CCQM-K154.c

Key Comparison Study – Organic Solvent Calibration Solution

Gravimetric preparation and value assignment of deoxynivalenol (DON) in acetonitrile (ACN)

Final Report

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SUMMARY

The CCQM-K154.c comparison was coordinated by the BIPM and NIM on behalf of the CCQM Organic Analysis Working Group (OAWG) for National Measurement Institutes (NMIs) and Designated Institutes (DIs) which provide measurement services in organic analysis under the ‘Comité International des Poids et Mesures’ Mutual Recognition Arrangement (CIPM MRA) and/or have participated in the BIPM’s Mycotoxin Metrology Capacity Building and Knowledge Transfer (MMCBKT) project as part of its "Metrology for Safe Food and Feed in Developing Economies" Capacity Building Programme. Gravimetrically prepared solutions having an assigned mass fraction of specified organic analytes are routinely used to calibrate measurement processes for the quantification of the same analytes in matrix samples. Appropriate assignments of the property value and associated uncertainty of calibration solutions thus underpin the traceability of routine analysis and are critical for accurate measurements. Evidence of successful participation in relevant international comparisons is needed to document calibration and measurement capability claims (CMCs) made by national metrology institutes and designated institutes. In total, ten NMIs/DIs participated in the Track C, Model II, Key Comparison CCQM-K154.c [Gravimetric preparation and value assignment of deoxynivalenol (DON) in acetonitrile (ACN)] for emerging areas of global interest and innovation. Participants were requested to gravimetrically prepare calibration solutions and value assign the mass fractions, expressed in mg/kg, of deoxynivalenol (DON) in the acetonitrile (ACN) solution. Study samples, with assigned values and associated uncertainties were prepared by the comparison participants and sent to the coordinating laboratory for comparison. The Key Comparison Reference Values (KCRVs) were assigned of all participant values that agreed within their expanded uncertainty with the values measured by the coordinating laboratory based on calibrations obtained from independent gravimetrically prepared calibrant solutions.

Successful participation in CCQM‐K154.c for MMCBKT participants was intended to demonstrate measurement capabilities for preparation and value assignment of deoxynivalenol (DON) calibration solutions in the mass fraction range of 10 mg/kg to 100 mg/kg, prepared from a mycotoxin stock solution of pre-assigned content or solid of known purity. Successful participation for other participants, having value assigned their pure Primary Reference Materials, was intended to demonstrate measurement capabilities for the purity value assignment capabilities of organic materials with molar mass in the range 100 g/mol to 500 g/mol and polarity (*pKow*) > -2, with relative uncertainties at or above the relative uncertainty achieved in the comparison for calibration solutions as well as for the preparation and value assignment of single component organic calibration solutions with polar analytes in the mass fraction range of 10 mg/kg to 100 mg/kg, polarity (*pKow*) > -2, with molar mass in the range of 100 g/mol to 500 g/mol.

TABLE OF CONTENT

ACRONYMS

ACN	Acetonitrile
ANOVA	Analysis of variance
CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CMC	Calibration and Measurement Capability
DI	Designated Institute
DoE	Degree of equivalence
DON	Deoxynivalenol
ESI	Electrospray ionization
GLS	Generalized Least Squares regression analysis
KCRV	Key Comparison Reference Value
LC-DAD-MS/MS	Liquid chromatography with (UV) diode array and tandem mass spectrometric detection
MMCBKT	Mycotoxin Metrology Capacity Building and Knowledge Transfer
NMI	National Metrology Institute
NMR	Nuclear magnetic resonance spectroscopy
OAWG	Organic Analysis Working Group
pKow	Negative log base 10 of the octanol-water partition coefficient
qNMR	Quantitative nuclear magnetic resonance spectroscopy
SRM	Selected reaction monitoring

SYMBOLS

D_i	Degree of equivalence
$D_{rel,\, i}$	Percent relative degree of equivalence
k	Coverage factor
n	Number of quantity values in a series of quantity values
$u(x_i)$	Standard uncertainty of quantity value x_i
$U(x_i)$	Expanded uncertainty of quantity value x_i
$U_{95}(x_i)$	Expanded uncertainty defined such that $x_i \pm U_{95}(x_i)$ is asserted to include the true value of the quantity with an approximate 95 % level of confidence
x	A quantity value
x_i	i^{th} member of a series of quantity values
w_i	Mass fraction of organic analyte in kg/kg or subunits thereof in a given matrix

INTRODUCTION

The CCQM-K154.c comparison, agreed by the CCQM, was organized to support National Metrology Institutes (NMIs) or Designated Institutes (DIs) that have developed capabilities to prepare and value assign mycotoxin calibration solutions to benchmark and demonstrate the comparability of their measurement services.

Calibration solutions prepared from well characterized, high purity compounds are the source of metrological traceability of most routine organic analysis results. The preparation and characterization of these solutions is therefore essential within the measurement infrastructure that supports the delivery of reliable results. It is particularly challenging in the case of the provision of standards to underpin mycotoxin testing in developing economies due to stringent export / import regulations, challenging logistics and high costs.

A number of NMIs/DIs have participated in the BIPM's Mycotoxin Metrology Capacity Building and Knowledge Transfer (MMCBKT) project as part of its "Metrology for Safe Food and Feed in Developing Economies" Capacity Building Programme. The project was designed to allow NMIs/DIs to work together to strengthen mycotoxin metrology infrastructure; provide knowledge transfer to scientists developing capabilities in this area, including periods as visiting scientists at the BIPM; and enable NMIs to provide mycotoxin calibrant and matrix reference materials and proficiency test materials to support mycotoxin testing laboratories within their countries [1].

The CCQM-K154.a and CCQM-K154.a.1 comparisons on the gravimetric preparation and value assignment of the *Fusarium* mycotoxin zearalenone (ZEN) in acetonitrile (ACN) [2, 3, 4, 5] and the CCQM-K154.b comparison on the gravimetric preparation and value assignment of the *Aspergillus* mycotoxin aflatoxin B_1 (AfB_1) in acetonitrile (ACN) were the first comparisons of a series of comparisons that allowed NMIs/DIs that have participated in the MMCBKT project to demonstrate the compatibility of the capabilities and services they have established in their laboratories [6, 7, 8]. The CCQM-K154.c comparison on the gravimetric preparation and value assignment of the *Fusarium* mycotoxin deoxynivalenol (DON) in acetonitrile (ACN) tests core skills and competencies required in gravimetric preparation and value assignment of organic solvent-based calibration solutions of mycotoxins. It is considered as a Track C, Model II comparison. Track C comparisons are for an emerging area of global interest and innovation. The aim of Track C key comparisons is to underpin future CMCs. Model II signifies that study samples are sent to the coordinator for comparison under repeatability conditions. In addition, the comparison is used to demonstrate the compatibility of laboratory capabilities to assign the mass fraction of single polar organic analytes in organic solutions. This study involved a comparison at the BIPM of a suite of DON calibration solutions prepared by each of the participating laboratories. Seven laboratories took part in the framework of the MMCBKT while three laboratories participated to demonstrate their in-house calibration solution production capabilities. The calibration solutions have been sent to the BIPM where an LC-DAD(-MS/MS) method was used to compare the value assignments of the mass fraction content of DON in the solutions provided by each participant.

Deoxynivalenol (DON) belongs to the large group of trichothecene mycotoxins [9]. It is produced by certain fungi of the genus *Fusarium* that predominantly infect wheat, corn, oats, barley, rice, and other grains in the field or during storage. The exposure risk to human is directly through foods of plant origin (flour, bread, breakfast cereals, noodles, infant foods, pancakes, malt and beer) or indirectly through foods of animal origin (carry-over). DON occurs as predominant mycotoxin in the northern part of the globe and due to the adverse effects in animals, DON is known as one of the most significant for animal production (decrease in feed intake and reduced weight gain). The main toxic effect of DON is the inhibition of protein synthesis and mitochondrial function leading to effects such as immunosuppression and cytotoxic effects. DON affects animal and human health causing acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever. DON is a polar and water-soluble chemical compound and it is heat-stable, being able to partially withstand temperatures within the range of 170 °C to 350 °C, with no reduction of DON concentration after 30 min at 170 °C [10, 11, 12].

The importance of monitoring DON content in primary products and derived foodstuffs is reflected in the existence of regulations controlling the maximum limits for DON more than thirty-five countries. A typical limit is 750 µg/kg in wheat (flour) for direct human consumption [13, 14].

The ability to undertake robust and reliable quantitative analysis to establish levels of contamination of primary produce with DON and related compounds, or conversely to demonstrate its absence, is required for ensuring compliance with health and food safety regulations and to avoid the potential for disruption and damage to international trade between producers and consumers of large quantities of cereal grains and wheat. The analytical difficulty and the economic importance of controlling DON in food and feed support the need for solution and matrix certified reference materials. They are invaluable tools to ensure comparability and traceability in DON measurements and are very useful for the implementation of written standards, legislation/regulations and laboratory accreditation.

MEASURAND, QUANTITIES AND UNITS

The measurand was the mass fraction of deoxynivalenol [DON] present in solution acetonitrile (ACN), with the assigned value expressed in mg/kg (or one of its multiples µg/g, mg/g or ng/g).

PARTICIPANTS AND SCHEDULE

This study involved a simultaneous comparison of a suite of ten calibration solutions of DON in ACN gravimetrically prepared and value assigned by each of the participating laboratories.

Seven laboratories (INM, INMETRO, INTI, LATU, NIM, NIMT and UME) took part in the CCQM-K154.c comparison within the framework of the MMCBKT, using a value assigned stock solution of DON in ACN supplied by the BIPM. Three laboratories (NIM, NRC and NMISA) took part in CCQM-K154.c using their own stock solution of DON. NIM participated in CCQM-K154.c using both their own calibration solution and the solution supplied by the BIPM within the framework of the MMCBKT.

The study schedule for CCQM-K154.c is given in Table 1.

Table 1: CCQM-K154.c Timetable

Action	Date
Initial discussion	April 2019 MMCBKT and OAWG meetings
Study Proposal and draft protocol	September 2020 OAWG meeting
Approval of study protocol and confirmation	April 2021 OAWG meeting
Stock solution distribution	beginning March 2021 (MMCBKT participants)
Call for participation	July 1st, 2021
Final date to register	August 1st, 2021
Samples and data due to coordinator	October 31st, 2021
Initial presentation and discussion of results	June 2022 OAWG meeting
Draft A report	September 2022
Draft B report	October 2022
Final report to OAWG Chair	November 2022

DON PRIMARY CALIBRATOR STOCK SOLUTION

The BIPM provided the MMCBKT participants with a stock solution of DON in acetonitrile (OGP.034) that was to be used for the preparation of DON calibration solution batches submitted for comparison CCQM-K154.c.

The DON mass fraction and associated expanded uncertainty ($k = 2$) of the DON stock solution OGP.034 was 627.1 ± 19.2 mg/kg. The uncertainty corresponding to the gravimetric value assignment the homogeneity and stability uncertainty contribution were combined to calculate the combined standard uncertainty of the stock solution mass fraction assignment. The details of the purity assessment, gravimetric preparation, homogeneity and stability studies and corresponding uncertainty evaluations are briefly described below.

DON purity characterization

An essential requirement of the MMCBKT project was to obtain and characterize a primary reference material for DON that could be used subsequently to establish a calibration hierarchy to underpin the metrological traceability of results linked through calibration to this material [15]. The characterization and purity assignment studies to assess identity and purity of a primary reference material for DON used to deliver the BIPM MMCBKT program are described in detail in the Purity Evaluation Guideline: DON [16]. The guideline is also intended to be of use to other NMIs/DIs and reference measurement service providers needing to characterize their own source material for DON analysis. Particular reliance was placed on nuclear magnetic resonance spectroscopy (NMR) studies both to confirm the qualitative identity of the main component of the material and to assign the mass fraction of DON it contained. Due to the relatively complex structure of DON, the assignment by qNMR only provides in the first instance an estimate of the total DON and related structure impurity mass fraction. This initial value needed to be corrected for the relevant related structure impurity mass fraction as assigned separately by an LC-UV-MS/MS method to give the final value for the true DON mass fraction of the material. Additional analyses for the assessment of other potential impurities were undertaken to support and confirm the value assigned through combination of the qNMR and LC data.

The direct qNMR value for the DON mass fraction in the material was estimated at 977.9 ± 3.0 mg/g. This value was corrected for the total contributions from the related structure impurities present in the material, an unidentified DON isomer, DOM-1 and 3-AcDON, expected to contribute to the H-10 signal (in total 1.30 ± 0.32 mg/g) as determined by LC-UV. This gave the qNMR value for the "true" DON mass fraction of OGO.179 by qNMR of 976.6 ± 3.1 mg/g.

It should be noted that LC-MS/MS measurements have demonstrated that the fragmentation of the observed DON isomer is similar to the fragmentation of DON. Consequently, the DON isomer was assumed to contribute to the H-10 signal in qNMR measurements. The calibration for the DON isomer was performed using DON standard due to the lack of original DON isomer standards. The DON isomer occurred at low mass fraction levels of less than 0.5 mg/g.

The mass balance value for the DON mass fraction and its corresponding expanded uncertainty of 980.55 ± 2.84 mg/g was calculated separately, based solely on the contributions from the DON related impurities as no significant contribution from water, VOCs or non-volatile components were identified as listed in Table 2.

Table 2: Impurity assignments

Impurity	Mass fraction (mg/g)	U (mg/g)	Assignment
DON related impurities	19.45	2.84	LC-UV (and LC-MS/MS)
VOCs	-	-	qNMR*
Water	-	-	KFT

* VOC detection based on ^{1}H-NMR and is qualitative only.

The two purity estimates based on qNMR and mass balance are plotted in Figure 1. As there was no basis for assigning greater confidence to one value over the other, and they are consistent within the expanded uncertainty of each, a conservative estimate for the assigned value for the mass fraction of DON in the OGO.179 material was obtained by combining the mass balance value and the qNMR value using a Hierarchical Bayes random effects model. This final value in this case for the OGO.179 material analyzed at the BIPM and used subsequently in the CCQM-K154.c comparison was 978.6 ± 9.2 mg/g [16].

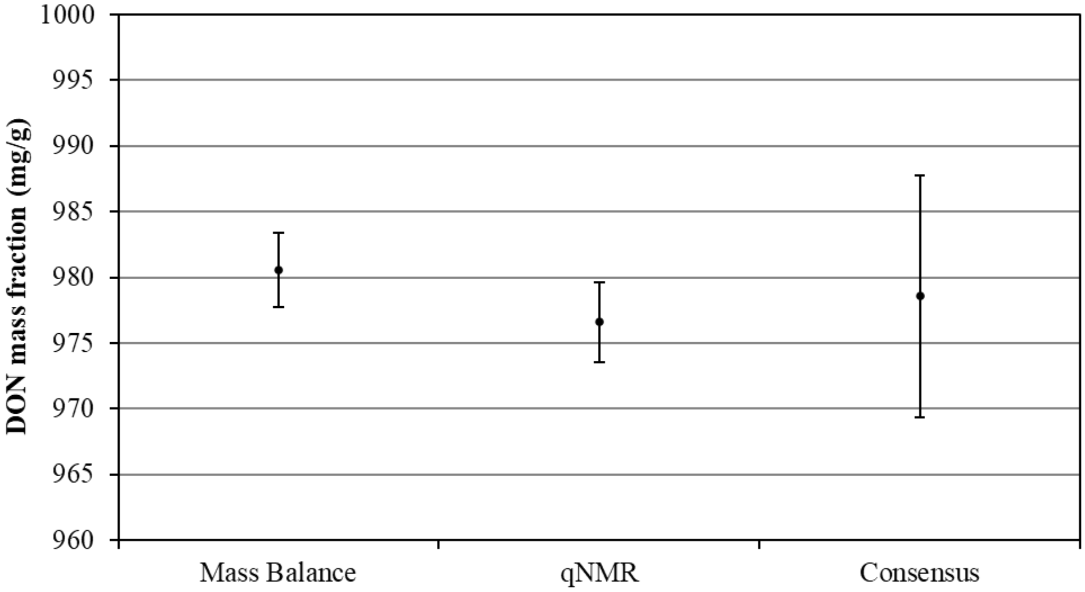

Figure 1: Estimates for the DON mass fraction in BIPM source material OGO.179

Gravimetric preparation of DON stock solution

The DON stock solution (OGP.034) was prepared gravimetrically by dissolving an accurately weighed sample of about 500 mg of DON powder material (OGO.179) in 1 L of acetonitrile. Mettler Toledo MX5 and XP10002S balances were used for the weighing of the DON powder and the final solution, respectively. Table 3 demonstrates the preparation of the stock solution and the mass fraction assignment, calculated according to Equation 1.

Table 3: Experimental data used for the preparation of the DON stock solution and the calculation using Eq. 1 of its DON mass fraction.

	Weighed mass (m)	Buoyancy (*b*)	m x b
DON powder (mg)	499.41	1.000685	499.752
Solution (g)	778.85	1.001386	779.925
Purity ± U (mg/g)	978.6 ± 9.2		
Final mass fraction (μg/g)	627.1		

$$w_{stock} = \frac{m_p \cdot b_p \cdot w_p}{m_{sol} \cdot b_{sol}} \qquad \text{Eq. 1}$$

where:
m_p: weighed mass of DON powder
b_p: buoyancy correction for powder weighing
w_p: purity of DON powder
m_{sol}: weighed mass of stock solution
b_{sol}: buoyancy correction for solution (ACN) weighing

The uncertainties from input quantities in Equation 1 were combined (Equation 2) and the final combined standard uncertainty was calculated as depicted in Table 4. A minor uncertainty component, u(V), was included to account for the potential solvent loss due to evaporation during sample preparation and weighing. The buoyancy mass correction and its uncertainty were calculated as described by the Calibrant Assessment Guideline: DON [17].

$$u(w_{stock}) = w_{stock} \cdot \sqrt{\left[\frac{u(m_p)}{m_p}\right]^2 + \left[\frac{u(b_p)}{b_p}\right]^2 + \left[\frac{u(w_p)}{w_p}\right]^2 + \left[\frac{u(m_{sol})}{m_{sol}}\right]^2 + \left[\frac{u(b_{sol})}{b_{sol}}\right]^2 + \left[\frac{u(V)}{V}\right]^2} \qquad \text{Eq. 2}$$

Table 4: Individual uncertainty components contributing to the combined uncertainty of the DON stock solution mass fraction

Uncertainty source	Value (%)
$\frac{u(m_p)}{m_p}$	0.00068
$\frac{u(b_p)}{b_p}$	0.0035
$\frac{u(w_p)}{w_p}$	0.470
$\frac{u(m_{sol})}{m_{sol}}$	0.0028
$\frac{u(b_{sol})}{b_{sol}}$	0.0020
$\frac{u(V)}{V}$	0.005
u_{rel} (%)	0.47
$u(w_{stock})$ µg/g	2.95
$U(w_{stock})$ µg/g (k = 2)	5.90

Filling of DON stock solution

The 1 L flask containing the stock solution was agitated thoroughly and about 50 mL were transferred to prepare a calibration solution. The rest of the stock solution was stored at 4 °C until ampouling, which took place within 24 h of the preparation.

A 500 mL bottle and 1-10 mL bottle-top dispenser (Dispensette, Brand GmbH) were rinsed twice with the DON stock solution and a stainless-steel flat tip syringe needle was fitted at the outlet of the dispenser to ensure that all solution is discharged at the bottom of the ampoule.

10 mL glass ampoules were selected for a filling volume of 4 mL to ensure that sufficient head space remains above the liquid and therefore minimize the risk of accidental ignition of the solvent during the sealing process. An Ampoulmatic (Bioscience Inc) system connected to propane and oxygen cylinders was used to ampoule the batch. The flow of both gases was adjusted to produce a bright blue flame at the neck of the ampoules.

The ampoules were filled with 4 mL of OGP.034, one at a time, to minimize the impact of evaporation of acetonitrile. A refrigerant (Jelt Refroidisseur 5320) was sprayed onto the lower part of the ampoule before being placed in the ampouling carousel to further reduce the ignition risk. After flame sealing, ampoules were allowed to adjust to room temperature in an upright position.

To test for possible leaks, ampoules were placed into a vacuum drying oven (Haraeus) in an upright position and vacuum (about 50 mbar) was applied for at least 4 hours. The ampoules then remained in the sealed oven overnight, after which they were visually inspected for changes in the solution levels. Leaking ampoules were recorded and discarded while the rest of the batch was stored at -20 °C [17].

Homogeneity studies of DON stock solution

The BIPM investigated the levels of within and between ampoule homogeneity of the main component and selected significant minor components and identified a minimum sample size which reduces to an acceptable level the effect of between bottle inhomogeneity of both the main component and the minor components. The homogeneity of the DON stock solution was studied using an LC-DAD-MS/MS method that allowed for the quantitative determination of DON by UV and of the other DON related impurities by MS/MS detection.

The results of the ANOVA are summarised in Table 5.

Table 5: Homogeneity results of the DON stock solution (OGP.034)

	DON (219 nm)	DON isomer	DOM-1
N	30	30	30
s_{wb} (%)	0.48	1.68	3.13
s_{bb} (%)	0.09	-$^{(2)}$	-$^{(2)}$
u^*_{bb} (%)	0.16	0.60	1.01
$\mathbf{u_{bb}}^{(1)}$ (%)	0.16	0.60	1.01
F	1.11	0.35	0.87
F_{crit}	2.39	2.39	2.39

(1) Higher value (u^*_{bb} or s_{bb}) was taken as uncertainty estimate for potential inhomogeneity

(2) Not calculable because $MS_{bb} < MS_{wb}$

Homogeneity evaluation was performed by single factor ANOVA, allowing for the separation of the variation associated with the method (s_{wb}) from the actual variation between ampoules (s_{bb}), which is an estimate of the uncertainty associated to batch heterogeneity. This uncertainty was 0.16 %, 0.60 % and 1.01 % for DON and the two major related impurities DON isomer and DOM-1, respectively (Table 5). The material was regarded to be homogeneous since the u_{bb} of 0.16 % is very small compared with the target uncertainties of < 2 % and in agreement with typical u_{bb} for similar materials [18].

Representative normalized results due to the analysis and filling sequences are presented for the main component DON (Figure 2) and the impurities DOM-1 (Figure 3) and DON isomer (Figure 4). The first, second and third replicates are represented by circles, grey filled circles and dots respectively.

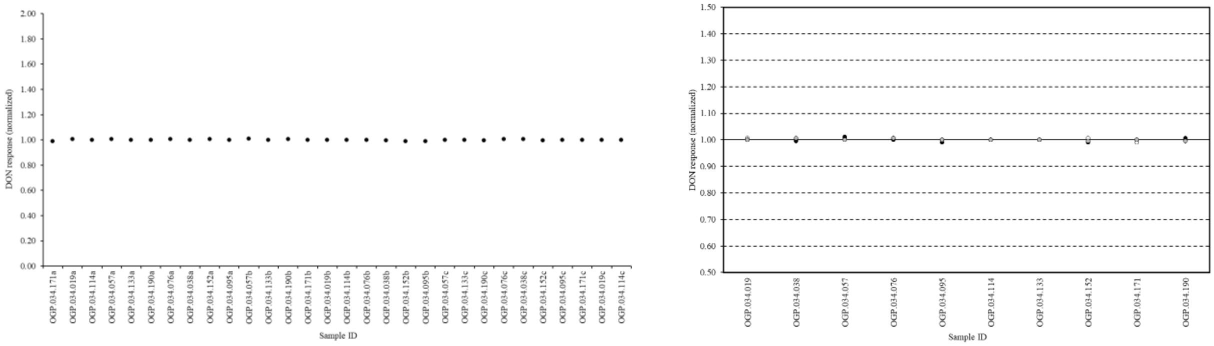

Figure 2: Homogeneity of DON by LC-DAD at 219 nm – Main component - Injection and filling sequence

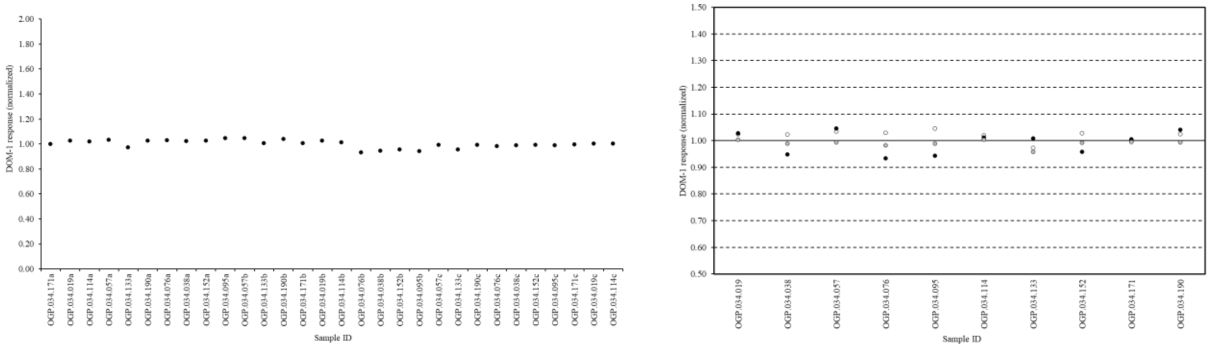

Figure 3: Homogeneity of DOM-1 by LC-MS/MS – Representative impurity - Injection and filling sequence

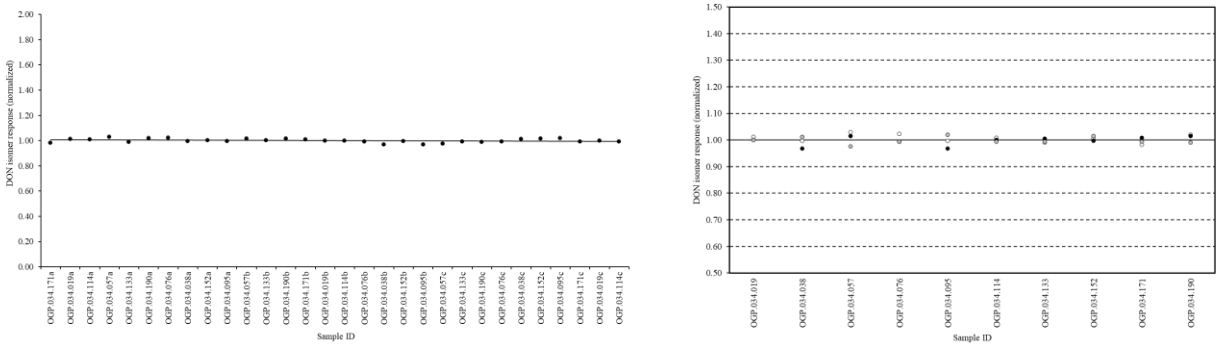

Figure 4: Homogeneity of DON isomer by LC-MS/MS – Representative impurity - Injection and filling sequence

Stability studies of DON stock solution

Isochronous stability studies were performed using a reference storage temperature of -20 °C and test temperatures of 4 °C, 22 °C and 40 °C. A set of units from the production batch were stored at each selected temperature over 8 weeks, with units transferred to reference temperature storage at 2 week intervals. The detected DON related impurities in the stock solution were DON isomer and DOM-1. They were measured in the tested ampoules by LC-MS/MS whereas the main component DON was measured by LC-DAD. Original impurity standards were used for external calibration of the LC-MS/MS method and the calculated mass fractions were normalized to the reference samples (stored at -20°C). For DON isomer the calibration was performed using DON as standard. For the main component DON, no calibration was performed as absorbance values were directly normalized to the main peak absorbance of the reference samples. Data were evaluated as a function of the storage time at each of the studied temperatures.

The DON mass fraction of the material was stable on storage at 4 °C, 22 °C and 40 °C as shown in Figure 5. The DOM-1 impurity mass fractions of the material were stable on storage at 4 °C, 22 °C and 40 °C as well (Figure 6). The DON isomer mass fraction of the material was stable on storage at 4 °C and 22 °C but did increase significantly after storage at about 4 weeks at 40 °C as shown in Figure 7. It was concluded that the stock solution (OGP.034) should be stored at maximum 4 °C in the dark for long-term storage and can be shipped at maximum 40 °C in the dark for not longer than 2 weeks. A conservative stability uncertainty contribution of 9.10 µg/g (1.45 %) was estimated based on the 4 °C stability data taking in consideration storage to cover the comparison period (20 months).

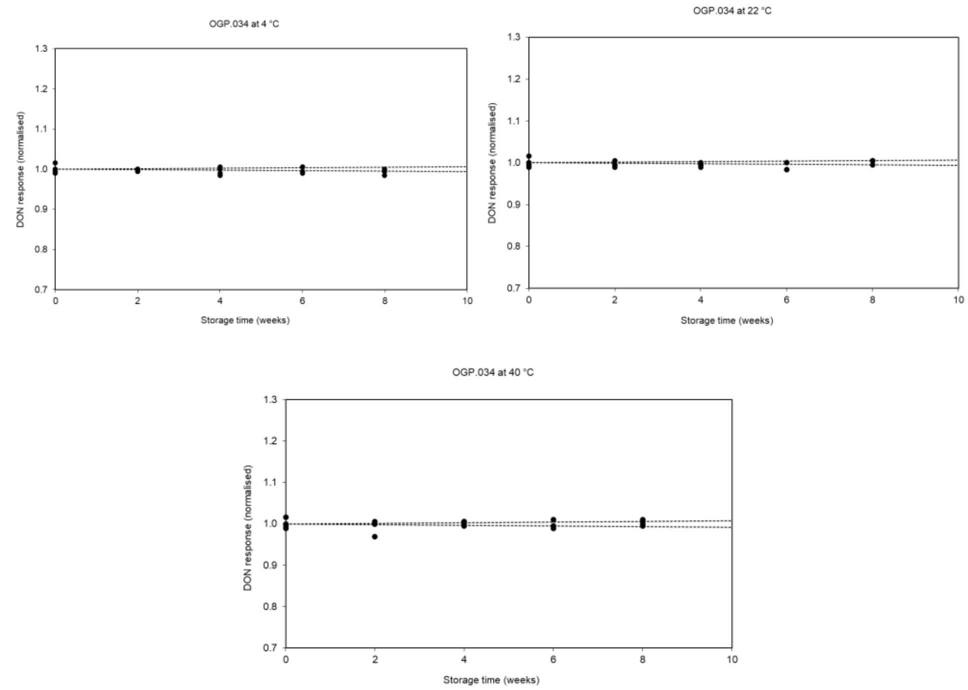

Figure 5: Stability study of DON by LC-DAD

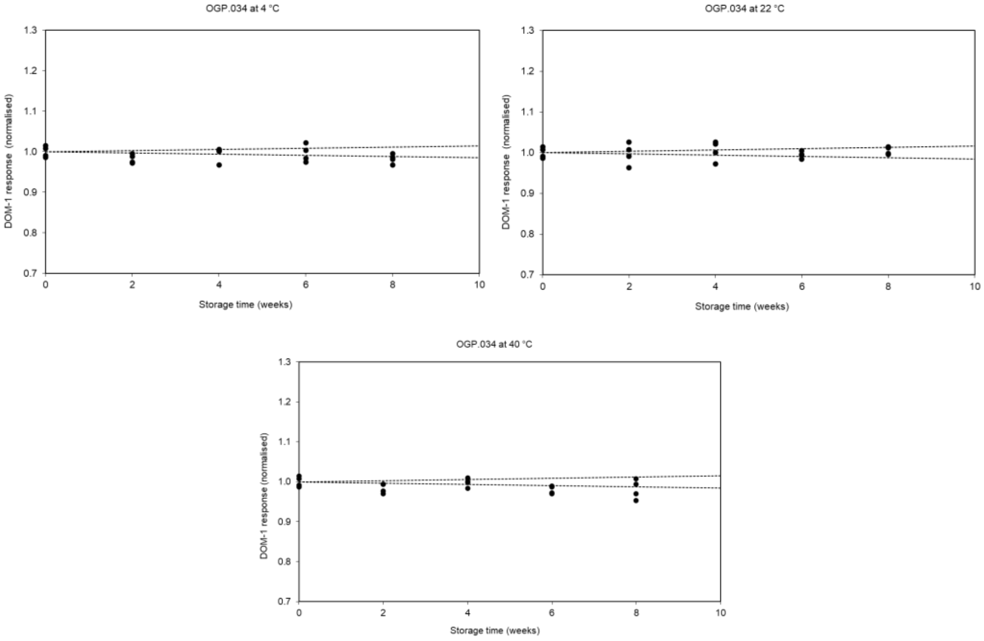

Figure 6: Stability study of DOM-1 by LC-MS/MS

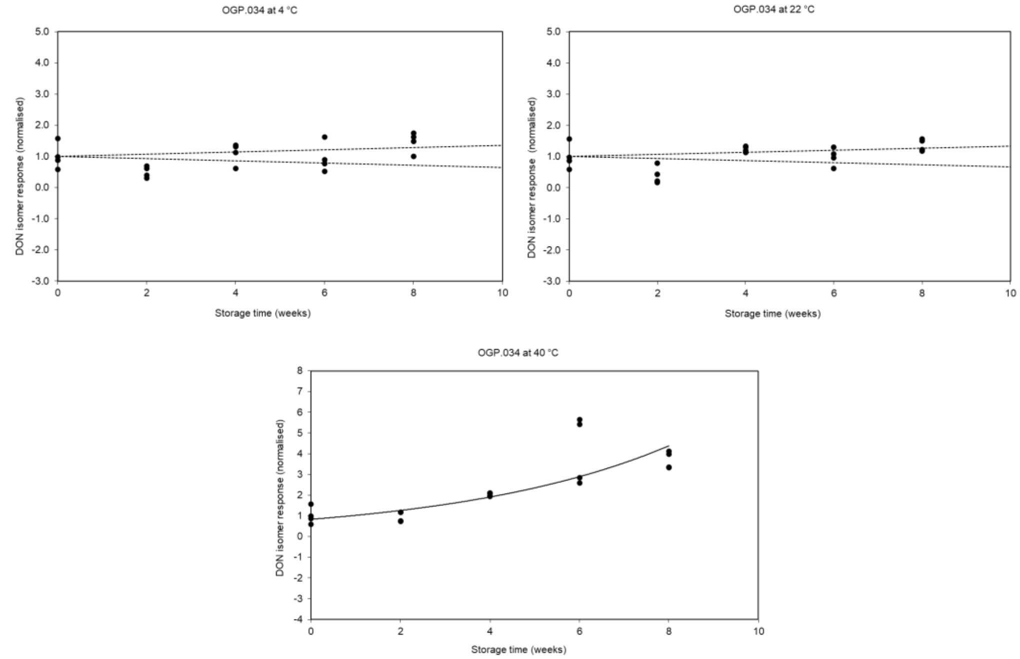

Figure 7: Stability study of DON isomer by LC-MS/MS

DON stock solution and corresponding uncertainty

A mass fraction of 627.1 µg/g was calculated for the DON stock solution based on the gravimetric preparation. The expanded uncertainty *(U)* with a coverage factor of $k = 2$, corresponding to a level of confidence of approximately 95 %, was estimated to be 19.2 µg/g. Uncertainty contributions arising from the gravimetric preparation, $u(w_{stock})$, as well as from homogeneity, u_{bb} and stability assessment, u_{lts} were taken into consideration. Details of the uncertainty contributions, mass fraction (w_{stock}) and corresponding combined (u_c) and expanded uncertainty *(U)* of the DON stock solution are summarized in Table 6.

Table 6: Uncertainty contributions, mass fraction (w_{stock}) and corresponding combined (u_c) and expanded uncertainty *(U)* of the DON stock solution

DON stock solution	
$u_{rel}(w_{stock})$ (%)	0.47
$u_{bb,rel}$ (%)	0.16
$u_{lts,rel}$ (%)	1.45
$u_{c,rel}$ (%)	1.53
w_{stock} (µg/g)	627.1
u_c (µg/g)	9.6
U (µg/g), $k = 2$	19.2

Sample distribution of DON stock solution

Six units of the DON stock solution, each containing a minimum of 4 mL of material, were distributed to each MMCBKT participant by express mail service in insulated boxes equipped with temperature indicators. Participants were asked to acknowledge receipt of the samples and to advise the coordinator if any obvious damage had occurred to the ampoules or if temperatures has exceeded 40 ºC during shipping. The shipping details are listed in Table 7. There was a delay in delivery (22 days) of the ampoules sent to INTI, Argentina. It has been decided that the ampoules were fit for purpose as they have been received in good condition without exceeding 40 ºC temperature during shipping. The ampoules sent to NIMT, Thailand have reached about 40 ºC temperature but the ampoules arrived at destination within 2 days only. It was decided that the NIMT ampoules were fit for purpose as well. Unfortunately, shipping to KEBS, Kenya was repeatedly rejected even though all international air freight standards (IATA) have been met.
All other samples were delivered to the NMIs/DIs without incident.

Table 7: Shipping details for the DON stock solution from the BIPM to MMCBKT participants

NMI/DI	Shipping date	Date of receipt	In transit (days)	Comments
INMETRO	23.03.2021	24.03.2021	1	-
INM	23.03.2021	24.03.2021	1	-
INRAP	23.03.2021	25.03.2021	2	-
INTI	23.03.2021	14.04.2021	22	Samples were retained at the customs
KEBS	23.03.2021	Shipping rejected	-	2nd Shipping on 07.05.2021 also rejected
LATU	23.03.2021	26.03.2021	3	-
NIM#	23.03.2021	25.03.2021	2	-
NIMT	23.03.2021	25.03.2021	2	Exposure to 40 ºC
NMISA*	23.03.2021	31.03.2021	8	-
UME	23.03.2021	25.03.2021	2	-

For their participation in the study NIM chose to submit DON calibrator solutions prepared using both in-house value-assigned materials and based on the MMCBKT DON stock solution OGP.034.

* As an MMCBKT participant NIMSA was supplied with the DON stock solution OGP.034. However, they chose to submit a DON calibrator solution prepared using in-house value-assigned materials for their participation in the study.

STUDY MATERIALS

The participants were required to gravimetrically prepare and ampoule their own (about 4 mL per ampoule) standard solutions of deoxynivalenol (DON) in acetonitrile and to send these to the BIPM for comparison measurements. The mass fraction values targeted (in the range 10 mg/kg to 100 mg/kg) are intended to be representative of the mass fraction content of DON in a standard solution provided as a reference standard used for calibrations in DON analyses.

Prior to sending samples to the BIPM, participants should have demonstrated that the levels of within and between vial inhomogeneity of the mass fraction of DON in acetonitrile were sufficiently small so as to not influence the validity of the comparison. Isochronous stability studies should have been completed to confirm that the material was sufficiently stable within the proposed time scale of the study. Participants should also have ensured that DON was stable in acetonitrile in the ampoule in the dark and under controlled temperature conditions. Appropriate conditions for storage, transport and handling of the solution should have been established by the participants.

STUDY GUIDELINE

Each participant provided the BIPM at least four ampoules with each ampoule containing at least 4 mL of solution (DON in acetonitrile). Two ampoules were required by the BIPM for analysis to obtain the comparison results and the additional ampoules were available as a reserve. The ampoules were stored at -20 °C in the dark until use. Participants were required to provide their estimate of the mass fraction of DON in the solution and its corresponding uncertainty based on the gravimetric preparation corrected for purity. Each participant provided results using the reporting sheet provided with the samples. The results were sent via e-mail to the study coordinator prior to the result submission deadline. Submitted results were considered final and no corrections or adjustments of analytical data were accepted.

It was proposed by the coordinator and decided by the CCQM OAWG that the CCQM-K154.c comparison was undertaken end of 2021. The deadline to submit the samples and to return the filled in data submission forms to the BIPM for participation in the comparison was 31st October 2021.

The details of the shipping of the comparison solutions from the NMIs/DIs to the BIPM are listed in Table 8. LATU, Uruguay had difficulties to meet the deadline as there was a prolonged delay in delivery (18 days) of the ampoules sent to the BIPM. All ampoules (no. 4, 17, 38, 54) were found to be empty on arrival at the BIPM. A second shipping could be arranged in the December 2021 and samples were again received with a delay in delivery (20 days) but at least ampoules were received in good condition. NMISA, South Africa had also difficulties to ship the samples in time because the shipping company has returned the samples to NMISA. A second shipping could be arranged immediately after the incident in the begin of November 2021 and samples were received within 5 days and in good condition. All samples of other NMIs/DIs were delivered within less than 14 days to the BIPM and were received in good condition for comparison.

Table 8: Shipping details of the CCQM-154.c DON comparison solutions from NMIs/DIs to the BIPM

NMI/DI	Shipping date	Date of receipt	In transit (days)	Comments
INTI	30.09.2021	11.10.2021	11	-
INMETRO	26.10.2021	08.11.2021	13	-
NRC	06.10.2021	08.10.2021	2	-
NIM	01.11.2021	09.11.2021	8	-
INM	28.10.2021	03.11.2021	6	-
NMISA	20.10.2021	-	-	Returned to NMISA
	04.11.2021	09.11.2021	5	-
NIMT	25.10.2021	28.10.2021	3	-
UME	19.10.2021	21.10.2021	2	-
LATU	29.10.2021	16.11.2021	18	Ampoules empty
	14.12.2021	03.01.2022	20	-

REPORTED MASS FRACTIONS OF DON AND IMPURITIES

The values reported by participating NMIs/DIs for the DON mass fractions of their DON comparison solutions and their corresponding uncertainties based on the gravimetric preparation (corrected for purity for non-MMCBKT participants) are given in Table 9. The details of the gravimetric preparation, calculation of the DON mass fraction values and assessment of corresponding expanded uncertainties are described in Annex A for each participating NMI/DI. If the uncertainty includes contributions deriving from other sources (for example, homogeneity and/or stability testing) details are also provided in Annex A.

NMISA reported the detection of five minor structurally related impurities in their material (nivalenol, nor-deoxynivalenol, diepoxy-deoxynivalenol, 15-o-acetyl-4-deoxynivalenol and 3-acetyldeoxynivalenol). The NMISA material was directly purity assigned by using quantitative NMR providing for the mass fraction and corresponding uncertainties of the minor structurally related impurities.

VALUE ASSIGNMENT PROCEDURE OF THE COORDINATING LABORATORY

The DON mass fraction assigned solutions provided by the NMIs/DIs were measured and compared at the BIPM under repeatability conditions by an in-house developed and validated LC-DAD-MS/MS method. UV detection was used for the quantification of DON. MS/MS detection served as a verification tool for the determination of potential related structure impurities. Preliminary experiments demonstrated that the UV response was linear over the mass fraction range of about 5 µg/g to 130 µg/g of DON.

Two-point calibrations with external bracketing using DON standards assigned at the BIPM were used for quantification and comparison. It was decided to split the CCQM-K154.c comparison in six groups (A, B, C, D, E and F) with separate calibrations for each of the comparison rounds to allow working at narrow and linear mass fraction ranges. Thus, injection sequences were short in order to minimize the extent of instrument drift.

Materials and calibrants

The DON bracketing standards were prepared immediately before use as solutions in acetonitrile (Hipersolv HPLC grade, VWR, France) of the pure BIPM DON material (OGO.197a) having a DON mass fraction of 978.6 ± 9.2 mg/g ($k = 2$) as outlined in the chapter ‘DON purity characterization’. The gravimetric preparation of the stock solutions were performed in the same way as described in detail in the chapter ‘Gravimetric preparation and filling of DON stock solutions’. Low and high level calibration solutions were gravimetrically prepared from the stock solutions according to the procedure described in detail in the Calibrant Assessment Guideline: DON [17]. The DON mass fractions and corresponding standard uncertainties for the stock and calibration solutions are listed in Table 10.

LC-DAD-MS/MS method

Liquid chromatographic (LC) separation and UV diode array detection (DAD)

An LC 1100 system (Agilent, Les Ulis, France) consisting of an 1100 Series G1312A binary pump, 1100 Series G1329A autosampler, 1100 temperature-controlled column compartment with cooling and 1200 diode-array detector was employed for LC-DAD analysis.
LC separation was performed on a Kinetex EVO C18 100 A column (250 mm × 4.6 mm, 2.6 µm from Phenomenex (Le Pecq, France) maintained at 30 °C. The mobile phases consisted of (A) acetonitrile and (B) purified water. The separation was performed by use of a gradient program with a constant flow rate of 600 µL/min. The gradient started with 6.5 % A and holding for 17 min. It was increased to 20 % A in 10 min and holding for additional 8 min. The column was then washed by increasing to 95 % A in 1 min, holding at 95 % A for 1 min and returning to starting conditions (6.5 % A) in 1 min. The column was re-equilibrated for a further 13 min at 6.5 % A. The total run time was 60 min and the injection volume was 2 µL. The detection wavelength of the UV diode array detector (DAD) was 219 nm. The wavelengths of 229 nm and 279 nm were recorded for verification.

Mass spectrometric detection (MS/MS)

Mass spectrometric detection was performed for verification purposes of potentially occurring impurities. A SCIEX QTRAP 4000 tandem mass spectrometer (Sciex, Villebon sur Yvette, France) fitted with an electrospray ionization (ESI) source was used. The MS parameters were optimized in negative electrospray ionization mode. A capillary voltage of -4500 V with source temperature of 700 ºC was employed for the negative ESI mode. Nitrogen was used as the ion source gas, curtain gas and collision gas. The Gas 1 and Gas 2 of the ion source were set at 50 psi and 70 psi, respectively. The curtain gas (CUR) was set at 20 psi. The collision gas (CAD) was set at ‘Mid’. Table 11 lists MS/MS transitions of DON and potential impurities with optimized dwell time, declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) settings.

Table 9: DON mass fraction values and corresponding uncertainties submitted by the NMIs/DIs CCQM-K154.c

Participant	Comparison round	Primary calibrator used	DON Mass fraction (µg/g)	Combined standard uncertainty (µg/g)	Coverage factor (*k*)	Expanded uncertainty (µg/g)
INM, Colombia	1	CBKT	10.00	0.16	2	0.31
INMETRO, Brazil	1	CBKT	30.2	0.669	2	1.3
INTI, Argentina	1	CBKT	54.81	0.9	2	1.8
LATU, Uruguay	2	CBKT	39.876	0.958	2	1.915
NIM, China	1	own	32.24	0.49	2	0.98
	1	CBKT	31.35	0.56	2	1.12
NIMT, Thailand	1	CBKT	10.04	0.195	2	0.39
NMISA, South Africa	1	own	119.2	1.96	2	3.9
NRC, Canada	1	own	56.6	0.8	2	1.6
UME, Turkey	1	CBKT	20.56	0.32	2	0.65

Table 10: Details of the gravimetric preparation of the BIPM bracketing calibration standards for DON

Comparison round	Calibration	Mass fraction range	DON Stock solution		High level calibration solution		Low level calibration solution	
			w (mg/kg)	u (mg/kg)	*w* (mg/kg)	u (mg/kg)	*w* (mg/kg)	u (mg/kg)
1	A	9-11 mg/kg	140.60	2.15	11.25	0.17	8.79	0.14
1	B	19-22 mg/kg	140.60	2.15	21.92	0.34	18.98	0.29
1	C	28-34 mg/kg	140.60	2.15	33.94	0.52	29.13	0.45
2	D	38-42 mg/kg	140.60	2.15	42.12	0.65	37.93	0.58
1	E	52-60 mg/kg	140.60	2.15	58.54	0.90	51.47	0.79
1	F	113-125 mg/kg	140.60	2.15	124.70	1.91	113.18	1.73

Table 11: Summary of selected precursor and product ions, optimized time, DP, CE, EP and CXP settings for the detection of DON and potential related structure impurities by electrospray ionization MS/MS

Compounds	Precursor ion Q1 (*m/z)*	Product ion Q3 (*m/z)*	Optimized parameters				
			Time (ms)	DP (V)	CE (V)	EP (V)	CXP (V)
DON	295.1	265.1	50	-47	-17	-5	-9
		247.1	50	-47	-18	-5	-9
NIV	311.1	281.1	50	-47	-17	-5	-9
		191.0	50	-47	-28	-5	-9
3-AcDON	337.1	307.1	50	-47	-17	-5	-9
		173.0	50	-47	-16	-5	-9
15-AcDON	337.1	150.0	50	-47	-22	-5	-9
		219.1	50	-47	-16	-5	-9
DOM-1	279.1	249.1	50	-47	-17	-5	-9
		231.1	50	-47	-25	-5	-9
norDON	265.1	247.1	50	-47	-22	-5	-9
		235.1	50	-47	-23	-5	-9

Samples, sequence preparation and measurement order

Two ampoules supplied by each participant were each measured in triplicate by LC-DAD-MS/MS. CCQM-K154.c was grouped in comparison round 1 and 2 with measurements performed end of 2021 and begin of 2022, respectively. Comparison round 1 measurements were undertaken in five batches (A, B, C, E and F) on different days involving one to three participants according to their target mass fraction ranges. Comparison round 2 measurements was performed in one batch (D) on one day involving one participant. Grouping in different mass fraction ranges provided for narrow and linear calibrations. Thus, injection sequences were short to minimize instrument drift. The grouping of CCQM-K154.c participant samples is listed in Table 12.

Table 12: Grouping of CCQM-K154.c participant samples

		Comparison round 1			Comparison round 2
Calibration A (9-11 mg/kg)	Calibration B (19-22 mg/kg)	Calibration C (28-34 mg/kg)	Calibration E (52-60 mg/kg)	Calibration F (113-125 mg/kg)	Calibration D (38-42 mg/kg)
INM (CBKT)	UME (CBKT)	INMETRO (CBKT)	INTI (CBKT)	NMISA (own)	LATU (CBKT)
NIMT (CBKT)		NIM (CBKT)	NRC (own)		
		NIM (own)			

About 300 µL of the NMI/DI samples, low and high mass fraction level calibrant solutions and control samples (BIPM) were transferred in LC vials and injected separately. Calibrants (Low and High), control samples (BIPM) and pure acetonitrile (Blank) vial were distributed and injected over the sequences. The results for blanks and control samples served to identify potential carry-over and instrument drifts, respectively. Neither carry-over nor significant instrument drifts were observed. The detailed injection sequences for CCQM-K154.c comparison round 1 (A, B, C, E and F) and round 2 (D) are given in Table 13.

Table 13: Detailed injection sequences for the different calibrations of CCQM-K154.c

Injection	Comparison round 1 Calibration A (9-11 mg/kg)	Calibration B (19-22 mg/kg)	Calibration C (28-34 mg/kg)	Calibration E (52-60 mg/kg)	Calibration F (113-125 mg/kg)	Comparison round 2 Calibration D (6-16 mg/kg)
1	Blank	Blank	Blank	Blank	Blank	Blank
2	Low-1	Low-1	Low-1	Low-1	Low-1	Low-1
3	INM-X-1	High-1	High-1	INTI-X-1	High-1	High-1
4	NIMT-X-1	Blank	Blank	NRC-X-1	Blank	Blank
5	High-1	Low-2	Low-2	High-1	Low-2	Low-2
6	Blank	UME-X-1	NIM-CBKT-X-1	Blank	NMISA-X-1	LATU-X-1
7	Low-2	High-2	NIM-OWN-X-1	Low-2	High-2	High-2
8	BIPM-D-1	Blank	INMETRO-X-1	BIPM-F-1	Blank	Blank
9	NIMT-Y-1	Low-3	High-2	NRC-Y-1	Low-3	Low-3
10	High-2	BIPM-C-1	Blank	High-2	BIPM-G-1	BIPM-H-1
11	Blank	UME-Y-1	Low-3	Blank	NMISA-Y-1	LATU-Y-1
12	Low-3	High-3	BIPM-E-1	Low-3	High-3	High-3
13	BIPM-D-2	Blank	NIM-OWN-Y-1	BIPM-F-2	Blank	Blank
14	INM-Y-1	Low-4	NIM-CBKT-Y-1	INTI-Y-1	Low-4	Low-4
15	High-3	BIPM-C-2	High-3	High-3	BIPM-G-2	BIPM-H-2
16	Blank	High-4	Blank	Blank	High-4	High-4
17	Low-4	Blank	Low-4	Low-4	Blank	Blank
18	NIMT-X-2	Low-5	BIPM-E-2	NRC-X-2	Low-5	Low-5
19	BIPM-D-3	UME-X-2	INMETRO-Y-1	BIPM-F-3	NMISA-X-2	LATU-X-2
20	High-4	High-5	NIM-CBKT-X-2	High-4	High-5	High-5
21	Blank	Blank	High-4	Blank	Blank	Blank
22	Low-5	Low-6	Blank	Low-5	Low-6	Low-6
23	INM-X-2	UME-Y-2	Low-5	INTI-X-2	NMISA-Y-2	LATU-Y-2
24	NIMT-Y-2	BIPM-C-3	NIM-OWN-X-2	NRC-Y-2	BIPM-G-3	BIPM-H-3
25	High-5	High-6	INMETRO-X-2	High-5	High-6	High-6
26	Blank	Blank	BIPM-E-3	Blank	Blank	Blank
27	Low-6	Low-7	High-5	Low-6	Low-7	Low-7
28	BIPM-D-4	BIPM-C-4	Blank	BIPM-F-4	BIPM-G-4	BIPM-H-4
29	INM-Y-2	High-7	Low-6	INTI-Y-2	High-7	High-7
30	High-6	Blank	NIM-OWN-Y-2	High-6	Blank	Blank
31	Blank	Low-8	INMETRO-Y-2	Blank	Low-8	Low-8
32	Low-7	UME-X-3	NIM-CBKT-Y-2	Low-7	NMISA-X-3	LATU-X-3
33	BIPM-D-5	BIPM-C-5	High-6	BIPM-F-5	BIPM-G-5	BIPM-H-5
34	NIMT-X-3	High-8	Blank	NRC-X-3	High-8	High-8
35	High-7	Blank	Low-7	High-7	Blank	Blank
36	Blank	Low-9	BIPM-E-4	Blank	Low-9	Low-9
37	Low-8	UME-Y-3	INMETRO-X-3	Low-8	NMISA-Y-3	LATU-Y-3
38	INM-X-3	High-9	NIM-OWN-X-3	INTI-X-3	High-9	High-9
39	NIMT-Y-3	Blank	High-7	NRC-Y-3	Blank	Blank
40	High-8	Low-10	Blank	High-8	Low-10	Low-10
41	Blank	BIPM-C-6	Low-8	Blank	BIPM-G-6	BIPM-H-6
42	Low-9	High-10	BIPM-E-5	Low-9	High-10	High-10
43	INM-Y-3	Blank	NIM-CBKT-X-3	INTI-Y-3	Blank	Blank
44	BIPM-D-6		INMETRO-Y-3	BIPM-F-6		
45	High-9		High-8	High-9		
46	Blank		Blank	Blank		
47	Low-10		Low-9	Low-10		
48	High-10		NIM-OWN-Y-3	High-10		
49	Blank		NIM-CBKT-Y-3	Blank		
50			BIPM-E-6			
51			High-9			
52			Blank			
53			Low-10			
54			High-10			
55			Blank			

Measurements and results

Subsequent to the LC-DAD-MS/MS analyses the UV absorption peak areas of DON at 219 nm were automatically integrated, manually verified and refined using the Analyst software (Sciex, Villebon sur Yvette, France).

XLGENLINEv1.1 (National Physics Laboratory, United Kingdom) an Excel-based software program was used for the further treatment of the data. It allows the undertaking of a Generalized

Least Squares (GLS) regression analysis that is fully compliant with the International Standard ISO 6143 [19, 20]. This approach is fully implemented and widely used for very similar applications in the field of gas mixture standards analysis and related Model II key comparisons of the CCQM Gas Analysis Working Group (GAWG) [21, 22] where typically mass fractions and corresponding uncertainties are of the same order of magnitude. Model II comparisons of the CCQM OAWG are until now mainly applied for the comparison of CRMs with mass concentrations that span several orders of magnitude [23]. Statistical approaches including GLS are discussed in detail by Duewer *et al*. [24].

In the present case, XLGENLINEv1.1 calculates the values and uncertainties of the 'unknowns', displays a plot of the fitted regression function, and outputs the parameters of the fit. Slope and y-intercepts of the calibrations were calculated by use of the UV absorption peak area responses. Regression lines were built by use of the bracketing low and high mass fraction level calibrants prepared by the BIPM. Input DON mass fractions and standard uncertainties of the bracketing low and high mass fraction level calibrants based on the gravimetrical preparation (Table 9) are compared with the arithmetic mean and corresponding standard deviation of the UV absorption peak area responses of ten replicates each. The ten replicates of each of the bracketing low and mass fraction level calibrant were strategically placed to cover the entire injection sequence (Table 13). The DON mass fractions and associated standard uncertainties of the NMI/DI solutions were evaluated inversely based on the UV absorption peak area responses and the standard deviation of its three replicates.

The mass fraction values assigned at the BIPM using this procedure for the NMI/DI solutions (w_{BIPM}), corresponding standard u(w_{BIPM}) and expanded uncertainties U(w_{BIPM}) are listed in Table 14. The bracketing calibrations with the values, standard uncertainties and UV peak area responses for the solutions submitted by NMIs/DIs, low and high mass fraction level calibrants and internal control samples for CCQM-K154.c A, B, C, D, E and F are depicted in Figures 8-13, respectively.

Table 14: DON mass fraction values and absolute corresponding and expanded uncertainties measured by the BIPM for CCQM-K154.c participants' ampoules

NMI/DI	Comparison round	w_{BIPM} *(mg/kg)*	$u(w_{BIPM})$ *(mg/kg)*	$U(w_{BIPM})$ *(mg/kg)*	*Quantification range (mg/kg)*
INM (CBKT) A	1	10.06	0.34	0.68	9-11
INM (CBKT) B	1	10.14	0.27	0.54	9-11
NIMT (CBKT) A	1	9.92	0.27	0.54	9-11
NIMT (CBKT) B	1	9.88	0.28	0.56	9-11
UME (CBKT) A	1	20.44	0.37	0.74	19-22
UME (CBKT) B	1	20.52	0.44	0.88	19-22
NIM (own) A	1	31.83	0.60	1.20	28-34
NIM (own) B	1	30.88	0.63	1.26	28-34
NIM (CBKT) A	1	31.02	0.49	0.98	28-34
NIM (CBKT) B	1	31.18	0.63	1.26	28-34
INMETRO (CBKT) A	1	30.09	0.61	1.22	28-34
INMETRO (CBKT) B	1	29.78	0.77	1.54	28-34
LATU (CBKT) A	2	40.83	0.75	1.50	38-42
LATU (CBKT) B	2	39.24	0.72	1.44	38-42
INTI (CBKT) A	1	53.55	0.98	1.96	52-60
INTI (CBKT) B	1	53.55	0.80	1.60	52-60
NRC (own) A	1	54.94	0.96	1.92	52-60
NRC (own) B	1	54.70	0.78	1.56	52-60
NMISA (own) A	1	123.45	2.03	4.06	113-125
NMISA (own) B	1	121.21	1.92	3.84	113-125

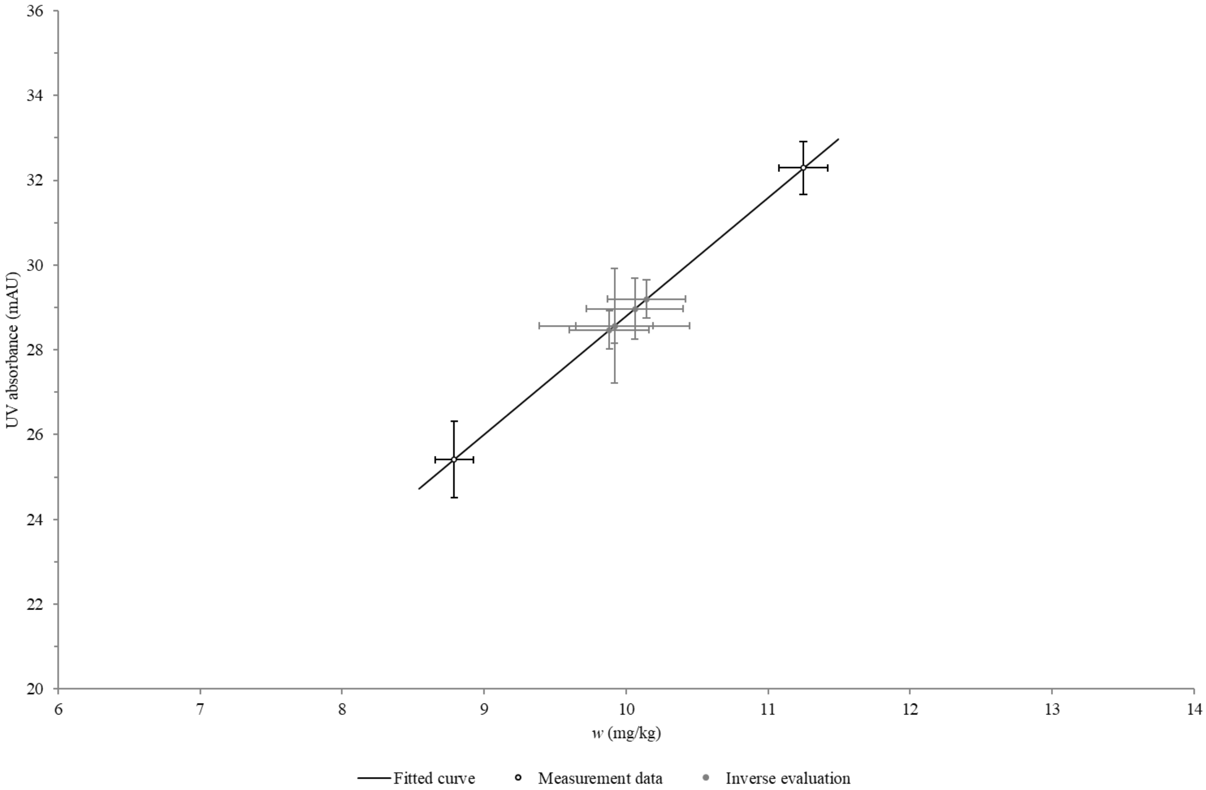

Figure 8: CCQM-K154.c – Comparison round 1 - Calibration A - Bracketing calibration for the DON mass fraction quantification range of 9-11 mg/kg. UV absorbance values (mAU) and corresponding mass fractions (mg/kg) plotted with standard uncertainties (u). BIPM measurement data are shown as black circles at the upper and lower end of the calibration line. Inverse evaluation data of INM (CBKT) and NIMT (CBKT) and internal control sample are depicted as grey dots.

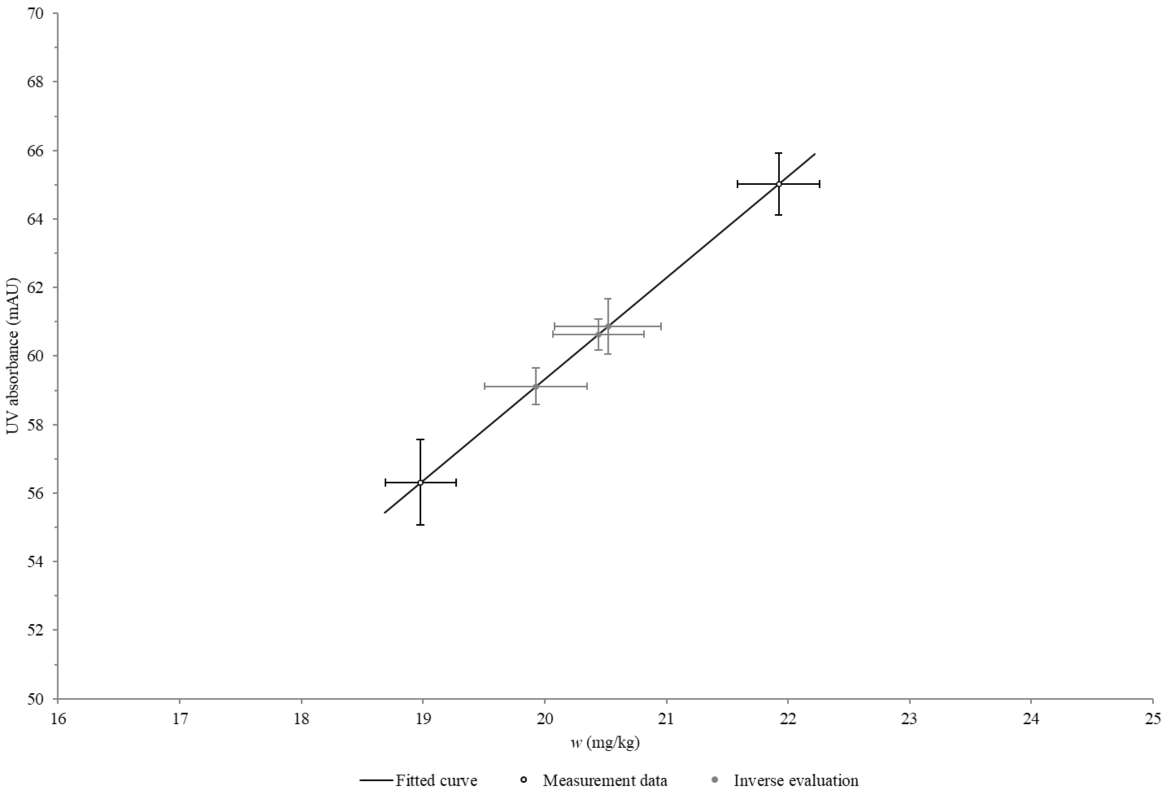

Figure 9: CCQM-K154.c – Comparison round 1 - Calibration B - Bracketing calibration for the DON mass fraction quantification range of 19-22 mg/kg. UV absorbance values (mAU) and corresponding mass fractions (mg/kg) plotted with standard uncertainties (u). BIPM measurement data are shown as black circles at the upper and lower end of the calibration line. Inverse evaluation data of UME (CBKT) and internal control sample are depicted as grey dots.

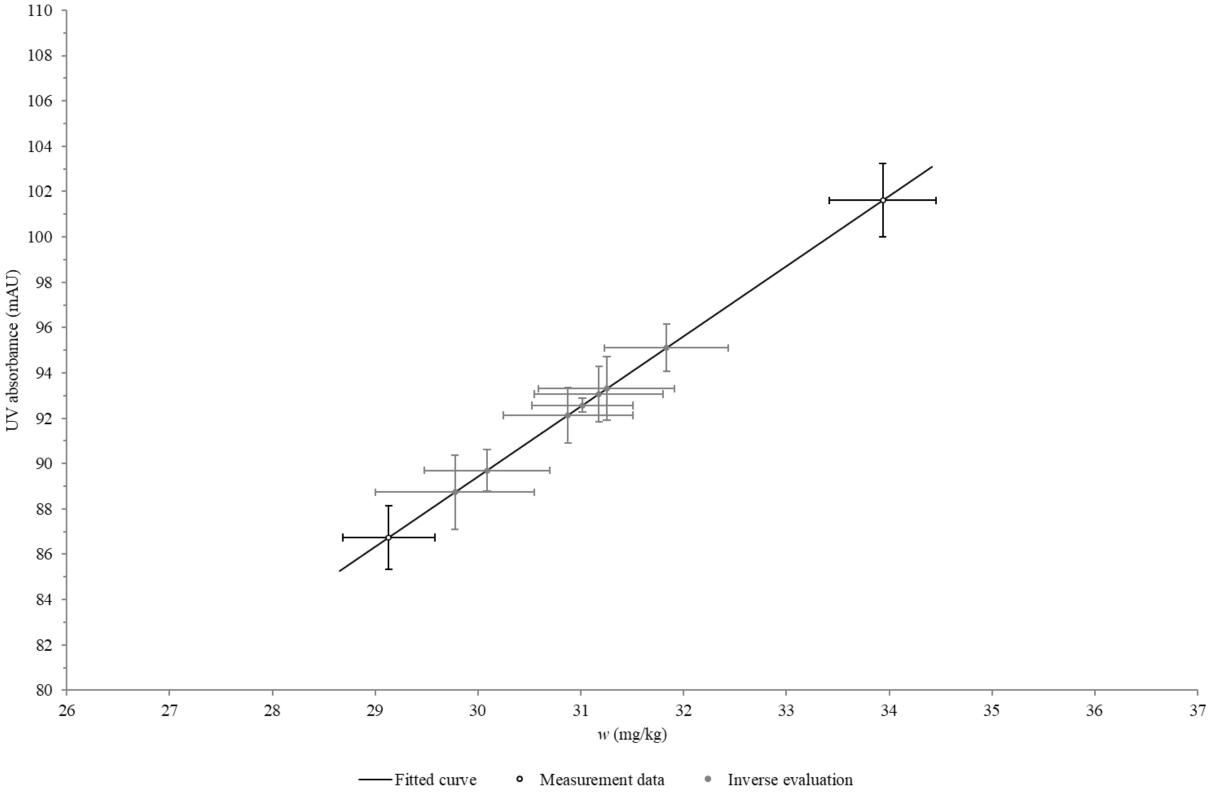

Figure 10: CCQM-K154.c – Comparison round 1 - Calibration C - Bracketing calibration for the DON mass fraction quantification range of 28-34 mg/kg. UV absorbance values (mAU) and corresponding mass fractions (mg/kg) plotted with standard uncertainties (u). BIPM measurement data are shown as black circles at the upper and lower end of the calibration line. Inverse evaluation data of NIM (own), NIM (CBKT) and INMETRO (CBKT) and internal control sample are depicted as grey dots.

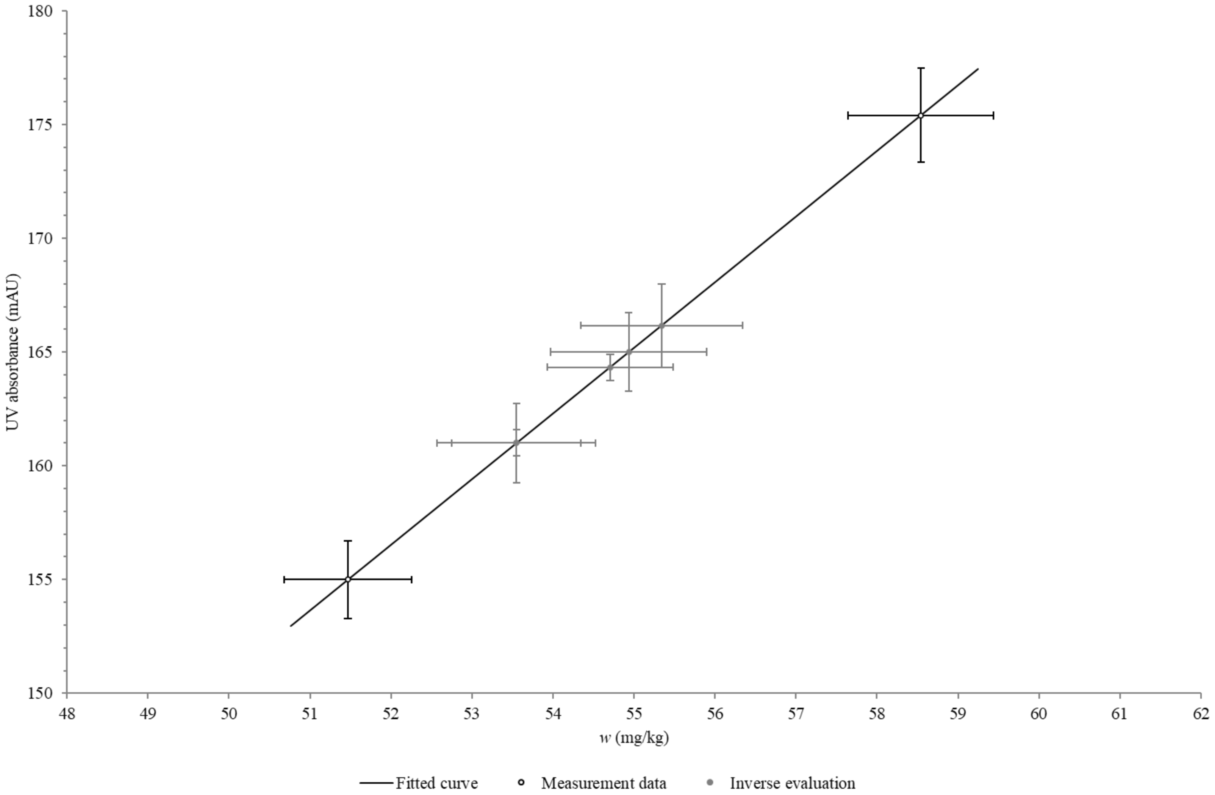

Figure 11: CCQM-K154.c – Comparison round 1 - Calibration E - Bracketing calibration for the DON mass fraction quantification range of 52-60 mg/kg. UV absorbance values (mAU) and corresponding mass fractions (mg/kg) plotted with standard uncertainties (u). BIPM measurement data are shown as black circles at the upper and lower end of the calibration line. Inverse evaluation data of INTI (CBKT) and NRC (own) and internal control sample are depicted as grey dots.

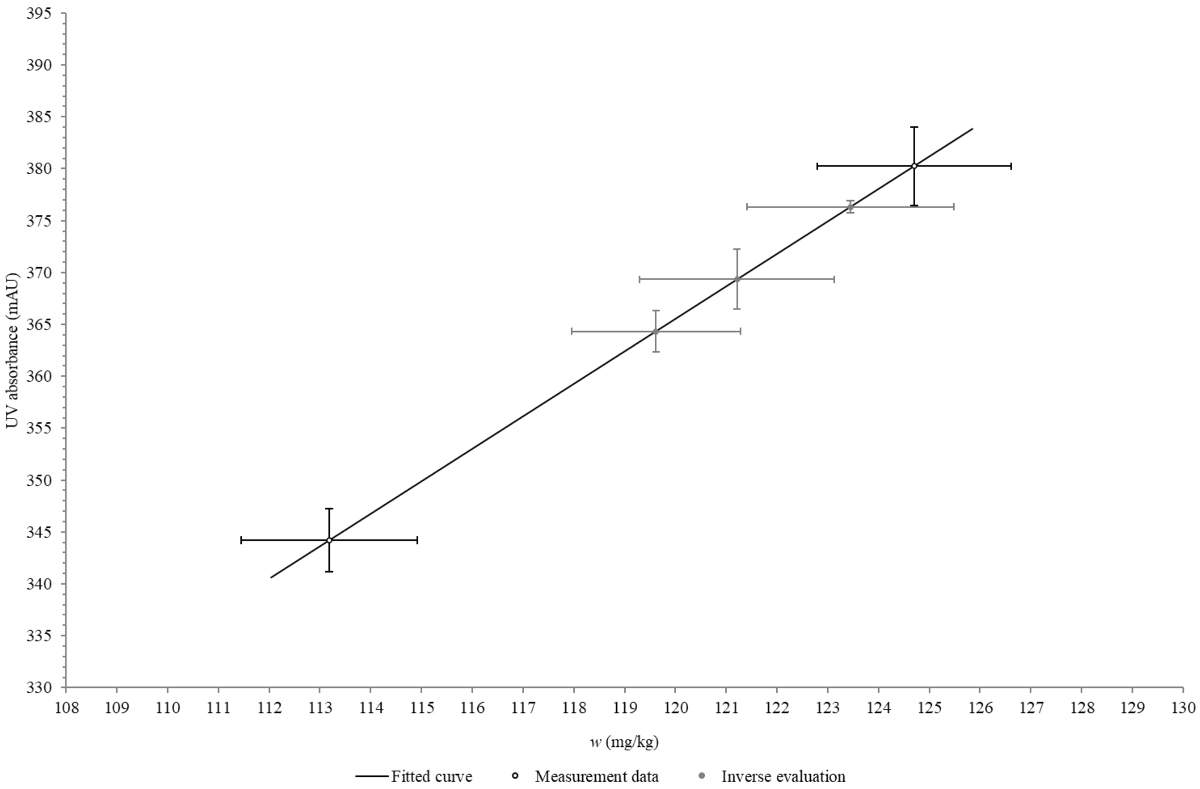

Figure 12: CCQM-K154.c – Comparison round 1 - Calibration F - Bracketing calibration for the DON mass fraction quantification range of 113-125 mg/kg. UV absorbance values (mAU) and corresponding mass fractions (mg/kg) plotted with standard uncertainties (u). BIPM measurement data are shown as black circles at the upper and lower end of the calibration line. Inverse evaluation data of NMISA (own) and internal control sample are depicted as grey dots.

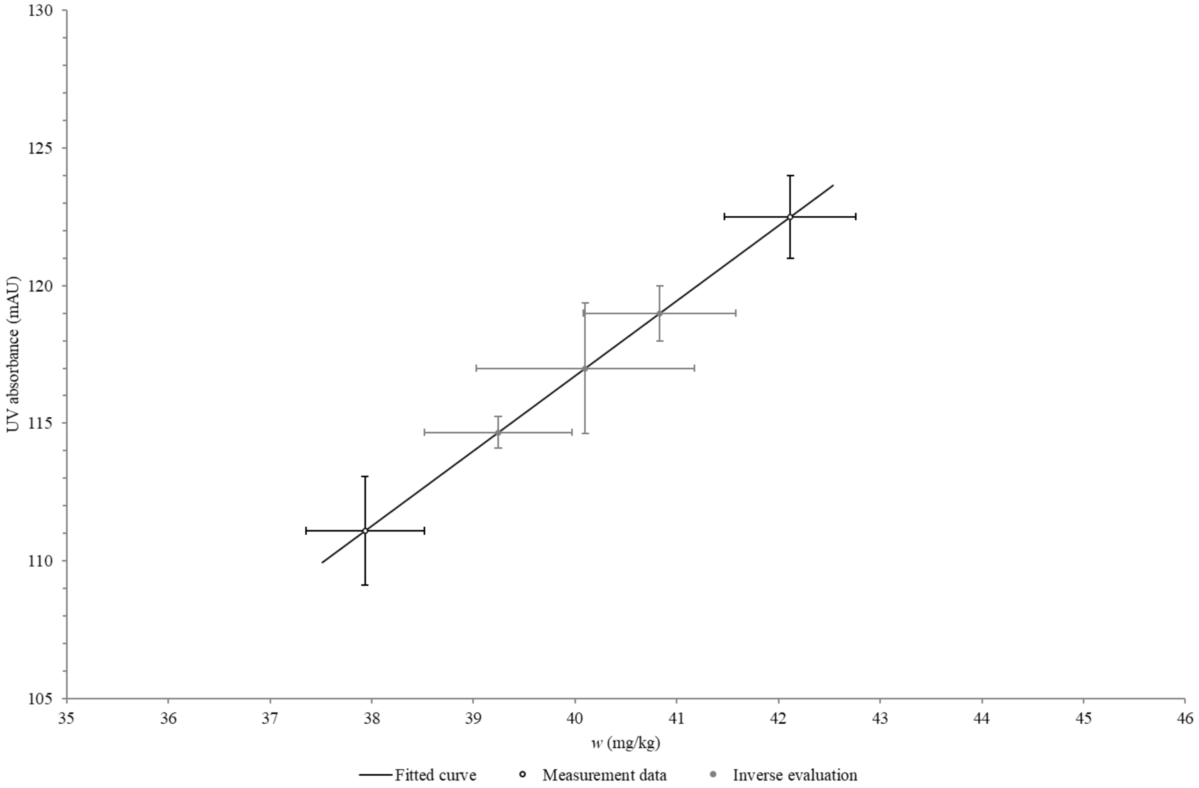

Figure 13: CCQM-K154.c – Comparison round 2 - Calibration D - Bracketing calibration for the DON mass fraction quantification range of 38-42 mg/kg. UV absorbance values (mAU) and corresponding mass fractions (mg/kg) plotted with standard uncertainties (u). BIPM measurement data are shown as black circles at the upper and lower end of the calibration line. Inverse evaluation data of LATU (CBKT) and internal control sample are depicted as grey dots.

KEY COMPARISON REFERENCE VALUES FOR CCQM-K154.c

The DON mass fraction values used to establish the Key Comparison Reference Values (KCRVs) for CCQM-K154.c were assigned by the BIPM following the above-mentioned calibration procedure ($w_{\mathrm{BIPM}} = w_{\mathrm{KCRV}}$). For each ampoule, the Key Comparison Reference Value is the DON mass fraction (w_{KCRV}) and its corresponding uncertainty ($u(w_{KCRV})$). All NMIs/DIs (i) participating in CCQM-K154.c were required to submit estimates for the DON mass fraction w_{i} and its corresponding uncertainty u(w_{i}) for their set of ampoules.

The degree of equivalence (D_i) of a participant's submitted value w_i with w_{KCRV} is given by:

$$D_i = w_i - w_{KCRV}$$

The expanded uncertainty U_i at a confidence level of about 95 % associated with the D_i was calculated as:

$$U(D_i) = 2 \cdot \sqrt{u(w_i)^2 + u(w_{KCRV})^2}$$

The relative degree of equivalence ($D_{rel,\,i}$) of a participant's submitted value w_i with the w_{KCRV} was calculated as participants worked at different mass fraction levels:

$$D_{rel,i} = 100 - \left(\frac{100 \cdot w_{KCRV}}{w_i}\right)$$

The expanded uncertainty $U_{rel}(D_{rel,\,i})$ at a confidence level of about 95 % associated with the ($D_{rel,\,i}$) was calculated as:

$$U_{rel}\left(D_{rel,i}\right) = 2 \cdot \sqrt{u_{rel}(w_i)^2 + u_{rel}(w_{KCRV})^2}$$

The DON mass fractions values and associated absolute uncertainties with degree of equivalences for CCQM-K154.c are listed in Table 15. Figure 14 indicates the degree of equivalence (D_i) of each key comparison participant's result with the w_{KCRV}.

The DON mass fractions values and associated relative uncertainties with relative degree of equivalences are listed in Table 16. Figure 15 indicates the relative degree of equivalence ($D_{rel,\,i}$) of each key comparison participant's result with the w_{KCRV}.

Table 15: DON mass fractions and absolute corresponding uncertainties with degree of equivalences for CCQM-K154.c

NMI/DI	w_{KCRV} *(mg/kg)*	$u(w_{KCRV})$ *(mg/kg)*	$U(w_{KCRV})$ *(mg/kg)*	w_i *(mg/kg)*	$u(w_i)$ *(mg/kg)*	$U(w_i)$ *(mg/kg)*	D_i	$U(D_i)$	*Quantification range (mg/kg)*
INM (CBKT) A	10.06	0.34	0.68	10.00	0.16	0.31	-0.06	0.75	9-11
INM (CBKT) B	10.14	0.27	0.54	10.00	0.16	0.31	-0.14	0.63	9-11
NIMT (CBKT) A	9.92	0.27	0.54	10.04	0.195	0.39	0.12	0.67	9-11
NIMT (CBKT) B	9.88	0.28	0.56	10.04	0.195	0.39	0.16	0.68	9-11
UME (CBKT) A	20.44	0.37	0.74	20.56	0.32	0.65	0.12	0.98	19-22
UME (CBKT) B	20.52	0.44	0.88	20.56	0.32	0.65	0.04	1.09	19-22
NIM (own) A	31.83	0.60	1.20	32.24	0.49	0.98	0.41	1.55	28-34
NIM (own) B	30.88	0.63	1.26	32.24	0.49	0.98	1.36	1.60	28-34
NIM (CBKT) A	31.02	0.49	0.98	31.35	0.56	1.12	0.33	1.49	28-34
NIM (CBKT) B	31.18	0.63	1.26	31.35	0.56	1.12	0.17	1.69	28-34
INMETRO (CBKT) A	30.09	0.61	1.22	30.2	0.669	1.3	0.11	1.81	28-34
INMETRO (CBKT) B	29.78	0.77	1.54	30.2	0.669	1.3	0.42	2.04	28-34
LATU (CBKT) A	40.83	0.75	1.50	39.876	0.958	1.915	-0.95	2.43	38-42
LATU (CBKT) B	39.24	0.72	1.44	39.876	0.958	1.915	0.64	2.40	38-42
INTI (CBKT) A	53.55	0.98	1.96	54.81	0.90	1.80	1.26	2.66	52-60
INTI (CBKT) B	53.55	0.80	1.60	54.81	0.90	1.80	1.26	2.41	52-60
NRC (own) A	54.94	0.96	1.92	56.6	0.8	1.6	1.66	2.50	52-60
NRC (own) B	54.70	0.78	1.56	56.6	0.8	1.6	1.90	2.23	52-60
NMISA (own) A	123.45	2.03	4.06	119.2	1.96	3.9	-4.25	5.64	113-125
NMISA (own) B	121.21	1.92	3.84	119.2	1.96	3.9	-2.01	5.49	113-125

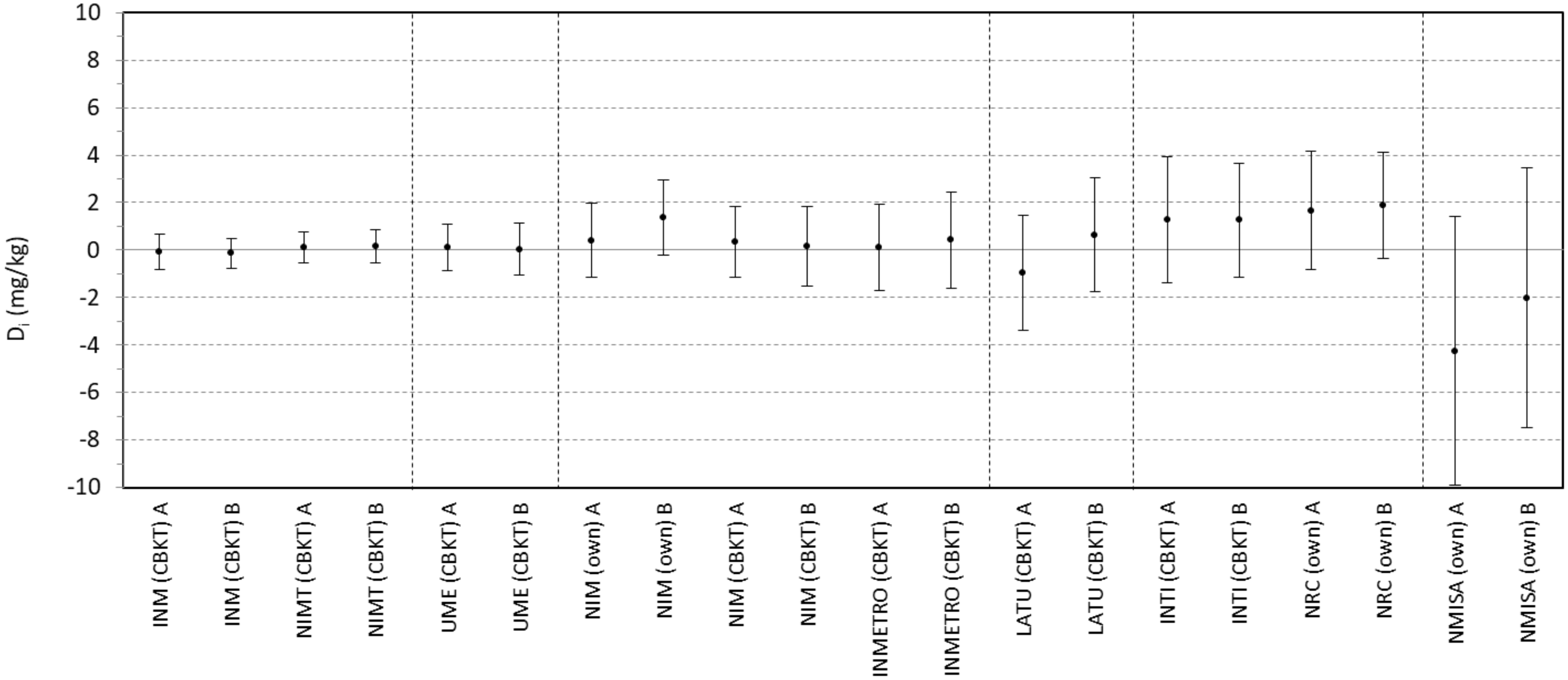

Figure 14: Absolute values for the degree of equivalence for CCQM-K154.c

Table 16: DON mass fractions and relative corresponding uncertainties with relative degree of equivalences for CCQM-K154.c

NMI/DI	w_{KCRV} (mg/kg)	$u_{rel}(w_{KCRV})$ (%)	$U_{rel}(w_{KCRV})$ (%)	w_i (mg/kg)	$u_{rel}(w_i)$ (%)	$U_{rel}(w_i)$ (%)	$D_{rel,\,i}$	$U_{rel}(D_{rel,\,i})$	*Quantification range (mg/kg)*
INM (CBKT) A	10.06	3.38	6.76	10.00	1.60	3.10	-0.60	7.48	9-11
INM (CBKT) B	10.14	2.66	5.33	10.00	1.60	3.10	-1.40	6.21	9-11
NIMT (CBKT) A	9.92	2.72	5.44	10.04	1.94	3.88	1.20	6.69	9-11
NIMT (CBKT) B	9.88	2.83	5.67	10.04	1.94	3.88	1.59	6.87	9-11
UME (CBKT) A	20.44	1.81	3.62	20.56	1.56	3.11	0.58	4.77	19-22
UME (CBKT) B	20.52	2.14	4.29	20.56	1.56	3.11	0.19	5.30	19-22
NIM (own) A	31.83	1.89	3.77	32.24	1.52	3.04	1.27	4.84	28-34
NIM (own) B	30.88	2.04	4.08	32.24	1.52	3.04	4.22	5.09	28-34
NIM (CBKT) A	31.02	1.58	3.16	31.35	1.79	3.57	1.05	4.77	28-34
NIM (CBKT) B	31.18	2.02	4.04	31.35	1.79	3.57	0.54	5.39	28-34
INMETRO (CBKT) A	30.09	2.03	4.05	30.2	2.22	4.30	0.36	6.01	28-34
INMETRO (CBKT) B	29.78	2.59	5.17	30.2	2.22	4.30	1.39	6.81	28-34
LATU (CBKT) A	40.83	1.84	3.67	39.876	2.40	4.80	-2.39	6.05	38-42
LATU (CBKT) B	39.24	1.83	3.67	39.876	2.40	4.80	1.59	6.05	38-42
INTI (CBKT) A	53.55	1.83	3.66	54.81	1.64	3.28	2.30	4.92	52-60
INTI (CBKT) B	53.55	1.49	2.99	54.81	1.64	3.28	2.30	4.44	52-60
NRC (own) A	54.94	1.75	3.49	56.6	1.41	2.83	2.93	4.49	52-60
NRC (own) B	54.70	1.43	2.85	56.6	1.41	2.83	3.36	4.02	52-60
NMISA (own) A	123.45	1.64	3.29	119.2	1.64	3.29	-3.57	4.65	113-125
NMISA (own) B	121.21	1.58	3.17	119.2	1.64	3.29	-1.69	4.57	113-125

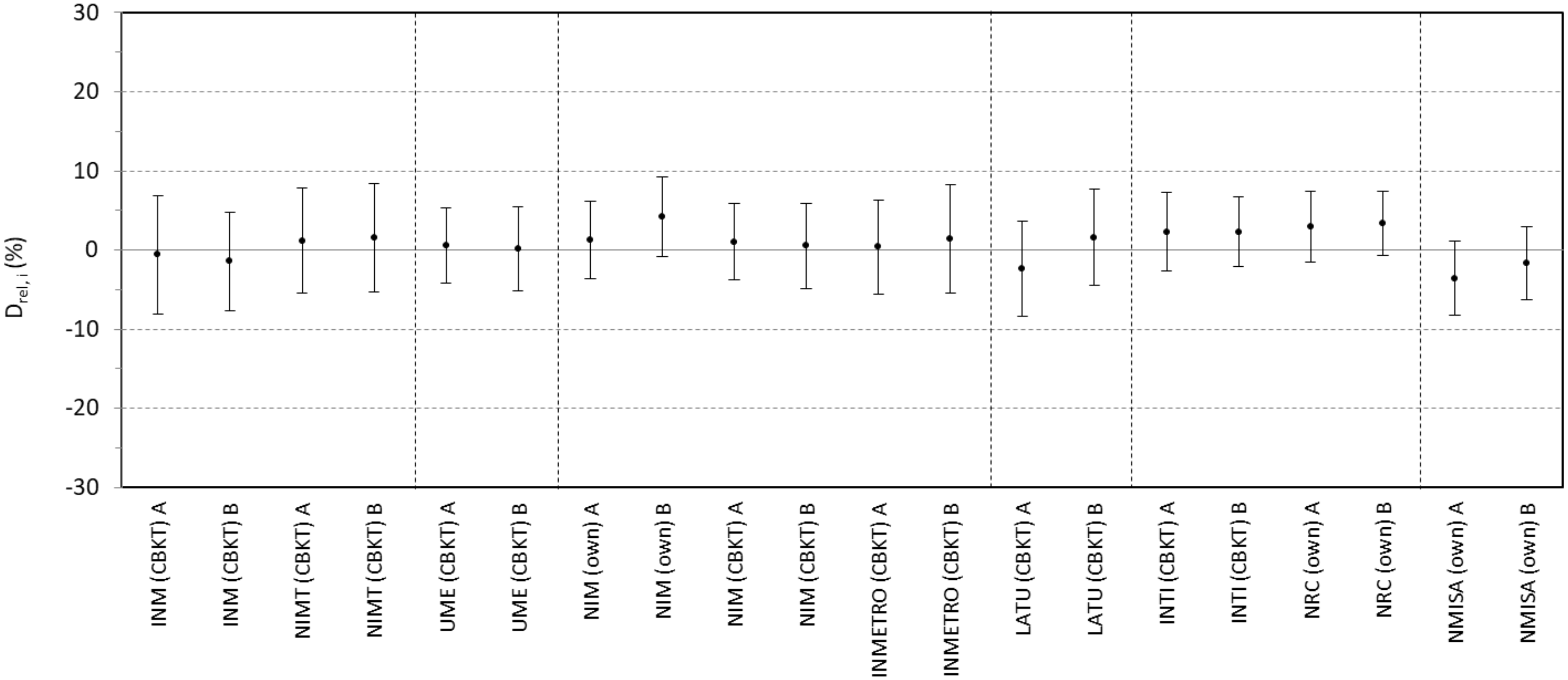

Figure 15: Relative values for the degree of equivalence for CCQM-K154.c

CONCLUSIONS

Deoxynivalenol (DON) was selected to be representative of polar *Fusarium* mycotoxins. It was anticipated to provide a challenge representative for the gravimetrical preparation and value assignment of calibration solutions in the mass fraction range of 10 mg/kg to 100 mg/kg of mycotoxins with broadly similar structural characteristics.

Seven participants of the MMCBKT programme were provided with a stock solution having a known DON mass fraction and expanded uncertainty to use to gravimetrically prepare and value assign a calibration solution. Three NMIs/DIs also participated using their own calibration solutions. The use of in-house solutions required an additional capacity to undertake a fit-for-purpose purity assessment. NIM was the only NMI participating using both the MMCBKT based and their own in-house assigned solutions in order to connect the two different groups.

It was decided to propose separate KCRVs for each of the two ampoules provided by the participating NMIs/DIs based on the DON mass fraction. This allowed participants to demonstrate the efficacy of their implementation of the approaches used to gravimetrically prepare calibrations solutions and to assess the DON mass fraction.

The majority of the DON mass fraction KCRVs (w_{KCRV}) for CCQM-K154.c spanned a mass fraction range of 9.88 mg/kg to 123.45 mg/kg. The relative expanded uncertainties $U(w_{\mathrm{KCRV}})$ ranged from 2.8 % to 6.8 %.

Inspection of the degree of equivalence plots (Figures 14 and 15) for the DON mass fraction assignments in CCQM-K154.c indicated that there was an excellent agreement of results.

HOW FAR THE LIGHT SHINES STATEMENT (HFTLS)

Successful participation in CCQM-K154.c for MMCBKT participants will support CMCs for:

a) Preparation and value assignment of deoxynivalenol calibration solutions in the mass fraction range of 10 mg/kg to 100 mg/kg, prepared from a mycotoxin stock solution or solid of known purity.

Successful participation in CCQM-K154.c for other participants (having value assigned their pure Primary Reference Materials) will support CMCs for:

a) purity value assignment capabilities of organic materials with molar mass in the range 100 g/mol to 500 g/mol and polarity (*pKow*) > -2, with relative uncertainties at or above the relative uncertainty achieved in the comparison for calibration solutions;
b) preparation and value assignment of single component organic calibration solutions in the mass fraction range of 10 mg/kg to 100 mg/kg, polarity (*pKow*) > -2, with molar mass in the range of 100 g/mol to 500 g/mol.

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ANNEX A – ADDITIONAL ANALYTICAL INFORMATION

Instituto Nacional de Metrologia (INM), Colombia

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)

NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

OGP 034 DON, stock solution (Provided by BIPM)
Metrological traceability: through DON, OGO.179a (BIPM)
Mass fraction: 627.1 µg/g ± 19.2 µg/g (k=2)

Amount of primary calibrator used for analysis

1.0387 g stock solution, 1.5013 g working solution

Gravimetry

Type of balance (make, model and resolution)

Make:	Mettler Toledo
Model:	XPE504 and XPE4002S
Resolution:	0.1 mg and 0.1 g

Balance repeatability

40 µg and 4 mg

Solution preparation procedure

The preparation was gravimetrically realized, 9.0904 g of the OGP034 solution was weighed and acetonitrile (LC-MS grade) was used as the solvent. 570.06 g of the solution was obtained with a mass fraction of 10.00 ug/g ± 0.15 ug/g (gravimetrical value).

Homogeneity and/or stability testing

The material uncertainty budget includes sources as:

Homogeneity: The homogeneity was evaluated using 12 units of the material. seven replicates by the unit were measured under repeatability conditions through LC-DAD
Short term stability: The short term stability was evaluated using an isochronous and accelerated design, The material was exposed to 50 °C for 29 days (the reference temperature was -20 °C), one ampoule per time were taken, for a total of five points. The DON determination was realized through LC-DAD, seven replicates by each ampoule were measured under repeatability conditions.

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

Mobile phase:	ACN:MeoH (1:1) (A)	Water (B)
Programm (Isocratic)	30	70
Column temperature (°C)	40	ThermoScientific Acclaim C18 100mm x 2.1 mm 2.2 um
Injection volume (ul)	10	
Flow (ml/min)	0.35	
Run time (min)	3	

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

Bracketing.

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

OGP 034 Stock solution
627.1 ug/g +/- 19.2 ug/g (k=2)
Metrological traceability: trought DON OGO 179a (BIPM)

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

-/-

Additional Comments or Observations

-/-

Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO), Brazil

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)

NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

OGP.034 standard DON solution provided by BIPM: (627.1 ± 19.2) µg/g ; k=2, 95%.

Amount of primary calibrator used for analysis

0.5 g

Gravimetry

Type of balance (make, model and resolution)

Balance 1: Mettler Toledo, model XS205, resolution 0.0001g (used for OGP.034.156 / OGP.034.041 /OGP.034.022); Balance 2: Mettler Toledo, model PR1203, resolution 0.001g (used for whole solution).

Balance repeatability

100 µg for model XS205 and 300 µg for model PR 1203

Solution preparation procedure

Solution was measured directly without further dilution.

Homogeneity and/or stability testing

Homogeneity study was performed with 10 ampoules randomly selected (batch of 59 ampoules). The heterogeneity contribution of 0,96% was included on uncertainty of assigned value. The short-term stability study showed that samples can be transported up to 50°C during 28 days, without changes on certified value, and its uncertainty contribution was also included on uncertainty of assigned value. Both studies were performed using HPLC-DAD (l = 219 nm) to measure the main compound (DON).

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

UPLC-MS/MS (ESI+)-PDA, XEVO TQ Waters

Column:	Acquity HSS T3 100 mm, 1.8 µm
Mobile phase:	A = H2O (0,01% acetic acid); B = Methanol (0,01% acetic acid). Solvent gradient: 99% A (initial); 5% A (5.5 min); 99% A (7.7 min).
Flow rate:	0.2 mL/min ; injection volume: 10 µL; run time: 10 min
Detector:	DAD 219 nm; Resolution: 4.8 nm.

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

Bracketing. Two calibration solutions was diluted using BIPM OGP.034.0186 to check the gravimetric preparation of DON batch.

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

BIPM OGP.034 (DON in acetonitrile): (627.1 ± 19.2) µg/g ; k=2, 95%.

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

DON (main compound): 297.2>248.9 for quantification, 297.2>231.0 for confirmation (ES+).
DOM-1 impurity: 281.0>233 for quantification, 281.0>215.0 for confirmation (ES+).

Additional Comments or Observations

The value assigned of Deoxynivalenol (DON) is the gravimetric preparation value 30.2 µg/g with relative combination of uncertainties from characterization (gravimetric preparation), homogeneity and short-term stability.

Instituto Nacional de Tecnología Industrial (INTI), Argentina

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)

NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

Deoxynivalenol (DON) in acetonitrile (ACN) stock solution. BIPM Reference: OGP.034. Purity of main component and uncertainty: 627.1 ± 19.2 µg/g (k = 2). Combination of DON gravimetric preparation 627.06 µg/g with uchar of 2.95 ug/g (0.47 %), homogeneity contribution of ubb of 1.00 µg/g (0.16 %) and stability contribution of ults of 9.09 µg/g (1.45 %). Used ampoules: OGP.034.147, OGP.034.135, OGP.034.073, OGP.034.013, OGP.034.039.

Amount of primary calibrator used for analysis

15.6469 g

Gravimetry

Type of balance (make, model and resolution)

Analytical Balance Sartorius LA230P.
Resolution: 0.1/0.2/0.5 mg (at 60/120/230 g respectively)

Balance repeatability

100/200/500 µg (at 60/120/230 g respectively)

Solution preparation procedure

The calibrant solution preparation was carried out weighing the content of 5 ampoules of stock solution into a 250 ml plastic bottle. The plastic bottle was filled up to 179.0-179.4 grams with acetonitrile. The exact final weight was considered. 76 clear glass ampoules containing 3 ml of calibrant solution were produced. Batch was called 2021-DON-DAI.

Homogeneity and/or stability testing

Homogeneity study was carried out considering 10 ampoules from the batch 2021-DON-DAI. Homogeneity was assessed by HPLC-DAD at 219 nm, 220 nm and 229 nm. The result obtained by HPLC-DAD at 219 nm was considered as inhomogeneity of batch 2021-DON-DAI (u*bb = 0.577 %).

Stability study was carried out following an isochronous experiment design. Three different conditions were considered: 4°C, 25°C and 40°C (all conditions in dark) during 1, 2, 4 and 6 weeks. The reference condition was -20°C in dark. Two ampoules were measured for each temperature and each period of time. HPLC-DAD was used to evaluate the stability. The results of the stability studies of 2021-DON-DAI did not show decreasing trends in any condition. All samples were analysed directly without dilution in a stratified random order. ISO Guide 35 (2017) was considered for all studies.

Optional: Analytical check method

Chromato graphic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

HPLC-DAD Conditions:
Column: Kromasil 100-5-C18 (4.6x250mm) (Batch/Serial: 0000139143/E173118)
Mobile Phase: (Water + ACN) (6.5 + 93.5)
Flow rate: 0.6 ml/min
Run Time: 22 minutes
Retention time of DON: 4.1 min
Oven temperature: 30°C
Injection Volume: 10 µl
Detector Wavelengths: 219 nm, 220 nm and 229 nm

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

-/-

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

-/-

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

-/-

Additional Comments or Observations

The assigned uncertainty is a combination of DON gravimetric preparation uncertainty (uchar) of 0.84 µg/g (1.53 %) and the homogeneity contribution of ubb of 0.32 µg/g (0.58 %).

Mass fraction assignment

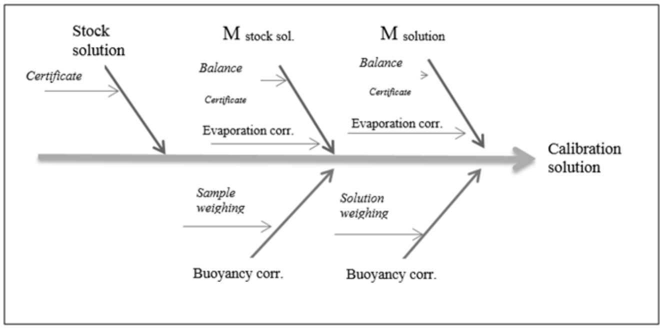
$$w_{DON\ calib\ solution} = \frac{w_{DON\ stock} \cdot m_{bal\ stock} \cdot b_{Sartorius\ LA230P} \cdot Ev}{m_{bal\ Sol} \cdot b_{Sartorius\ LA230P} \cdot Ev}$$

Calibrant Solution Preparation	
Stock Solution (mg) ($m_{bal\ stock}$) (mg)	15646.9
Whole Solution ($m_{bal\ sol}$) (mg)	179019.5
Stock Solution Concentration ($W_{DON\ stock\ solution}$)	**627.1**
Buoyancy Sartorius LA230P ($b_{Sartorius\ LA230P}$)	1.001387
Evaporation Correction (Ev)	1
Calibrant Solution Concentration ($W_{DON\ calibratio}$	**54.811**

Uncertainty assignment (Gravimetric Procedure)

$$u_{c,rel}(W_{DON-calib\ sol}) = \sqrt{\left\{\frac{u_c(w_{DON\ stock})}{w_{DON\ stock}}\right\}^2 + \left\{\frac{u_c(m_{bal\ stock\ sol})}{m_{bal\ stock\ sol}}\right\}^2 + \left\{\frac{u_c(b_{Sartorius\ LA230P})}{b_{Sartorius\ LA230P}}\right\}^2 + \left\{\frac{u_c(m_{bal\ sol})}{m_{bal\ sol}}\right\}^2 + \left\{\frac{u_c(b_{Sartorius\ LA230P})}{b_{Sartorius\ LA230P}}\right\}^2 + \left\{\frac{u_c(E_v)}{E_v}\right\}^2 + \left\{\frac{u_c(E_v)}{E_v}\right\}^2}$$

Resource of Uncertainty	Value
$\frac{u_c(w_{DON\ stock})}{w_{DON\ stock}}$	0.01531
$\frac{u(m_{bal\ stock\ sol})}{m_{bal\ stock\ sol}}$	0.00065
$\frac{u_c(m_{bal\ sol})}{m_{bal\ sol}}$	0.00066
$\frac{u_c(b_{Sartorius\ LA230P})}{b_{Sartorius\ LA230P}}$	0.0000117
$\frac{u_c(b_{Sartorius\ LA230P})}{b_{Sartorius\ LA230P}}$	0.0000117
$\frac{u(Ev)}{Ev}$	0.00005
$u_{c,rel}(w_{DON-calib\ sol})$	0.01531
$U_{c,rel}(w_{DON-calib\ sol})$(k=2)	0.03062
$u_c(w_{DON-calib\ sol})$ µg/g	0.84
$U_c(w_{DON-calib\ sol})$ µg/g(k=2)	1.68
U_c (%)	**3.06**



Laboratorio Technologico del Uruguay (LATU), Uruguay

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)
NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

OGP.034 standard DON solution provided by BIPM.

Amount of primary calibrator used for analysis

12.4371 g

Gravimetry

Type of balance (make, model and resolution)

SARTORIUS
model MSE 524S
resolution 0.0001g

Balance repeatability

100 µg

Solution preparation procedure

1. Balance performance check, tare, 2. weigh empty flask, 3. transfer stock solution, 4. weighing stock solution, 5. ACN LC MS grade addition, 6. weighing final mass of calibration solution.

Homogeneity and/or stability testing

Homogeneity testing have been done by HPLC DAD analysis.

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

HPLC DAD, oven temperature 30ºC
Phenomenex Gemini 5 u C18 110 A, 250 x 4,6 mm column

Mobile phase: A: ACN 5: H2O 95; B: ACN 95 : H2O 5
Flow: 0,6 mL/min, gradient .

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

-/-

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

-/-

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

-/-

Additional Comments or Observations

The uncertainty shown in the `RESULTS´tab is a combination of gravimetric preparation (u = 1,531 %) and the homogeneity contribution (ubb = 1,847 %). Stability contribution estimated on a 8 week basis is not included in `RESULTS´tab.

National Institute of Metrology (NIM), China (CBKT)

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)
NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

OGP.034 standard DON solution provided by BIPM, 627.1 ± 19.2 µg/g

Amount of primary calibrator used for analysis

6.0496 g

Gravimetry

Type of balance (make, model and resolution)

Sartorius ME235S 0.1 mg

Balance repeatability

200 µg

Solution preparation procedure

A 6.0496 g portion of the 627.1±19.2 µg/g stock solution (about 8 mL at 21 °C) was transferred to a 250 mL flask. Then about 152 mL of acetonitrile was transferred to the same flask, and the mixed solution (both stock solution and acetonitrile) was weighted to 121.0011 g. The solution with a final concentration of 31.35 µg/g DON in acetonitrile was obtained. A volume of 2 mL of the calibration solution was dispensed into 5 mL amber glass ampoules using a manual dispenser. The ampules were sealed with an ampule sealer. A total of 74 ampules were produced, labelled and stored in a freezer at -18 °C.

Homogeneity and/or stability testing

Homogeneity of the DON solutions was tested by selecting 10 ampules of the 74 ampules. Three aliquots (> 500 µL) per ampule were transferred into three HPLC vials and were analysed directly without dilution in randomly stratified order by UPLC-DAD method. The results of homogeneity test were subject to an analysis of variance (ANOVA). The results from homogeneity test of DON solutions were summarised in Table 1 as follows.

The isochronous stability study of DON solutions was tested at 50 °C for 0, 1, 3, 6, 10 days (3 ampules at each study time point). Three aliquots (> 500 µL) per ampule were transferred into three HPLC vials and were analysed directly without dilution in randomly stratified order by UPLC-DAD method. The results of stability test were evaluated using trend analysis. The results from stability test of DON solutions were summarised in Table 2 as follows.

Table 1

Homogeneity	**DON in acetonitrile**
Mean	31.39 µg/g
SD	0.35 µg/g
N	30
F	0.308
*F*crit	2.39
Significance? (*F*>*F*crit)	No
s_{wb}	0.40 µg/g
s_{bb}	/
u'_{bb}	0.13 µg/g
u_{bb}	0.13 µg/g
u_{bb}	0.41%

Table 2

Stability 50 °C	**DON in acetonitrile**
β_1	-0.0042
β_0	31.40
F	0.173
*F*crit	4.196
Significance? (*F*>*F*crit)	No
s(β1)	0.0100
T	0.416
*T*crit	2.048
Significance? (*T*>*T*crit)	No
u_{st} (10 days)	0.15 µg/g
u_{st} (10 days)	0.48%

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

Waters UPLC

Column:	Waters BEH C18 (100 × 2.1 mm, 1.7 µm)	
Wavelength:	219 nm	
Mobile phase:	A) H2O B) acetonitrile	
Gradient：	Time(min)	Mobile phase A
	0	5%
	7	5%
	8	95%
	10	95%
	10.5	5%
	13	5%
Flow rate:	0.3 mL/min	
Injection volume:	0.5 µL	

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

Single-point method was applied to assign the mass fraction value of comparison sample. Based on the BIPM OGP.034 DON stock solution, another calibration solution of 30.13 µg/g was gravimetrically prepared as the calibrator. .

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

OGP.034 DON stock solution provided by BIPM, 627.1 ± 19.2 µg/g

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

None.

Additional Comments or Observations

None.

Uncertainty budget			
Source of uncertainty	Parameter x	Uncertainty	
		u(x)	u(x)/(x)
Stock solution (µg/g)	627.1	9.6	1.53%
m_{DON} (mg)	6049.6	0.50	0.0082%
$m_{DON+ACN}$ (mg)	121001.1	0.86	0.0007%
Inhomogeneity (µg/g)	31.35	0.13	0.41%
Instability (µg/g)	31.35	0.15	0.48%
Bias caused by filliing (µg/g)	31.35	0.21	0.67%
Combined standard uncertainty (µg/g)	31.35	0.56	1.79%
Expanded uncertainty (µg/g) (k=2)	31.35	1.12	3.57%

National Institute of Metrology (NIM), China (own)

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)

The DON pure material was obtained from First Standard company. The purity value of DON material was assigned by NIM with mass balance method and qNMR method. The main component of DON material was assigned directly by qNMR method. HPLC-MS/MS and HPLC-DAD-CAD methods were applied to measure the content of organic impurites of DON material; the moisture and inorganic impurities were determined by Karl Fischer and ICP-MS methods. VOC content was detected by GC-ECD. Finally, a purity of 979 mg/g with an uncertainty of 9 mg/g (k=2) was attributed to the DON pure material.

Amount of primary calibrator used for analysis

0.006429 g

Gravimetry

Type of balance (make, model and resolution)

Mettler-Toledo	XP E56Q	0.001 mg
Mettler-Toledo	XPR10002S	0.01 mg

Balance repeatability

4 µg	XP E56Q
10 mg	XPR10002S

Solution preparation procedure

6.429 mg of the DON pure material was weighted and transferred to a 250 mL flask. The flask was diluted to the mark with acetonitrile and weighed to 195.21 g (250 mL at 20 °C). The solution with a final concentration of 32.24 µg/g DON in acetonitrile was obtained. A volume of 2 mL of the calibration solution was dispensed into 5 mL amber glass ampoules using a manual dispenser. The ampules were sealed with an ampule sealer. A total of 109 ampules were produced, labelled and stored in a freezer at -18 °C.

Homogeneity and/or stability testing

Homogeneity of the DON solutions was tested by selecting 10 ampules of the 109 ampules. Three aliquots (> 500 µL) per ampule were transferred into three HPLC vials and were analysed directly without dilution in randomly stratified order by UPLC-DAD method. The results of homogeneity test were subject to an analysis of variance (ANOVA). The results from homogeneity test of DON solutions were summarised in Table 1 as follows.

The isochronous stability study of DON solutions was tested at 50 °C for 0, 1, 3, 6, 10 days (3 ampules at each study time point). Three aliquots (> 500 µL) per ampule were transferred into three HPLC vials and were analysed directly without dilution in randomly stratified order by UPLC-DAD method. The results of stability test were evaluated using trend analysis. The results from stability test of DON solutions were summarised in Table 2 as follows.

Table 1

Homogeneity	**DON in acetonitrile**
Mean	32.20 µg/g
SD	0.49 µg/g
N	30
F	1.784
Fcrit	2.39
Significance? (F>Fcrit)	No
s_{wb}	0.44 µg/g
s_{bb}	0.22 µg/g
u'_{bb}	0.17 µg/g
u_{bb}	0.22 µg/g
u_{bb}	0.69%

Table 2

Stability 50 °C	**DON in acetonitrile**
β_1	-0.0100
β_0	32.40
F	0.172
Fcrit	4.196
Significance? (F>Fcrit)	No
s(β1)	0.0242
T	0.414
Tcrit	2.048
Significance? (T>Tcrit)	No
u_{st} (10 days)	0.24 µg/g
u_{st} (10 days)	0.74%

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

Waters UPLC
Column: Waters BEH C18 (100 × 2.1 mm, 1.7 µm)
Wavelength: 219 nm
Mobile phase: A) H2O B) acetonitrile
Gradient：

Time(min)	Mobile phase A
0	5%
7	5%
8	95%
10	95%
10.5	5%
13	5%

Flow rate: 0.3 mL/min
Injection volume: 0.5 µL

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

Single-point method was applied to assign the mass fraction value of comparison sample. Based on the BIPM OGP.034 DON stock solution, another calibration solution of 30.13 µg/g was gravimetrically prepared as the calibrator.

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

OGP.034 DON stock solution provided by BIPM, 627.1 ± 19.2 µg/g

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

None.

Additional Comments or Observations

None.

Uncertainty budget			
Source of uncertainty	Parameter x	Uncertainty	
		u(x)	u(x)/(x)
Purity of DON material (mg/g)	979	4.5	0.46%
m_{DON} (mg)	6.429	0.006	0.092%
$m_{DON+ACN}$ (g)	195.21	0.043	0.022%
Inhomogeneity (µg/g)	32.24	0.22	0.68%
Instability (µg/g)	32.24	0.24	0.74%
Bias caused by filliing (µg/g)	32.24	0.33	1.02%
Combined standard uncertainty (µg/g)	32.24	0.49	1.51%
Expanded uncertainty (µg/g) (k=2)	32.24	0.98	3.02%

National Institute of Metrology Thailand (NIMT), Thailand

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)
NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

OGP.034.034 BIPM stock solution

Amount of primary calibrator used for analysis

3.0986 g

Gravimetry

Type of balance (make, model and resolution)

Mettler-Toledo XPR2004SC, 0.0001 g Resolution

Balance repeatability

95.42 μg

Solution preparation procedure

Deoxynivalenol (DON) solution was gravimetrically prepared. An ampoule from BIPM OGP.034.034 stock solution was used. The stock solution was diluted with acetonitrile to the final volume of 250 mL.

Homogeneity and/or stability testing

Homogeneity testing: Homogeneity was based on the data from High-performance liquid chromatography–photodiode array detection (HPLC–PDA). Eleven randomly selected ampoules were analyzed in triplicates. The data set was then statistically analyzed using One-way ANOVA.
Stability testing: Data set from HPLC-PDA was used for stability evaluation. Twenty-six ampoules, stored at reference temperature of -20 °C, were randomly selected and analyzed in triplicates. Stability study was carried out using isochronous approach at 4 °C, 22 °C and 40 °C for 0,1,2,3 and 4 weeks. Trend analysis was performed to statistically test according to the conditions during the stability study, in which the slope at each study temperature was tested for its significance at 95 % confidence level.

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

LC-PDA conditions
Mobile phase: Isocratic elution with water:acetonotrile of 10:90
Total flow rate: 0.5 mL/min
Column temperature: 30 °C
Injection volume: 10 µL

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

Single point bracketing calibration.

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

Verification of the prepared standard solution was carried out by analyzing the prepared solution using a single point, bracketing external calibration. Pure DON in acetonitrile obtained from IRMM was used as calibration solution. Details of DON:

	Certified value:	Expanded Relative Uncertainty (%, *k=2*)
Sorce:	IRMM 25.1 µg/g	1.2

* The certified value of the standard was traceable to the SI unit of kg through gravimetric preparation and to the stated purity of the solid raw material.

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

-/-

Additional Comments or Observations

Reported value is based on gravimetric value. Measurement uncertainty was estimated from gravimetric preparation, homogeneity and stability studies.

Measurement Equation:

$$w\left(xi\right)=\frac{w_{z}*m_{z}}{m_{total}}$$

where;

w(xi)	mass fraction of the prepared solution, µg/g
w_z	mass fraction of the stock solution prepared from (OGP.034), µg/g
m_z	mass of the stock solution (OGP.034) added (g)
m_{total}	mass of the total solution (g)

Expanded Meaurement Equation:

$$w\left(xi\right)=\frac{w_{z}*m_{z}}{m_{total}}.F_{stb}\,.F_{\text{homo}}$$

where;

w(xi)	mass fraction of the prepared solution, µg/g
w_z	mass fraction of the stock solution prepared from (OGP.034), µg/g
m_z	mass of the stock solution (OGP.034) added (g)
m_{total}	mass of the total solution (g)
F_{stb}	stability factor, given a value of 1
F_{homo}	homogeneity factor, given a value of 1

$$\frac{u(w_{xi})}{w_{xi}}=\sqrt{\left(\frac{u(w_{z})}{w_{z}}\right)^{2}+\left(\frac{u(m_{z})}{m_{z}}\right)^{2}+\left(\frac{u(m_{total})}{m_{total}}\right)^{2}+\left(\frac{u(F_{\text{homo}})}{F_{\text{homo}}}\right)^{2}+\left(\frac{u(F_{stb})}{F_{stb}}\right)^{2}}$$

where;

$u(w_{xi})$	standard uncertainty of the prepared standard solution
$u(w_z)$	standard uncertainty of the stock standard solution obtained from the certificate (OGP.034)
$u(m_z),u(m_{total})$	standard uncertainties of masses estimaetd from the bias and the precision of balance
$u(F_{homo})$	standard uncertainty due to homogeneity factor, estimated from one-way ANOVA
$u(F_{stb})$	standard uncertainty due to stability testing at 4 °C, estimated from trend analysis

uncertainty source	Xi	u(xi)	u(xi/xi)	(uxi/xi)^2
Preparation				
DON stock	627.1	9.6	1.53E-02	2.3435E-04
mass DON stock (g)	3.0986	0.0002	7.00E-05	4.9013E-09
mass total (g)	193.5544	0.0011	5.46E-06	2.9798E-11
combined gravimetric	10.0393	0.1537	1.53E-02	**2.34E-04**
Homogeneity	1	0.0102	0.0102	1.045E-04
Stability @4 °C	1	0.0061	0.0061	3.690E-05
			sum (uxi/xi)^2	0.00038
			(uxi/xi)	0.0194
			u(cx) /Cx	0.0194
			ux	0.1946
			U (k=2)	0.39
			U(%)	3.88

National Metrology Institute of South Africa (NMISA), South Africa

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)

Crystalline Deoxynivalenol (DON) material was locally produced and procured from CSIR Biosciences.The material was purity assigned by NMISA using quantitative NMR. QNMR of DON purity was performed in deuterated acetone, using Fluka TraceCERT dimethylterphthalate as internal standard with traceability established in-house to NIST PS1 Benzoic acid using QNMR. The purity value determined was 932 ± 23 mg/g (k=2).

The OBP.034 primary calibrator from BIPM was also used as a calibrator to verify the NMISA value assignment. Although not used in the final value assignment of the solution, the mass concentration of NMISA CRM 0010b using the BIPM calibrator was within the expanded uncertainty of the NMISA DON CRM 0010b solution submitted for this study.

Amount of primary calibrator used for analysis

0.0501447 g

Gravimetry

Type of balance (make, model and resolution)

Mettler Toledo	XPR36	0.001 mg
Mettler Toledo	MS12002TS	0.01 g

Balance repeatability

2 µg

Solution preparation procedure

1. A 100 µg/mL solution (CRM0010b) was prepared by weighing ~50 mg in house value assigned DON (CRM0009) and diluting in 500 mL acetonitrile.
2. The solution was ampouled (2 mL) using the Ampulmatic 10 ampoule sealer, with the purge gas (nitrogen), and liquid filler accessories into 5 mL amber ampoules. The bulk solution was kept cool in an ice bath and continuously stirred with a magnetic stir bar during the dispensing process.

Homogeneity and/or stability testing

Of the 183 ampoules prepared, 10 were selected at regular intervals across the batch for the homogeneity assessment. Three repeat aliquots, without dilution, from each ampoule were evaluated using LC-UV at 219 nm. The relative standard homogeneity uncertainty determined using ANOVA, was estimated as 0.41 % and is included in the combined uncertainty reported. An isochronous stability assessment was carried out over 6 weeks, where 2 ampoules were stored at 4˚C, 20˚C, 40˚C and 60˚C, for 1, 2, 4 and 6 weeks, and 2 ampoules were stored at a reference temperature of -80˚C. All samples stored in the dark. The stability of the solutions was evaluated using LC-UV, monitoring the change in concentration of DON. No significant trends were observed at 4˚C, 20˚C or 40˚C over the 6 week period of the stability study. A significant trend was however observed at 60˚C, with an estimated relative u(Its) of 1.2% for storage at 60˚C for 6 weeks. A conservative, standard relative uncertainty of 1 % was included into the uncertainty estimate for stability of the solution.

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

A 2 µL injection of the undiluted aliquot of the DON solution (CRM 0010b) was injected onto a Phenomenex Synergi RP Polar column 80 Å, 150 x 4.6 mm, 4 µm (ANA0956) column. DON and impurities were separated in a 15 min isocratic phase (0.5 mL/min) of 80:20 aqueous mobile phase: acetonitrile/methanol (50/50) both containing 0.1 % formic acid. The isocratic phase was followed by a gradient and column wash with high organic solvent where some minor impurities were detected at 219 and 229 nm. The 219 nm wavelength trace was used for homogeneity and stability assessment. The samples were simultaneously analysed by MS/MS with numerous MRM transitions (12) to tentatively identify potential structurally related impurities to verify the QNMR purity value assignment.

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

The OBP.034 primary calibrator from BIPM was also used as a calibrator to verify the NMISA value assignment. Although not used in the final value assignment of the solution, the mass concentration of NMISA CRM 0010b using the BIPM calibrator was within the expanded uncertainty of the NMISA DON CRM 0010b solution submitted for this study. Concentration of the impurities was determined by relative peak area percentage assuming relative response factors of 1, used to support/verify qNMR data.

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

NMISA DON CRM0009 purity 932 mg/g with expanded uncertainty 23 mg/g (k=2, 95% level of confidence).
Mass fraction concentration traceable to SI through DMTP internal standard (Purity 997.0 mg/g with expanded uncertainty of 6.2 mg/g (k=2, 95% level of confidence) value assigned using QNMR internal standard NIST PS1 Benzoic acid (Purity 999.92 mg/g with expanded uncertainty -0.06 and + 0.04 mg/g (k=2, 95% confidence interval).

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

The identity and presence of structurally related impurities was evaluated using LC-MS/MS. Some of the MS/MS transitions in ESI negative mode that were used to detect potential Deoxynivalenol impurities were:
nor deoxynivalenol 265.1>235.1, 265.1>247.1; deepoxy deoxynivalenol 279.1>231.1,
279.1>249.1; deoxynivalenol 295.1>247.1, 295.1>265.1; nivalenol 311.1>191.0,
311.1>281.1; 15-o-acetyl-4-deoxynivalenol 337.1>150.0, 337.1>219.1;
3-acetyldeoxynivelanol 337.1>173.0, 337.1>307.1

Additional Comments or Observations

In the original NMISA DON crystalline material (CRM0009), 5 structurally related impurities were detected in the crystalline material namely: nivalenol; nor deoxynivalenol; deepoxy deoxynivalenol; 15-o-acetyl-4-deoxynivalenol and 3-acetyldeoxynivalenol.

National Research Council of Canada (NRC), Canada

Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)
NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

The purity of the solid 4-deoxynivalenol material was determined to be 0.896 ± 0. 013 g/g (k=2) by qHNMR and qH{^{13}C}NMR with dimethyl terephthalate as the internal standard. The purity value is SI-traceable to NIST benzoic acid PS1, which was the primary calibrator for the qNMR purity certification of dimethyl terephthalate. Four independent samples of 4-deoxynivalenol with dimethyl terephthalate as the internal calibrant were prepared. Two were dissolved in $CDCl_3$ and two in acetone-D_6. Each sample was analyzed in triplicate by qHNMR and qH{^{13}C}NMR, respectively. For each sample, about 4.5-5.5 mg of 4-deoxynivalenol and about 5 mg of dimethyl terephthalate were used. It is important to note that 4-deoxynivalenol tautomerizes in solution. Extensive NMR experiments (COSY, TOCSY and HSQC) were performed on a 900 MHz spectrometer to identify all the relevant tautomer signals for integration purposes prior to performing actual qNMR measurements.

Amount of primary calibrator used for analysis

0.005 g

Gravimetry

Type of balance (make, model and resolution)

Mettler Toledo, XP-6U, with a readability of 0.0001 mg

Balance repeatability

0.0007 mg obtained from the standard deviation of 10 readings of a calibrated 5g test weight

Solution preparation procedure

Solid 4-deoxynivalenol was gravimetrically weighed, dissolved in a gravimetrically known amount of acetonitrile and the solution sonicated for 30 min for homogeneous mixing. The solution was dispensed in minimum 1.0 mL aliquots in pre-washed and argon-filled ampules, which were immediately flame sealed.

Homogeneity and/or stability testing

Nine boxes, each containing about 100 ampules were obtained. One ampule from each box was sampled for homogeneity testing. The solution was determined to be homogeneous. An isochronous stability study was performed by monitoring the solution after week 1, 2, 4 and 8 at the following temperatures: +60 °C, +40 °C, 20 °C, 4 °C, -20 °C and -40 °C. After 8 weeks, a slight degradation trend was observed at +40 °C and a clear degradation trend was observed at +60 °C. As such an uncertainty due to stability was included in the final uncertainty as a conservative estimate. Please see table 1 below for a break-down of uncertainty components.

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

The homogeneity and stability studies were performed by LC-UV on an Agilent 1290 instrument fitted with an ACE-C18-PFP column (2.1 µm, 100 x 2.1 mm), heated to 40 °C. The injection volume of the samples was 2 µL, with elution proceeding at 0.4 mL/min with water and methanol as mobile phases A and B respectively. The following gradient was applied: 0-2 min (2 % B), 2-8 min (2-40 % B), 8-13 min (40 % B), 13-13.1 min (40-2 % B) and 13.1-17 min (2 % B). UV absorbance was monitored at 220, 225 and 253 nm.

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

To obtain a separate assessment of the concentration of the solution, an external calibration curve was prepared from a fresh sample of in-house 4-deoxynivalenol. A five point calibration curve was prepared and the concentration of the calibration solution was assessed against it. According to the calibration curve, the concentration of the calibration solution is in agreement with the reported value.

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

To prepare the external calibration curve, solid 4-deoxynivalenol that was produced and purified in-house was used. The purity of this material was determined by qNMR as described above.

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

-/-

Additional Comments or Observations

An analytical standard of 4-deoxynivalenol in acetonitrile purchased from Aldrich was used as quality control sample during the homogeneity and stability assessments by LC-UV. The QC samples were generally spread out throughout the sequence after every six samples. The measurements obtained showed that instrument drift was not a factor. However, the analytical standard lacked SI-traceability. Therefore, it was not used as an independent check for the concentration value of the calibration solution. Secondly, the 4-deoxynivalenol that we produced and purified in-house, did not contain any structurally related impurity that would have interfered with qNMR, LC-MS or LC-UV analyses. Thus, the measurements that we obtained did not require any impurity corrections and simplified the certification. Commercially available sources of 4-deoxynivalenol did not meet our purity requirements for preparing the calibration solution.

Table 1. Uncertainty components for the certified value of the calibration solution for DON.

Substance	U(k=2) µg/g	u_c µg/g	u_{char} µg/g	u_{hom} µg/g	$u_{stability}$ µg/g
4-deoxynivalenol	1.6	0.8	0.5	0	0.6

Uncertainty due to stability = ustability
Uncertainty due to homogeneity = uhom
Uncertainty due to characterization of calibration solution = uchar
Combined uncertainty = uc
Expanded uncertainty (U) = 2*uc

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Solution preparation procedure

Calibrator

Primary calibrator (e.g., source, purity, assignment method, establishment of traceability)
NOTE: For MMCBKT participants, the primary calibrator is the OGP.034 standard DON solution provided by BIPM

BIPM OGP.034

Amount of primary calibrator used for analysis

3.0816 g

Gravimetry

Type of balance (make, model and resolution)

Sartorius MSA524S-100-DA 0.1 mg

Balance repeatability

60 µg

Solution preparation procedure

BIPM stock solution transferred from -20 ˚C to the room temperature and kept in dark condition until the solution reached to room temperature. An empty 250 mL volumetric flask placed on the balance and tared. BIPM stock added to flask (3.0816 g) then capped and tared. Acetonitrile (90.1531 g) added into the flask, capped and weighed. The solution was mixed and chilled. Solution filled into 10 mL clear glass ampules as 4 mL by ampulmatic, purged with nitrogen then sealed.

Homogeneity and/or stability testing

Totally 30 ampoules were prepared and stored at -20 ˚C after preparation. Totally 6 ampoules were selected randomly by TRANS program for homogeneity testing. Isochronous stability testing was performed at 25 ˚C for 0, 1, 2 and 4 week time points. Two ampoules were selected by TRANS program randomly for each time point. Reference temperature was -20 ˚C. Homogeneity results were statistically evaluated by ANOVA and 0.21 % uncertainty reported for homogeneity. Stability results were evaluated by significance test on slope and 0.19 % uncertainty was reported for stability at 25˚C.

Optional: Analytical check method

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

Agilent 1290 HPLC with Agilent 1260 DAD detector used at 219 nm. Fortis Universil HS C18 250 mm x 4.6 mm 5 µm column used at 30 ˚C for seperation. Gradient program with A (Acetonitrile), B (water) at flow of 0.75 mL/min and program is given below. 5 µL sample injected into column.

Retention time (min)	Flow (mL/min)	A%	B%
0	0.75	11	89
30	0.75	11	89
35	0.75	95	05
50	0.75	95	05
51	0.75	11	89
60	0.75	11	89

Calibration type / details
(e.g., single-point, bracketing /external calibration, internal standard calibration, IDMS)

Six-point external calibration used (10-100 µg/g).

Calibration and/or Internal standards
(e.g., source, purity, and traceability of standards)

BIPM OGP.034

Indicate ion/MRM monitored in Mass Spectrometer (if applicable)

-/-

Additional Comments or Observations

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2}$$

Uncertainty Budget			
		Uncertainty	
Source of uncertainty	Parameter (x)	u(x)	u(x)/x
BIPM Stock Purity	627.1	9.6	0.015308563
m_{stock} *(g)*	3.0816	0.000370	0.0001201
m_{ACN} (g)	90.1531	0.000370	0.0000041
Repeatability	20.56	0.026	0.0012
Homogeneity	20.56	0.043	0.0021
Stability	20.56	0.039	0.0019
Combined Standard Measurement Uncertainty, %			1.56
Expanded Measurement Uncertainty, % ($k=2$)			3.12
Concentration Value (µg/g)			**20.56**
Standard uncertainty, *u* (µg/g)			0.321
Expanded Uncertainty, U (µg/g)			0.642
Expanded Uncertainty reported, U (µg/g)			**0.65**