

Certificate of analysis

Certified reference material code: CRM-00-DON

Name: Certified Reference Material of 4-deoxynivalenol.

Batch: 24-001

Description: This Certified Reference Material (CRM) is a solution of 4-deoxynivalenol (CAS Number 51481-10-8) in acetonitrile.

Intended use: Calibration of equipment or a measurement procedure. Establishing metrological traceability. Method validation. Quality control of a measurement or measurement procedure. For laboratory use only.

Certified values and uncertainties: The certified concentration given below is based on results obtained from the gravimetric preparation of this solution previous quantity determination by ^1H -qNMR in Varian 500 MHz equipment (Universidad de Santiago de Compostela).

Concentration of 4-deoxynivalenol (95% Confidence Interval)	$(434 \pm 43) \mu\text{mol} / \text{kg}$
	$(129 \pm 13) \mu\text{g} / \text{g}$

The starting material is measured by ^1H -qNMR against a Benzoic acid standard for quantitative NMR, certified reference material followed by gravimetric preparation using high precision balances calibrated with SI-traceable weights. Consequently, the quantified values are traceable to the International System of Units (SI) via an unbroken chain of comparisons through validated methodology.

Homogeneity was evaluated by analysis of variance on 2% of stratified randomly selected ampoules over the entire batch (6 replicate analyses per ampoule). A possible heterogeneity is hidden by the method repeatability. Therefore, a maximum possible heterogeneity was calculated as u_{bb} , 1.28%. The Stability studies after storage of selected units were performed at -20°C for a storage time of 12 months. Using the data from the long-term study, the uncertainty due to possible degradation was calculated as u_{ts} , 1.79%. Both studies were verified against an independently prepared calibration solution.

The components of Standard Uncertainty include related uncertainties due to characterization, heterogeneity, long term instability, short term instability (dispatch), and bulk assay, Table 1. The results are expressed as the certified value $\pm$ the expanded uncertainty (calculated by combination of the squared contribution values). Estimated expanded uncertainty with a coverage factor $k = 2$, corresponding to a level of confidence of about 95 %, as defined in the Guide to the expression of uncertainty in measurement, ISO33405.

The certificate is a summary of an extensive program of work involving selection and purification of the material, assessing its suitability and measuring the properties to be certified.

Expiration of Certification: *This certificate of CRM-00-DON, 24-001 batch is valid 12 months after purchase date within the measurement uncertainties specified, provided the CRM is handled and stored in accordance with the instructions given in this certificate. This validity may be extended if further evidence of stability becomes available.*



CRM-00-DON, 24-001,
rev.2, 12/01/2026

F-01-08 Edición 6 Página 1 de 4

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Maintenance of Certification: The material will be subjected to regular Laboratorio CIFGA stability monitoring programme to control its further stability over the period of its certification. If substantive changes occur that affect the certification before the expiration date, Laboratorio CIFGA will notify the purchaser.

Instructions for proper handling use: This material contains toxic material, and should be handled with care. Read MSDS before using. Material Safety Data sheet for information regarding CRM-00-DON is enclosed in the package. Use proper disposal methods.

Before opening an ampoule, it must be guaranteed that the content is a liquid and properly mixed to ensure homogeneity. Sample aliquots for analysis should be handled at 15°C to 25°C immediately after opening the ampoules and should be processed without delay for the certified value to be valid within the stated uncertainty. Ampoule should not remain open longer than five minutes due to solvent volatility and air contamination.

Storage conditions: The original sealed ampoules should be stored at temperatures below/at -20 °C (freezer) until use. Unopened ampoules should be stored upright under normal laboratory conditions inside the original container supplied.

Source and Preparation of Material: 4-deoxynivalenol is a toxin isolated and purified from *Fusarium graminearum*. The solution was prepared by weighing and mixing DON into acetonitrile until completely homogenization. Identity of the material was confirmed by ¹H-qNMR and LC-MS/MS. Finally, 1.0 mL aliquots of DON solution were dispensed into 2-mL amber argon-filled glass ampoules, which were then flame sealed and ready to use for calibration.

Non-certified and non-accredited data:

Taking into account the density of acetonitrile at 20 °C ($\rho_{ACN}^{20^{\circ}C} = 0.7822 \text{ g/mL}$), non-certified volumetric concentration values are:

Concentration of 4-deoxynivalenol (95% Confidence Interval)	(339 ± 34) µM
	(100.6 ± 10.1) µg / mL

NOTE: concentration of 4-deoxynivalenol and impurities are neglected in order to apply solution density value.

Purity analysis was performed by combining ¹H-qNMR and UPLC MS/MS, ≥ 98 % (molar ratio purity). *No purity correction over certified concentration should be done.*

Additional lectures:

1. UNE-EN ISO 17034 *General Requirements for the competence of reference material producers.*
2. ISO 33405 *Reference materials - Approaches for characterization and assessment of homogeneity and stability.*



CRM-00-DON, 24-001,
rev.2, 12/01/2026

F-01-08 Edición 6 Página 2 de 4

The marked activities (#) are not covered by ENAC accreditation

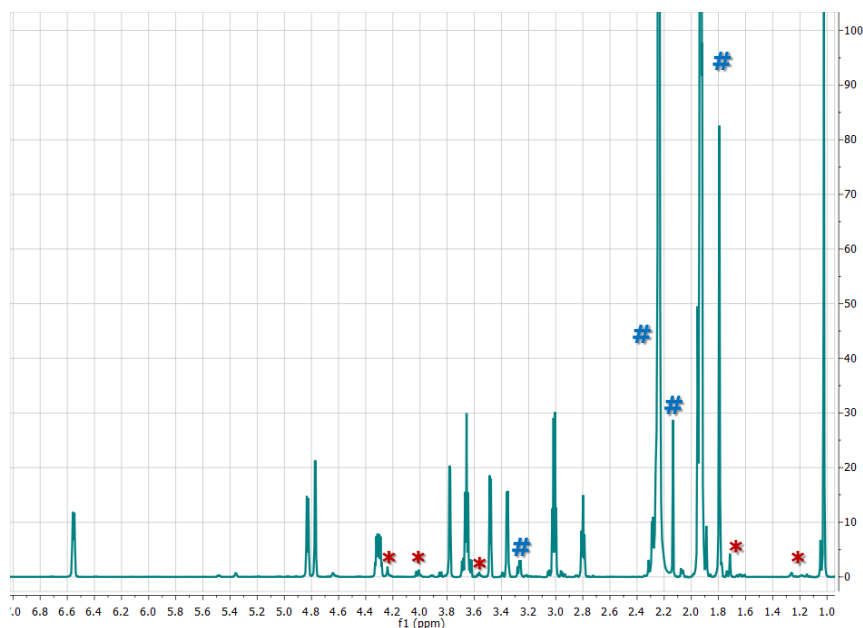


Figure 1. ^1H -NMR spectrum of 4-deoxynivalenol solution, VARIAN 500 WB, 20 mM of DON in CD_3CN used in preparation of CRM-00-DON batch 24-001. The most intense peaks correspond to the keto form, while the least intense peaks are related to hemiacetal tautomer. ^1H -qNMR integrals of all isomeric and tautomeric signals belonging to H-10 were added to obtain the most accurate value. No identified impurities are highlighted (*). As the structure of these impurities is not known, their exact amount cannot be calculated. Because the impurities usually are smaller molecules (otherwise, more NMR signals would arise), it can be estimated from the corresponding integrals, but this purity is not relative to close-related impurities to 4-deoxynivalenol analogues. (#) Signals arise from solvent residual peaks.

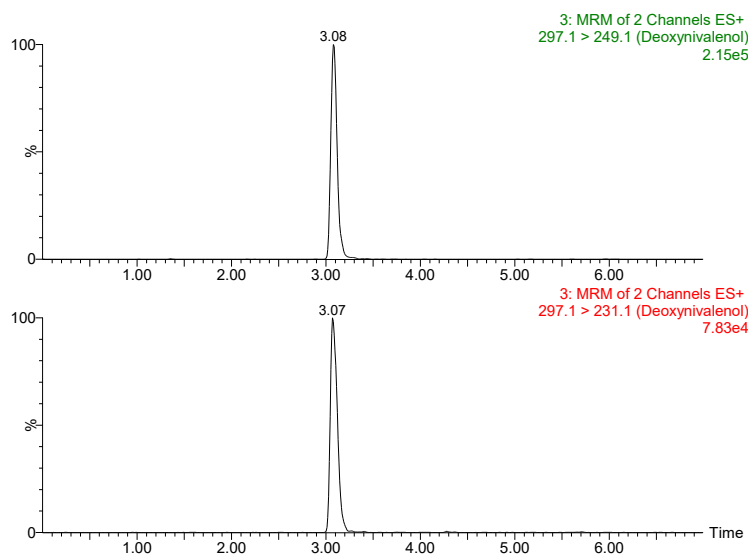


Figure 2. Analysis of the CRM-00-DON (24-001) by UPLC-MS/MS. Conditions: ACQUITY UPLC HSS T3 1.7 μm , 100x2.1 mm, UPLC column. Main elution conditions, gradient mode with mobile phases: (A) Water and (B) acetonitrile, both with 0.1% formic acid; Gradient: 0 min 5% B; 0.5 min 5% B; 4.5 min 95% B; 5.5 min 95% B; 5.6 min 5% B; 8.0 min 5% B; flow rate: 0.3 mL/min. Temperature: 30°C. MS detector: Waters XEVO TQ-MS using the typical MRM transitions for 4-deoxynivalenol and main expected impurities.



Table 1. Uncertainties contribution for the certification of **CRM-00-DON (24-001)**. *Char*: *qNMR quantification*; *ba*: *bulk assay*; *bb*: *homogeneity test*; *lts*: *long term stability test*; *sts*: *short term stability test*.

Uncertainty contribution parameter	Uncertainty values
	DON
$u_{char}/\%$	2.88
$u_{ba}/\%$	1.25
$u_{bb}/\%$	1.28
$u_{lts}/\%$	1.79
$u_{sts}/\%$	3.23
Expanded uncertainty ($k=2$) / %	10.03

Short-term stability study. The uncertainty due to transport instability was estimated from the test for slope significance at 40°C. The results indicated continued stability, no change is expected in the property values of the CRM. The combined uncertainty budget of the assigned value was upgraded to incorporate short-term stability at 40°C for 9 days.

Inquiries concerning this Reference Material should be directed to:

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RM PRODUCER'S APPROVING OFFICER:

Certification Manager: Álvaro Antelo

This Certificate is only valid if the product was obtained directly from Laboratorio CIFGA S.A. or one of our authorized distributors.

Certificate Revision History:

February, 13rd 2025 (Original certificate issue date, revision 1).

January, 12th 2026. Revised and added associated uncertainties following additional testing. Minor typographical errors (*certificate rev. 2*).

NOTE: This certificate of analysis shall not be reproduced, except in full, without written approval of Laboratorio CIFGA S.A.



CRM-00-DON, 24-001,
rev.2, 12/01/2026

F-01-08 Edición 6 Página 4 de 4

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