.32 mm x 0.10 µm	Hydrogen; 2.57 mL/min / 11 kPa	80 °C to 365 °C	On column; 83 °C	FID; 390 °C	See chapter 6.3
9	CP-SimDist UltiMetal 5 m x 0.53 mm x 0.17 µm	Helium; 14 mL/min	80 °C for 0.5 min; with 50 °C/ min to 190 °C; with 6 °C/min to 350 °C; 350 °C for 5 min	On column; 60 °C for 0.5 min, with 75 °C/min to 190 °C; with 9 °C/min to 380 °C 380 °C for 10.40 min.	FID; 370 °C.	See chapter 6.3
10	CP SimDist Ultimatl 5 m x 0.53 mm x 0.17 µm	Helium; 14 mL/min / 13.9 kPa	70 °C for 1 min; with 40 °C/min to 210 °C; with 6 °C/min to 350 °C; 350 °C for 5 min	Cold on column; (oven track +3 °C)	FID; 370 °C	See chapter 6.3

Annex 6. Results of the characterisation measurements

Results are expressed as relative mass fractions [g/100 g], i.e. the mass fraction of each triglyceride (C24, C26, C28, C30, C32, C34, C36, C38, C40, C42, C44, C46, C48, C50, C52, C54) and cholesterol is expressed as a percentage of the total triglycerides.

Table 6.1. Data from the characterisation for C24.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	0.0881	0.0926	0.0877	0.0865	0.0862	0.0861	0.0878	0.0049
D02	0.0854	0.0882	0.0893	0.0857	0.0730	0.0739	0.0826	0.0145
D04	0.0632	0.0627	0.0637	0.0565	0.0581	0.0544	0.0598	0.0079
D05	0.0772	0.0757	0.0798	0.0681	0.0741	0.0838	0.0764	0.0106
D06	0.0720	0.0795	0.0865	0.0891	0.0819	0.0886	0.0829	0.0131
D08	0.0867	0.0891	0.0880	0.0892	0.0850	0.0936	0.0886	0.0058
D09	0.0681	0.0684	0.0707	0.0714	0.0661	0.0683	0.0688	0.0038
D10	0.0774	0.0787	0.0813	0.0786	0.0763	0.0790	0.0785	0.0033
Results not used for value assignment								
D03	0.1124	0.1051	0.0926	0.0846	0.0901	0.1027	0.0979	0.0210
D07	0.0615	0.0638	0.0594	0.0632	0.0687	0.0675	0.0640	0.0071

Figure 6.1. Characterisation data for C24, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

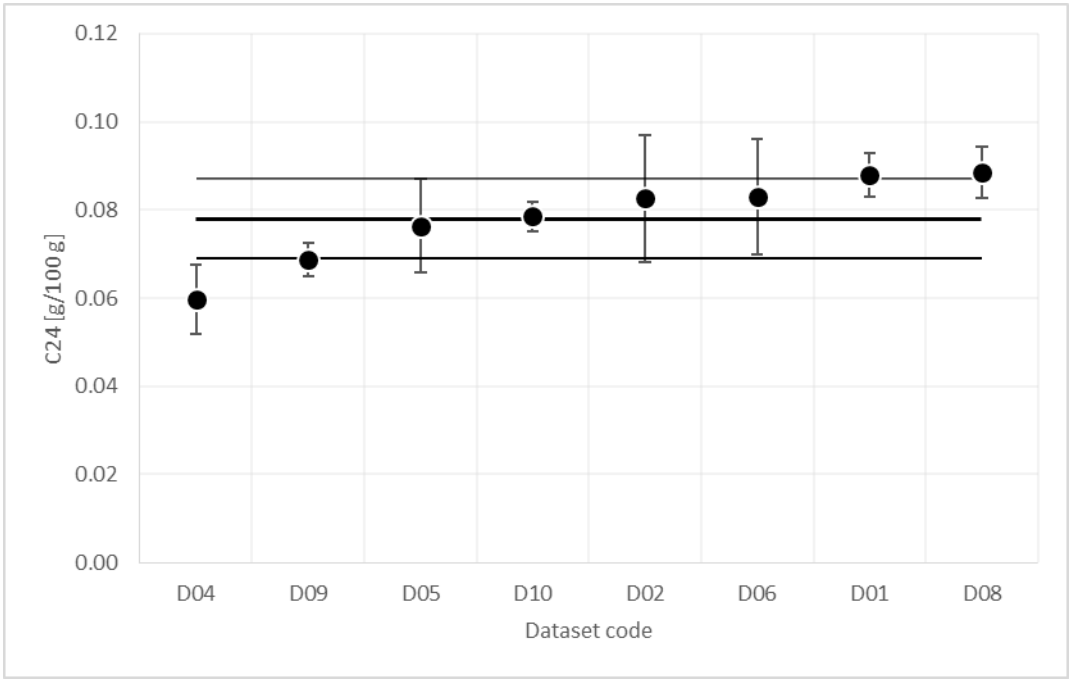


Table 6.2. Data from the characterisation for cholesterol.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	0.2866	0.2917	0.2826	0.2811	0.2835	0.2797	0.2842	0.0087
D02	0.2748	0.2740	0.2736	0.2760	0.2690	0.2699	0.2729	0.0056
D04	0.3476	0.3382	0.3260	0.3169	0.3324	0.3336	0.3325	0.0209
D05	0.2734	0.2729	0.2904	0.2884	0.2838	0.2831	0.2820	0.0148
D06	0.3658	0.3707	0.3665	0.3673	0.3402	0.3414	0.3586	0.0279
D08	0.2873	0.2860	0.2934	0.2975	0.3029	0.3063	0.2956	0.0164
D09	0.3066	0.3139	0.2829	0.2882	0.2656	0.2914	0.2915	0.0345
D10	0.3063	0.3093	0.3094	0.3076	0.3053	0.3086	0.3078	0.0034
Results not used for value assignment								
D03	0.6489	0.6191	0.4707	0.3605	0.4452	0.5885	0.5222	0.2273
D07	0.4064	0.4091	0.3921	0.3981	0.4128	0.4078	0.4044	0.0155

Figure 6.2. Characterisation data for cholesterol, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

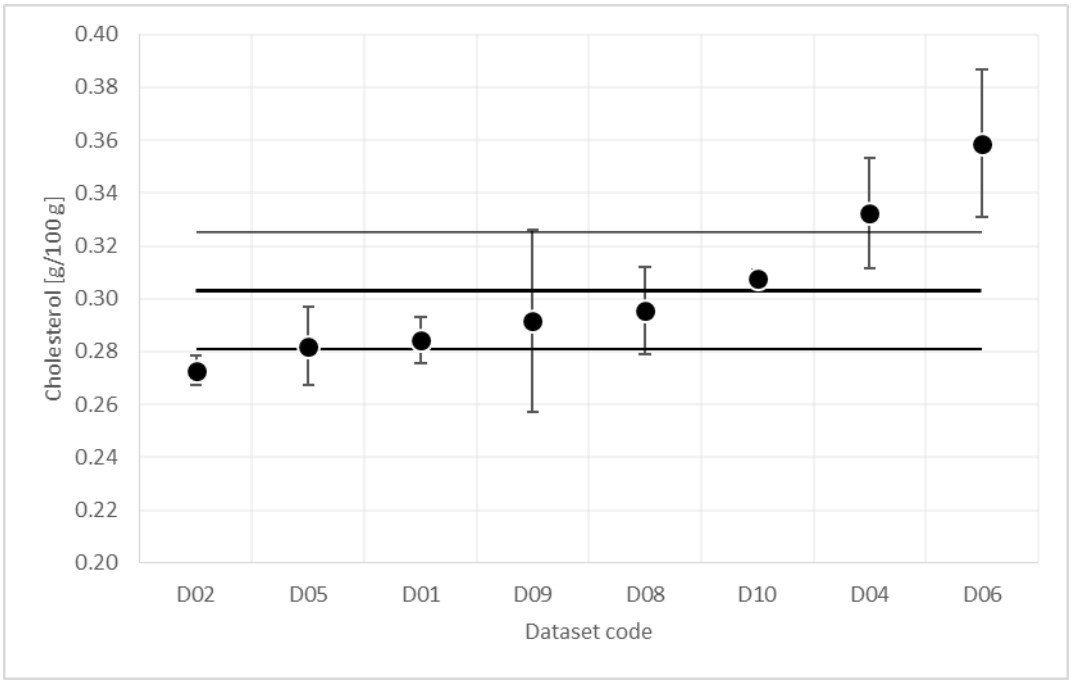


Table 6.3. Data from the characterisation for C26.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	0.3165	0.3327	0.3182	0.3177	0.3156	0.3184	0.3198	0.0128
D02	0.3689	0.3715	0.3775	0.3704	0.3175	0.3154	0.3535	0.0578
D04	0.3094	0.3023	0.3073	0.3063	0.3069	0.2961	0.3047	0.0096
D05	0.3193	0.3210	0.3152	0.3121	0.3264	0.3209	0.3192	0.0100
D06	0.4218	0.4211	0.4244	0.4219	0.3582	0.3506	0.3997	0.0703
D08	0.3901	0.3666	0.3895	0.3687	0.3487	0.3718	0.3726	0.0311
D09	0.2955	0.3169	0.2978	0.3327	0.3063	0.2980	0.3079	0.0290
D10	0.3216	0.3267	0.3428	0.3357	0.3192	0.3287	0.3291	0.0177
Results not used for value assignment								
D03	0.4939	0.4893	0.4915	0.4038	0.4498	0.4681	0.4661	0.0699
D07	0.4685	0.4660	0.4411	0.4581	0.4603	0.4495	0.4572	0.0207

Figure 6.3. Characterisation data for C26, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

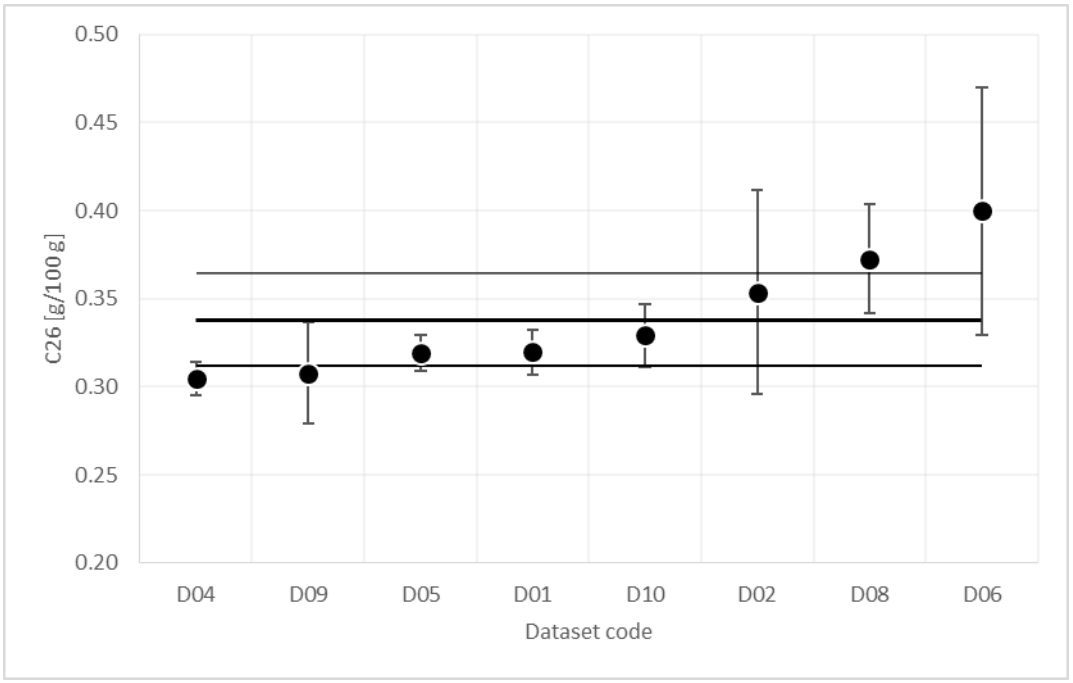


Table 6.4. Data from the characterisation for C28.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	0.7258	0.7401	0.7210	0.7206	0.7219	0.7216	0.7252	0.0151
D02	0.8056	0.8099	0.7699	0.7864	0.7224	0.7247	0.7698	0.0772
D04	0.7512	0.7506	0.7480	0.7497	0.7469	0.7378	0.7474	0.0099
D05	0.7429	0.7481	0.7365	0.7275	0.7545	0.7423	0.7420	0.0187
D06	0.8594	0.8679	0.8583	0.8599	0.7722	0.7581	0.8293	0.1000
D08	0.8144	0.7983	0.8362	0.8127	0.7824	0.8089	0.8088	0.0359
D09	0.7011	0.7100	0.7370	0.7526	0.7089	0.7032	0.7188	0.0420
D10	0.7527	0.7603	0.7830	0.7795	0.7544	0.7649	0.7658	0.0256
Results not used for value assignment								
D03	0.9087	0.8681	0.9113	0.7879	0.8866	0.9301	0.8821	0.1018
D07	0.8994	0.8996	0.8678	0.8760	0.8864	0.8765	0.8843	0.0264

Figure 6.4. Characterisation data for C28, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

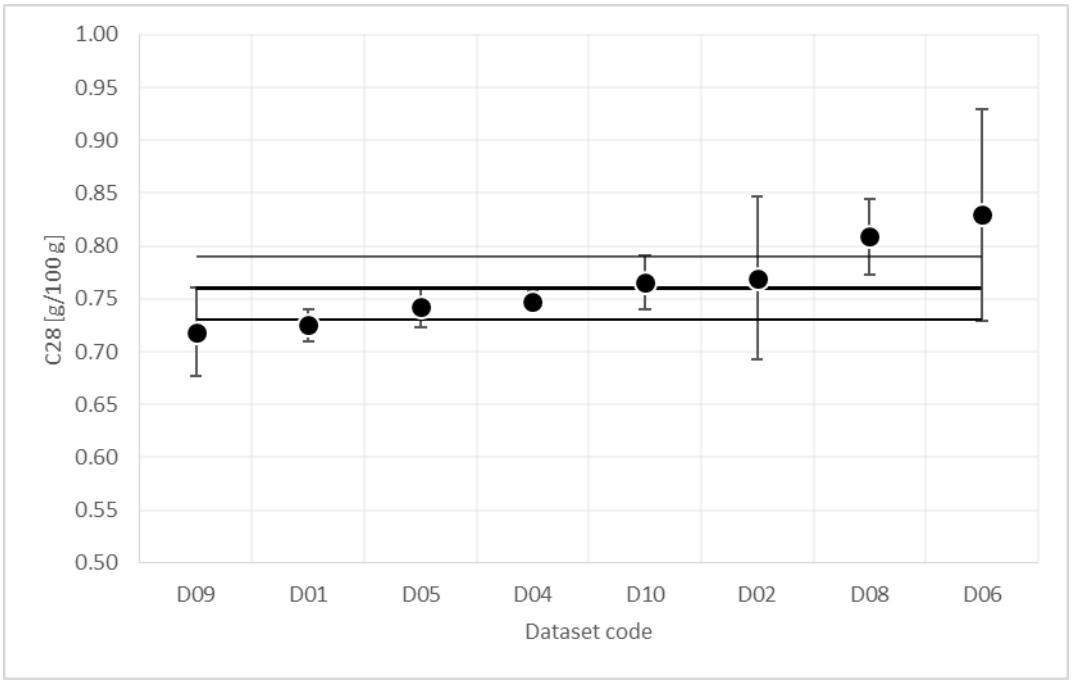


Table 6.5. Data from the characterisation for C30.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	1.4104	1.4153	1.3968	1.3922	1.3971	1.3909	1.4004	0.0200
D02	1.4812	1.4819	1.4292	1.4516	1.3987	1.4040	1.4411	0.0732
D04	1.4350	1.4303	1.4453	1.4422	1.4179	1.4103	1.4302	0.0274
D05	1.4291	1.4385	1.4193	1.4076	1.4410	1.4279	1.4272	0.0248
D06	1.5018	1.5116	1.4975	1.5007	1.4150	1.3858	1.4687	0.1079
D08	1.5268	1.5243	1.5761	1.5481	1.5006	1.5472	1.5372	0.0517
D09	1.3920	1.4018	1.4876	1.4633	1.3974	1.3908	1.4222	0.0844
D10	1.4787	1.4819	1.5181	1.5133	1.4779	1.4889	1.4931	0.0360
Results not used for value assignment								
D03	1.4667	1.4809	1.4937	1.3783	1.5083	1.5878	1.4859	0.1354
D07	1.5949	1.5942	1.5435	1.5594	1.5648	1.5542	1.5685	0.0428

Figure 6.5. Characterisation data for C30, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

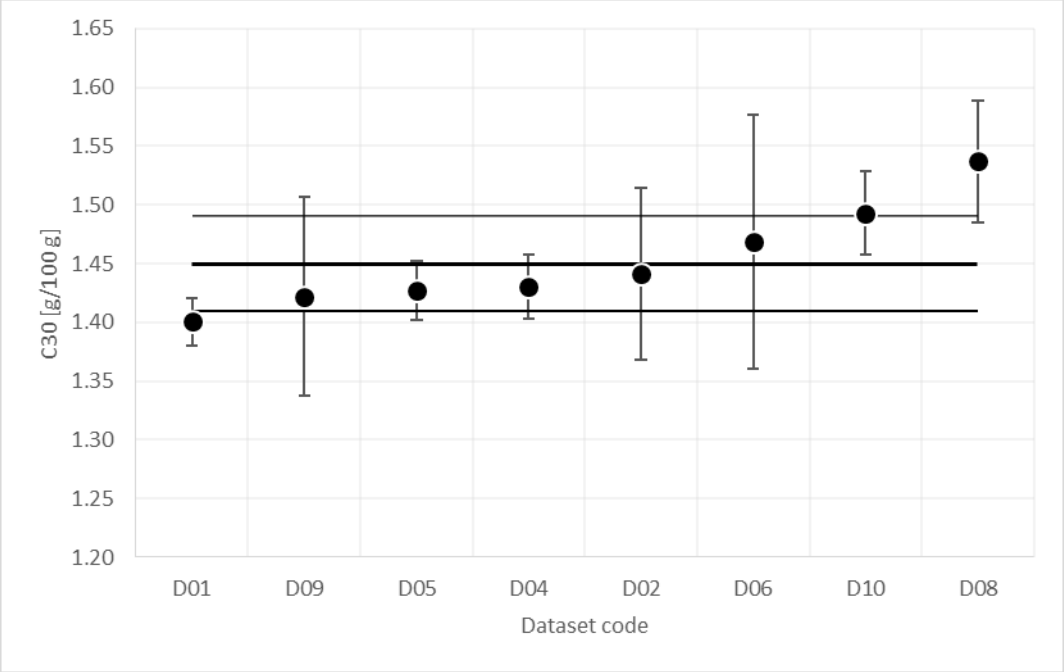


Table 6.6. Data from the characterisation for C32.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	2.9809	2.9933	2.9669	2.9628	2.9729	2.9538	2.9718	0.0280
D02	3.0409	3.0481	2.9630	2.9981	2.9806	2.9828	3.0023	0.0693
D04	2.9447	2.9366	2.9801	2.9762	2.9012	2.8820	2.9368	0.0788
D05	3.0233	3.0319	3.0037	2.9977	3.0282	3.0200	3.0175	0.0275
D06	2.9810	2.9857	2.9858	2.9879	2.8960	2.8831	2.9532	0.0991
D08	3.0601	3.0884	3.1284	3.1127	3.0603	3.1174	3.0945	0.0594
D09	3.0805	3.0825	3.2100	3.1659	3.0743	3.0913	3.1174	0.1133
D10	3.1344	3.1437	3.1800	3.1914	3.1371	3.1537	3.1567	0.0474
Results not used for value assignment								
D03	2.8267	2.8258	2.7898	2.7296	2.9182	2.9303	2.8367	0.1531
D07	3.1933	3.1957	3.1276	3.1339	3.1424	3.1315	3.1541	0.0634

Figure 6.6. Characterisation data for C32, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

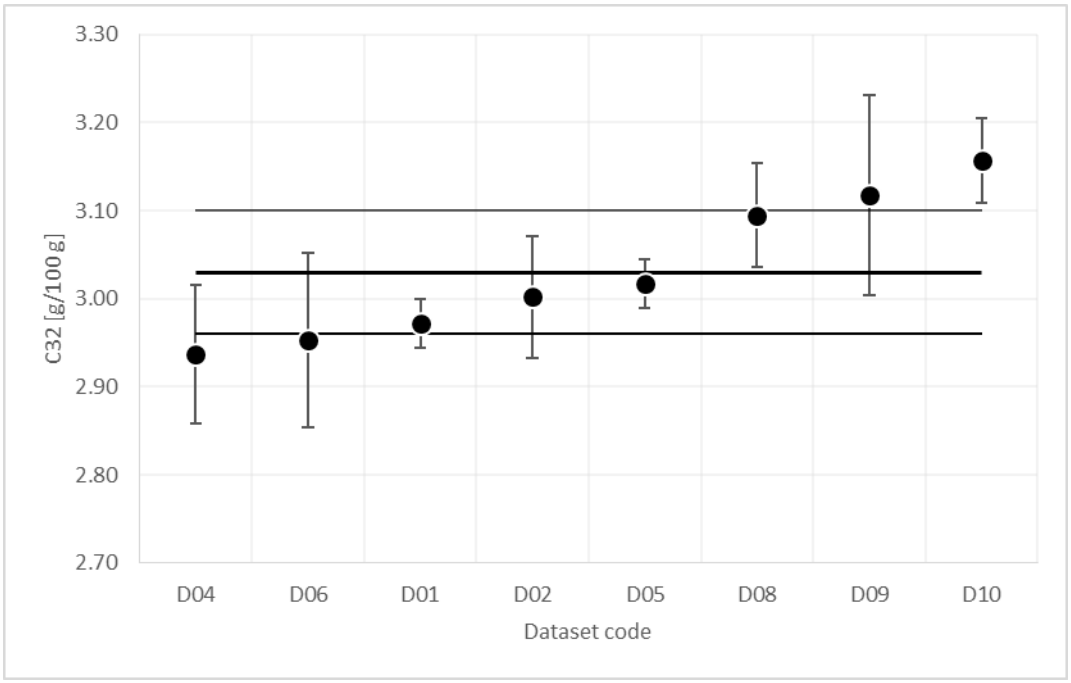


Table 6.7. Data from the characterisation for C34.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	6.5499	6.5495	6.5141	6.5077	6.5187	6.4837	6.5206	0.0511
D02	6.5180	6.5329	6.4058	6.4838	6.5039	6.4810	6.4876	0.0893
D04	6.6160	6.5973	6.6715	6.6844	6.5769	6.5280	6.6124	0.1176
D05	6.5708	6.5800	6.5547	6.5609	6.5548	6.5585	6.5633	0.0202
D06	6.3818	6.4108	6.3717	6.3702	6.3186	6.2807	6.3556	0.0946
D08	6.6084	6.4903	6.6527	6.6641	6.5980	6.6526	6.6110	0.1297
D09	6.7837	6.7797	6.9583	6.9127	6.8098	6.7619	6.8344	0.1622
D10	6.7630	6.7772	6.8170	6.8447	6.7744	6.7537	6.7883	0.0702
Results not used for value assignment								
D03	5.9086	5.9150	5.7391	5.7874	5.9950	6.0953	5.9067	0.2619
D07	6.7805	6.7900	6.7747	6.7676	6.7755	6.7601	6.7747	0.0206

Figure 6.7. Characterisation data for C34, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

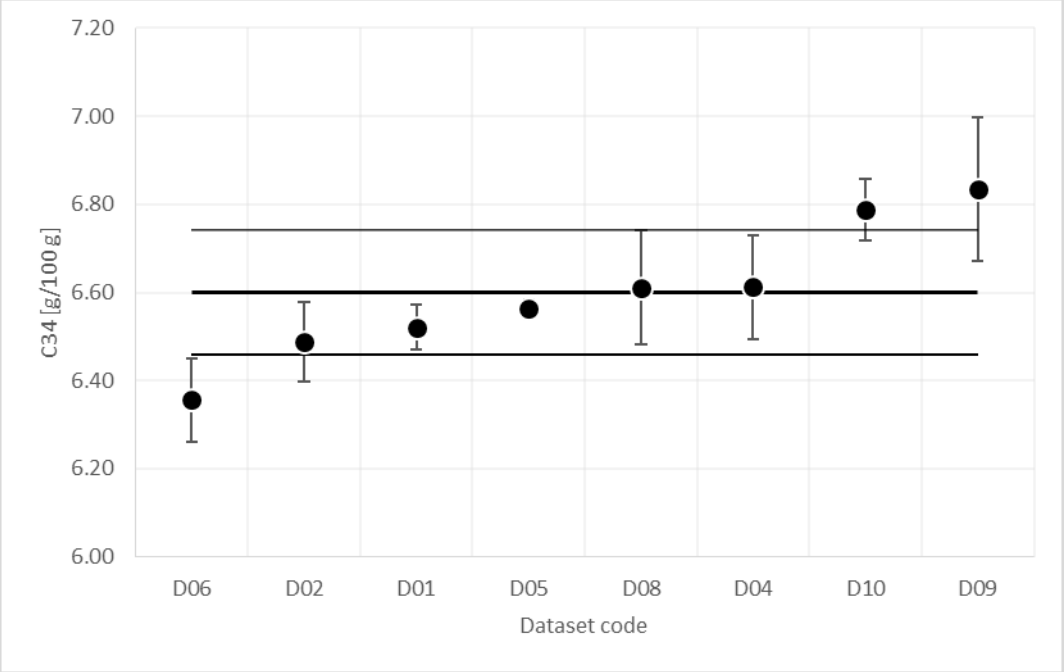


Table 6.8. Data from the characterisation for C36.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	11.2268	11.2023	11.1821	11.1832	11.1758	11.1356	11.1843	0.0605
D02	11.0901	11.0529	10.9340	11.0818	11.0954	11.0998	11.0590	0.1269
D04	11.4313	11.3586	11.4797	11.5115	11.4363	11.4013	11.4364	0.1089
D05	11.0577	11.0745	11.1128	11.1259	11.0016	11.0207	11.0655	0.0985
D06	11.0177	11.0287	10.9915	11.0180	11.0401	10.9661	11.0104	0.0540
D08	11.1927	11.3397	11.1734	11.2669	11.1752	11.2080	11.2260	0.1308
D09	11.4779	11.4951	11.7300	11.6284	11.5098	11.4427	11.5473	0.2188
D10	11.4836	11.4875	11.5016	11.5143	11.4344	11.4144	11.4726	0.0789
Results not used for value assignment								
D03	10.2887	10.3056	9.9673	10.1998	10.4034	10.3846	10.2582	0.3203
D07	11.5777	11.5756	11.6014	11.5738	11.5780	11.5498	11.5760	0.0328

Figure 6.8. Characterisation data for C36, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

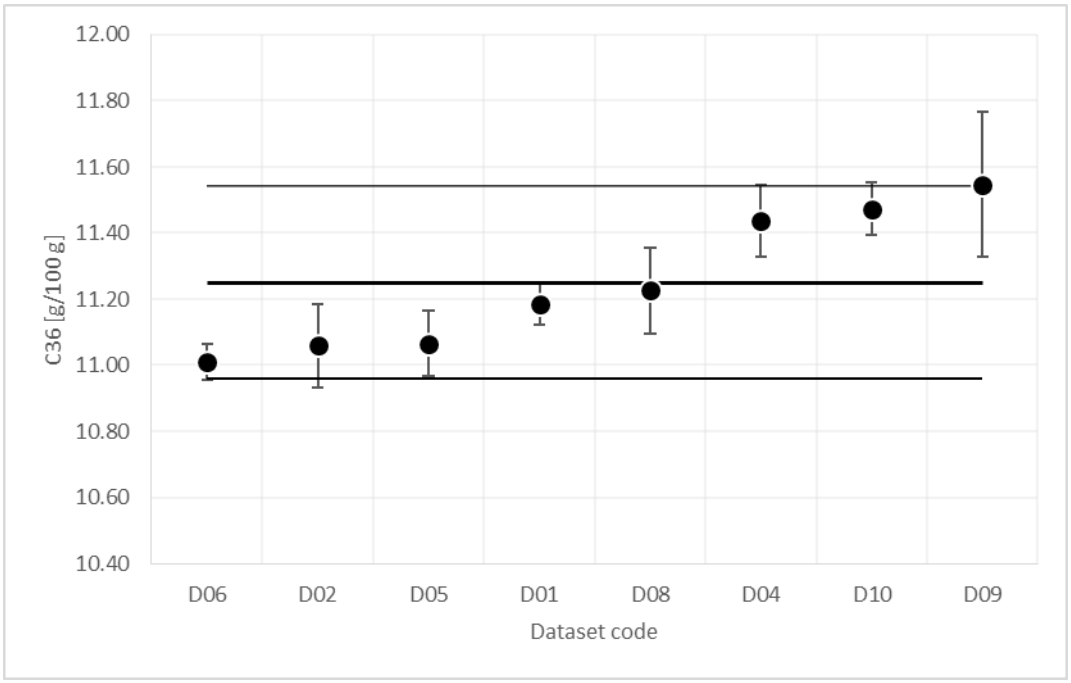


Table 6.9. Data from the characterisation for C38.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	12.3467	12.3431	12.3224	12.3163	12.3350	12.3098	12.3289	0.0300
D02	12.1547	12.1392	12.0132	12.2241	12.1627	12.1401	12.1390	0.1382
D04	12.5961	12.5886	12.7664	12.7965	12.7179	12.6953	12.6934	0.1721
D05	12.3626	12.3739	12.3996	12.4143	12.3090	12.3299	12.3649	0.0804
D06	12.2735	12.2742	12.2632	12.2895	12.3388	12.3238	12.2938	0.0612
D08	12.4699	12.4535	12.5042	12.5173	12.4591	12.4946	12.4831	0.0520
D09	12.6205	12.6314	12.7712	12.7414	12.6603	12.6130	12.6730	0.1344
D10	12.7063	12.7198	12.7116	12.7589	12.7570	12.7007	12.7257	0.0515
Results not used for value assignment								
D03	11.5595	11.6028	11.3678	11.6063	11.7357	11.5909	11.5772	0.2383
D07	13.1708	13.1485	13.0510	12.9971	13.0003	12.9640	13.0553	0.1715

Figure 6.9. Characterisation data for C38, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

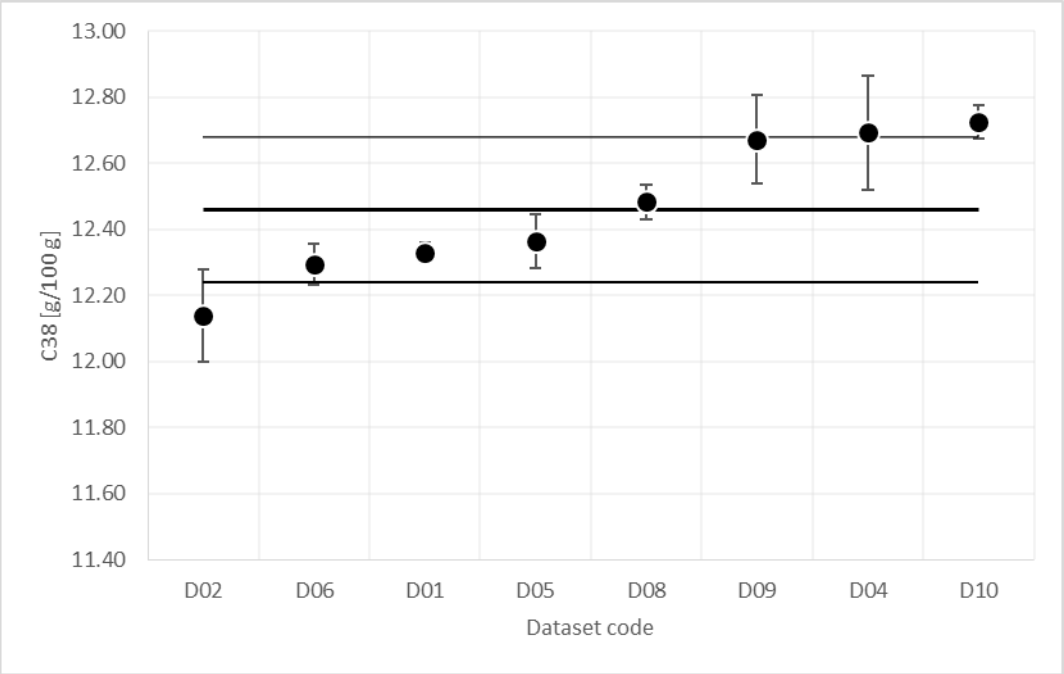


Table 6.10. Data from the characterisation for C40.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	9.8339	9.8534	9.8321	9.8325	9.8231	9.8217	9.8328	0.0227
D02	9.6622	9.7088	9.5524	9.6449	9.6774	9.6484	9.6490	0.1054
D04	9.8370	9.8432	9.9275	9.9552	9.8838	9.9029	9.8916	0.0931
D05	9.8832	9.8843	9.8938	9.8973	9.8395	9.8511	9.8749	0.0476
D06	9.9317	9.9271	10.0021	9.9370	10.0345	10.0616	9.9823	0.1169
D08	9.9021	9.9115	9.8965	9.9126	9.9151	9.8866	9.9041	0.0222
D09	10.0620	10.0696	10.1809	10.1972	10.0807	10.0735	10.1106	0.1225
D10	9.9976	10.0226	10.0376	10.0290	10.0234	9.9996	10.0183	0.0324
Results not used for value assignment								
D03	9.3241	9.3225	9.1733	9.3799	9.4278	9.4717	9.3499	0.2086
D07	10.5172	10.4964	10.4373	10.4399	10.4589	10.4241	10.4623	0.0736

Figure 6.10. Characterisation data for C40, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

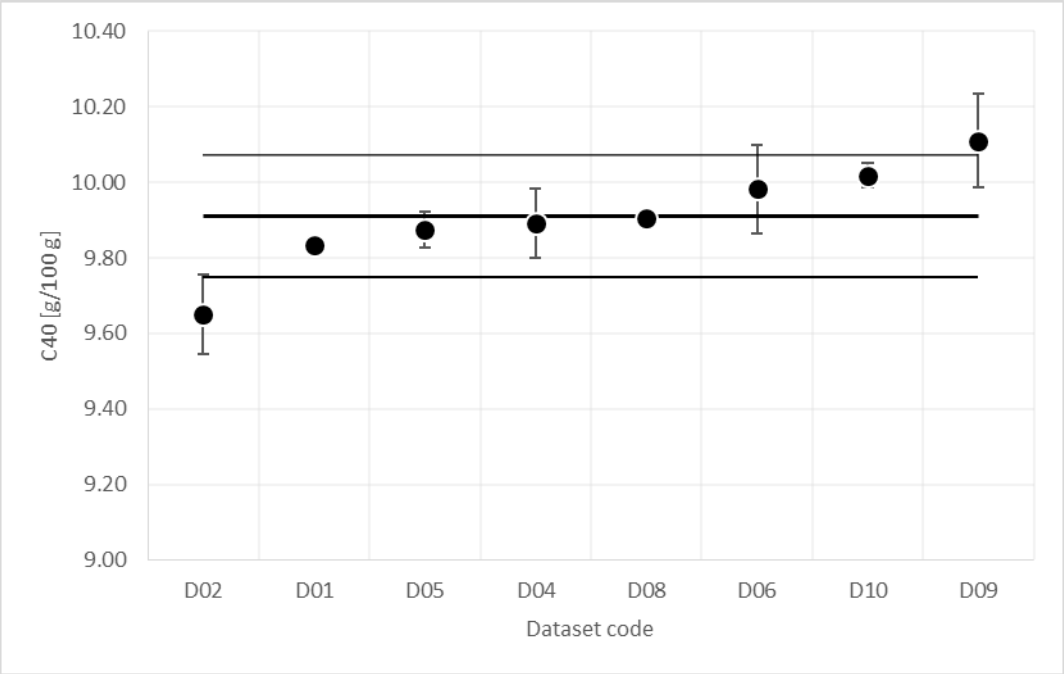


Table 6.11. Data from the characterisation for C42.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	7.4411	7.4241	7.4040	7.4159	7.4054	7.4086	7.4165	0.0283
D02	7.3020	7.3469	7.1960	7.3202	7.2899	7.2774	7.2887	0.1031
D04	7.2403	7.2430	7.3823	7.3737	7.2166	7.2281	7.2807	0.1521
D05	7.4779	7.4850	7.4759	7.4679	7.4507	7.4465	7.4673	0.0311
D06	7.3598	7.3161	7.3799	7.3676	7.4301	7.4350	7.3814	0.0902
D08	7.4546	7.4498	7.4266	7.4259	7.4509	7.4371	7.4408	0.0255
D09	7.5036	7.4936	7.5963	7.6570	7.5035	7.5305	7.5474	0.1310
D10	7.3865	7.4075	7.4186	7.4135	7.4111	7.4146	7.4086	0.0229
Results not used for value assignment								
D03	7.3486	7.3586	7.4132	7.4510	7.4582	7.4273	7.4095	0.0926
D07	7.7630	7.7570	7.6591	7.6638	7.6696	7.6519	7.6941	0.1029

Figure 6.11. Characterisation data for C42, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

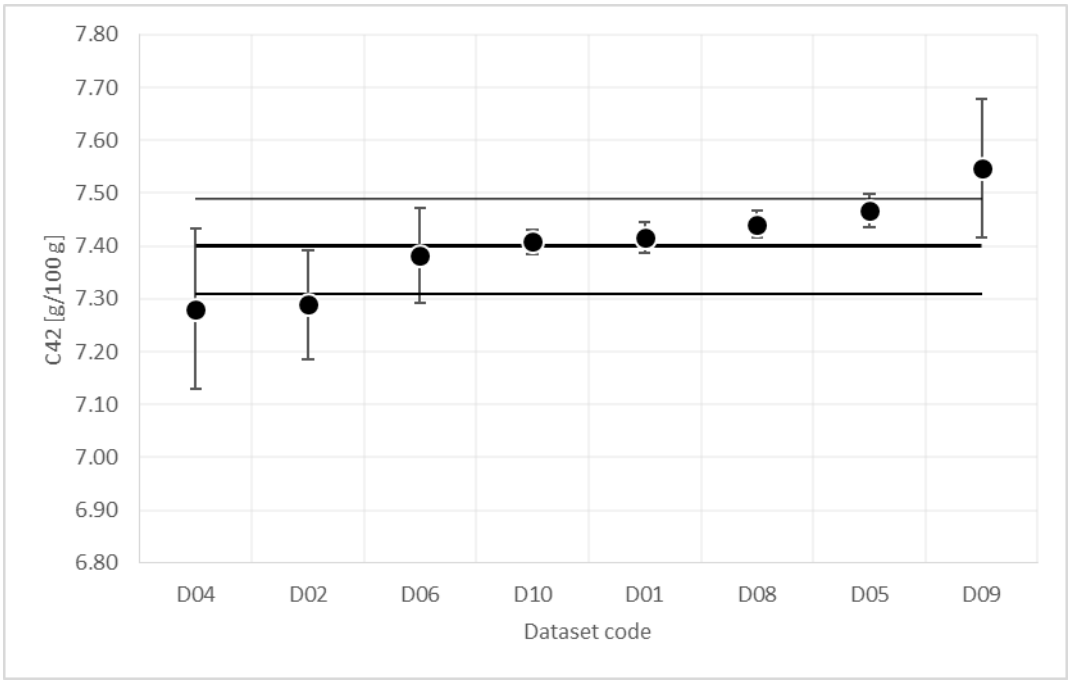


Table 6.12. Data from the characterisation for C44.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	7.1382	7.1548	7.1467	7.1558	7.1505	7.1553	7.1502	0.0137
D02	7.1027	7.0950	6.9827	7.0785	7.0685	7.1012	7.0714	0.0910
D04	7.0136	7.0109	7.1002	7.1254	7.0146	7.0122	7.0461	0.1045
D05	7.1427	7.1456	7.1237	7.1221	7.1280	7.1143	7.1294	0.0246
D06	7.0856	7.0701	7.1315	7.1042	7.1190	7.1440	7.1091	0.0559
D08	7.1104	7.1465	7.1037	7.1214	7.1026	7.0844	7.1115	0.0420
D09	7.1176	7.1065	7.1384	7.2233	7.1254	7.1746	7.1476	0.0878
D10	7.0514	7.0514	7.0334	7.0366	7.0299	7.0400	7.0404	0.0182
Results not used for value assignment								
D03	7.2948	7.2922	7.3819	7.4219	7.3938	7.3705	7.3592	0.1074
D07	7.0738	7.0773	7.0354	7.0181	7.0267	7.0367	7.0447	0.0497

Figure 6.12. Characterisation data for C44, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

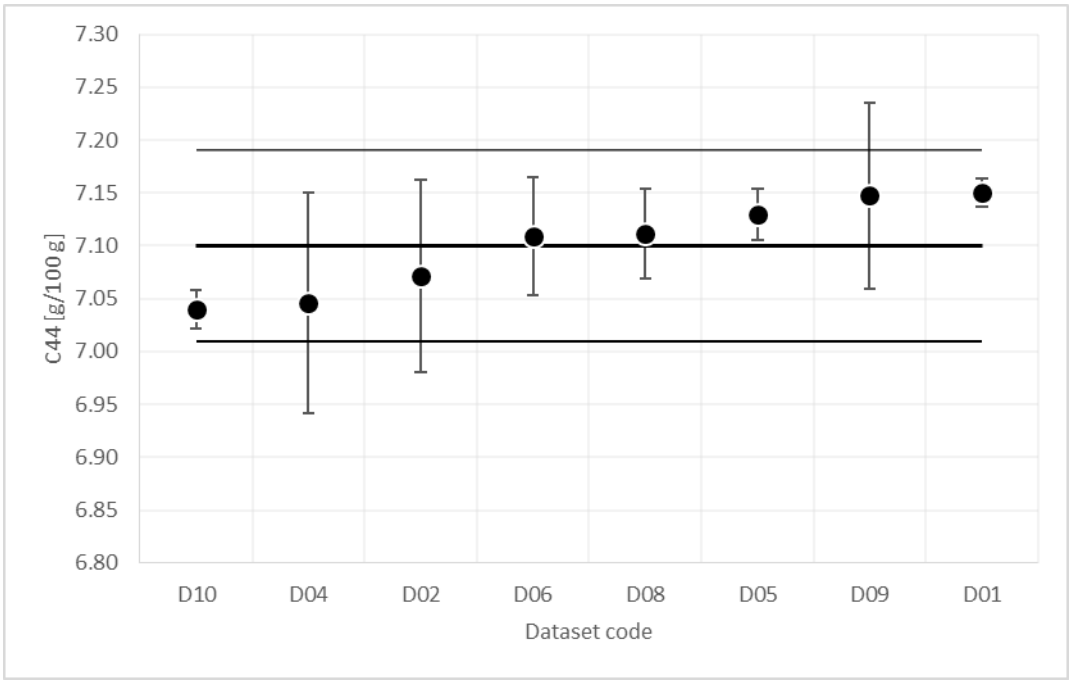


Table 6.13. Data from the characterisation for C46.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	7.8025	7.8150	7.8102	7.8087	7.8239	7.8307	7.8152	0.0208
D02	7.8274	7.8330	7.8563	7.8278	7.8075	7.8203	7.8287	0.0322
D04	7.7912	7.7667	7.7218	7.7334	7.7295	7.7514	7.7490	0.0526
D05	7.7506	7.7493	7.7176	7.7267	7.7384	7.7402	7.7371	0.0258
D06	7.7584	7.7146	7.6993	7.7217	7.7340	7.8037	7.7386	0.0751
D08	7.7528	7.7365	7.7237	7.6894	7.7402	7.7034	7.7243	0.0479
D09	7.6626	7.6674	7.6358	7.7823	7.6837	7.6998	7.6886	0.1013
D10	7.6282	7.6284	7.6142	7.5943	7.6138	7.6190	7.6163	0.0251
Results not used for value assignment								
D03	7.8976	7.9878	8.0601	8.1744	8.1394	8.0942	8.0589	0.2042
D07	7.5081	7.5137	7.5159	7.5260	7.5055	7.5229	7.5154	0.0161

Figure 6.13. Characterisation data for C46, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

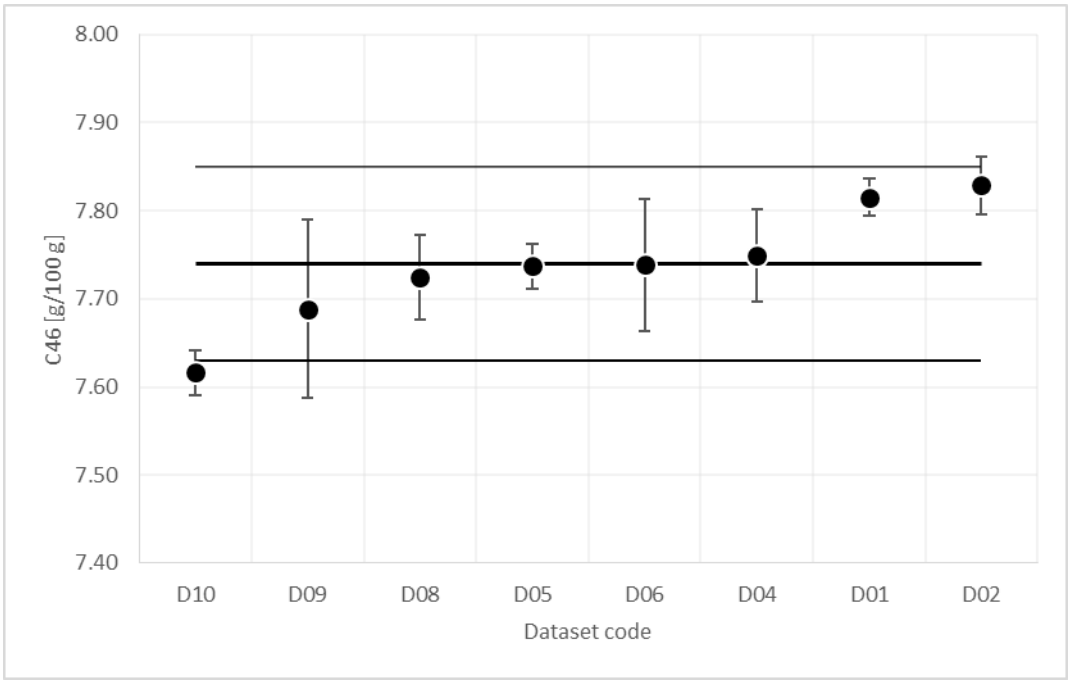


Table 6.14. Data from the characterisation for C48.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	9.1622	9.1280	9.1875	9.1776	9.1818	9.2082	9.1742	0.0542
D02	9.1385	9.0813	9.3292	9.1667	9.2072	9.1941	9.1862	0.1664
D04	9.0825	9.1269	8.9700	8.9652	9.0833	9.1002	9.0547	0.1387
D05	9.1323	9.1208	9.1088	9.1095	9.1376	9.1322	9.1235	0.0248
D06	9.2348	9.2664	9.2108	9.1818	9.2892	9.2841	9.2445	0.0858
D08	9.0340	9.0550	9.0420	9.0210	9.0396	9.0235	9.0358	0.0252
D09	8.9310	8.8905	8.7954	8.8544	8.8890	8.9474	8.8846	0.1096
D10	8.8853	8.8704	8.8174	8.8247	8.8459	8.8414	8.8475	0.0523
Results not used for value assignment								
D03	8.8796	8.8384	9.2165	9.1731	9.3016	9.2491	9.1097	0.3982
D07	8.7601	8.7630	8.8491	8.8552	8.8614	8.8851	8.8290	0.1073

Figure 6.14. Characterisation data for C48, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

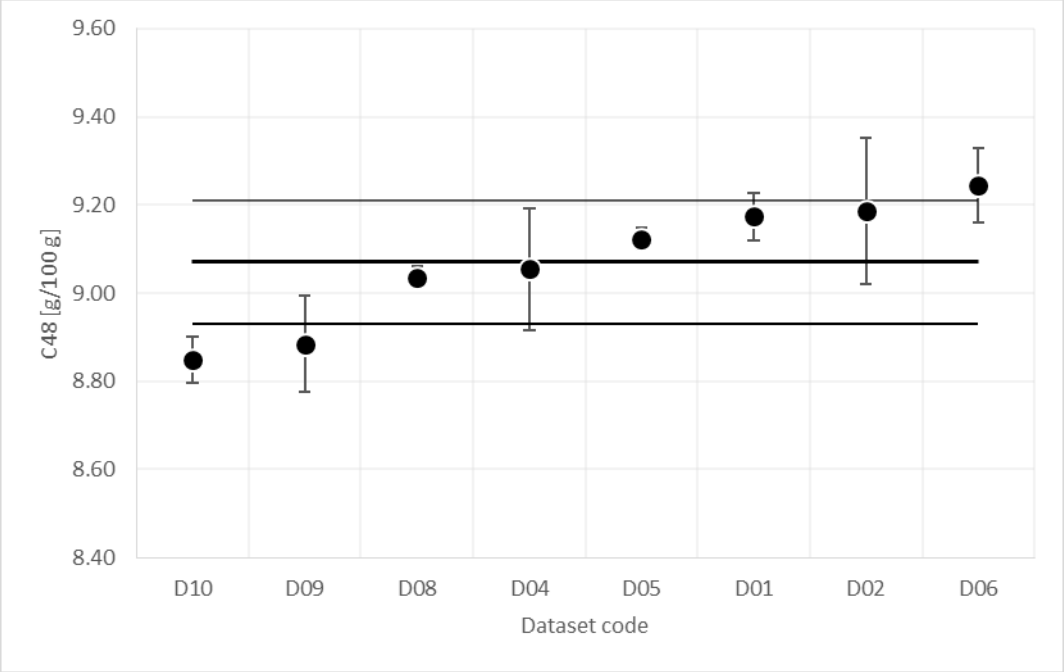


Table 6.15. Data from the characterisation for C50.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	10.4488	10.4393	10.5163	10.5052	10.5236	10.5485	10.4969	0.0869
D02	10.6148	10.6439	11.0202	10.6975	10.7405	10.7547	10.7453	0.2903
D04	10.3926	10.4658	10.1914	10.1725	10.3835	10.3994	10.3342	0.2433
D05	10.4701	10.4474	10.4189	10.4314	10.4696	10.4816	10.4532	0.0494
D06	10.3679	10.3589	10.3299	10.3602	10.3753	10.3716	10.3606	0.0326
D08	10.2403	10.2633	10.2384	10.2417	10.2572	10.2282	10.2448	0.0260
D09	10.1176	10.1455	9.8811	9.8315	10.0928	10.1549	10.0373	0.2854
D10	10.1235	10.0797	10.0509	10.0179	10.1057	10.1132	10.0818	0.0817
Results not used for value assignment								
D03	10.0947	10.0072	10.3101	10.5118	10.3922	10.3519	10.2780	0.3806
D07	9.7042	9.7088	9.8211	9.8058	9.7956	9.8357	9.7785	0.1149

Figure 6.15. Characterisation data for C50, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

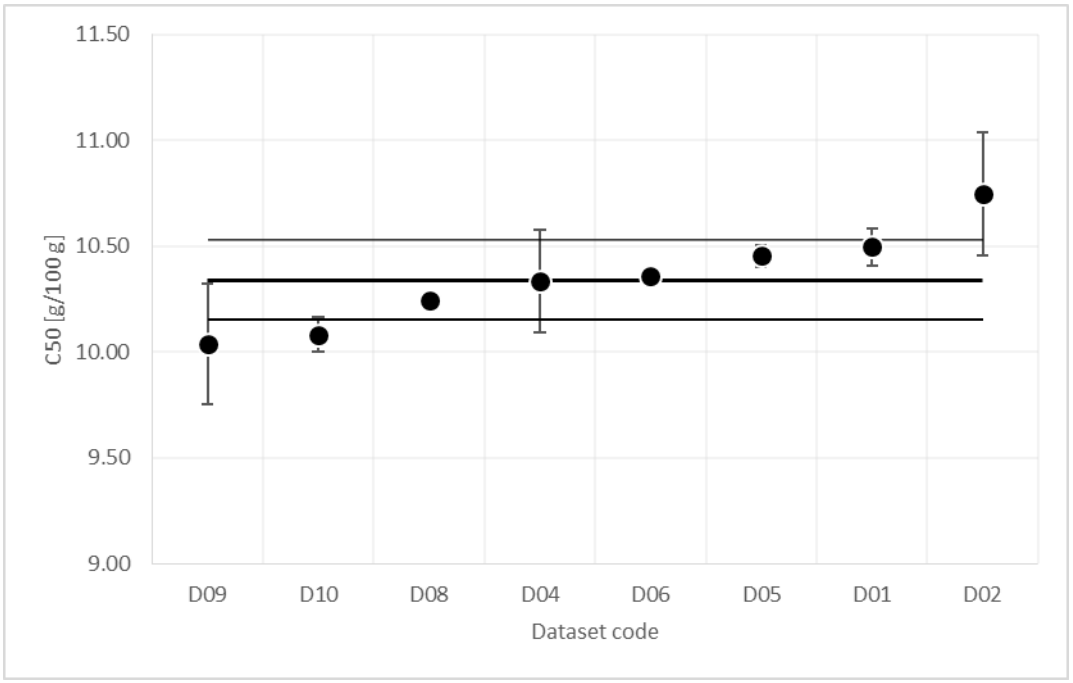


Table 6.16. Data from the characterisation for C52.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	8.4169	8.3963	8.4614	8.4714	8.4552	8.4889	8.4484	0.0698
D02	8.6941	8.6673	8.8668	8.6642	8.8244	8.8103	8.7545	0.1789
D04	8.3124	8.3298	8.1591	8.1140	8.3723	8.4166	8.2840	0.2413
D05	8.3601	8.3374	8.4211	8.4119	8.4371	8.4433	8.4018	0.0864
D06	8.3880	8.4299	8.3784	8.4324	8.4655	8.4742	8.4281	0.0781
D08	8.2795	8.2382	8.2328	8.2060	8.3291	8.2167	8.2504	0.0921
D09	8.1328	8.1024	7.7199	7.6421	8.1311	8.0913	7.9699	0.4515
D10	8.1049	8.0833	8.0291	8.0328	8.1600	8.1591	8.0949	0.1159
Results not used for value assignment								
D03	8.9175	9.0382	9.1612	9.0252	8.8288	8.6781	8.9415	0.3433
D07	7.4818	7.4887	7.6673	7.6864	7.6554	7.7045	7.6140	0.2023

Figure 6.16. Characterisation data for C52, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).

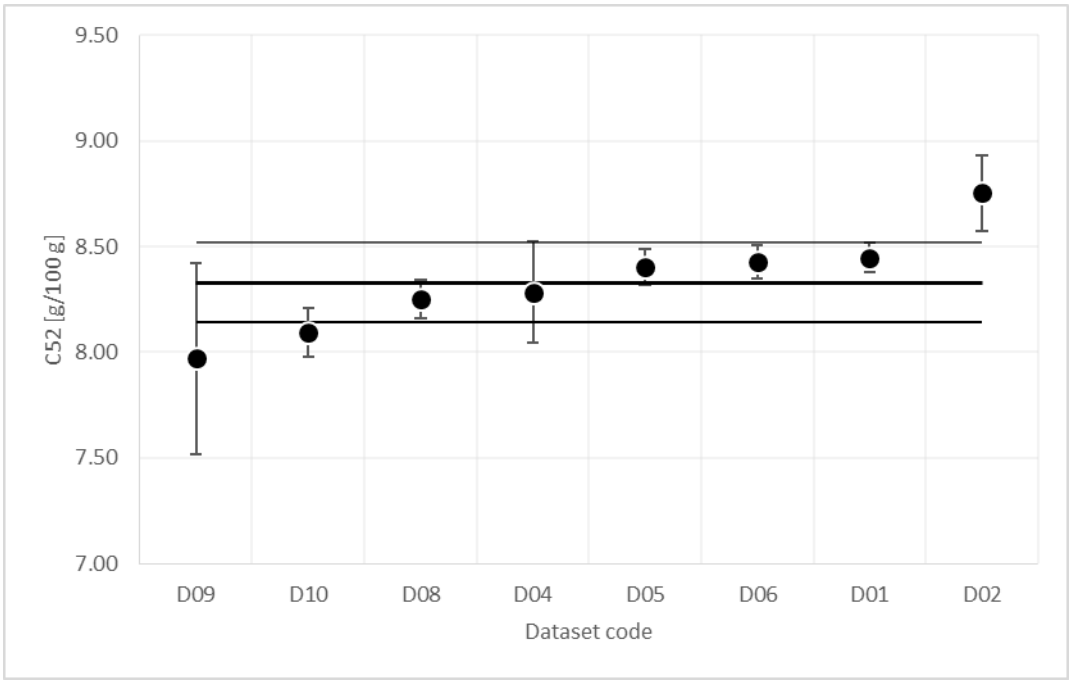
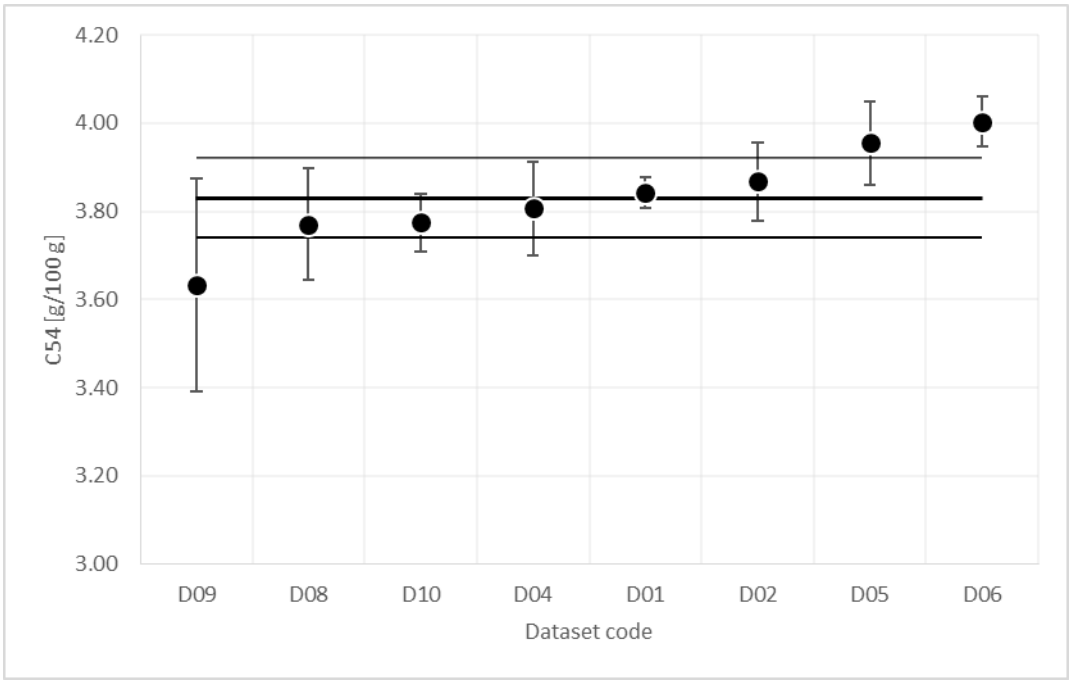


Table 6.17. Data from the characterisation for C54.

Dataset code	replicate 1 [g/100 g]	replicate 2 [g/100 g]	replicate 3 [g/100 g]	replicate 4 [g/100 g]	replicate 5 [g/100 g]	replicate 6 [g/100 g]	mean [g/100 g]	Expanded uncertainty [g/100 g]
D01	3.8248	3.8287	3.8499	3.8648	3.8297	3.8585	3.8427	0.0344
D02	3.8386	3.8252	3.9410	3.8424	3.8615	3.9020	3.8684	0.0889
D04	3.8361	3.8485	3.7600	3.7203	3.8220	3.8504	3.8062	0.1073
D05	3.9267	3.9135	3.9283	3.9306	4.0255	4.0037	3.9547	0.0946
D06	3.9989	3.9667	4.0228	3.9908	3.9914	4.0478	4.0031	0.0567
D08	3.7898	3.7629	3.6944	3.7048	3.8533	3.8196	3.7708	0.1258
D09	3.7467	3.7245	3.5067	3.4556	3.6951	3.6672	3.6326	0.2429
D10	3.7986	3.7716	3.7539	3.7272	3.7742	3.8203	3.7743	0.0655
Results not used for value assignment								
D03	6.0289	5.9435	5.9600	5.5244	4.6259	4.6791	5.4603	1.3014
D07	3.0389	3.0528	3.1565	3.1777	3.1374	3.1781	3.1236	0.1245

Figure 6.17. Characterisation data for C54, bold lines resembling the certified value and its uncertainty (Error bars reflect twice the standard deviation of the six results obtained in a laboratory).



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