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Bureau International des Poids et Mesures

Purity Evaluation Guideline: Deoxynivalenol

BIPM PEG-03

Authors: Steven Westwood (BIPM), Ralf Josephs (BIPM), Gustavo Martos (BIPM), Tiphaine Choteau (BIPM), Xianjiang Li (NIM China);Ilker Un (TÜBITAK UME, Türkiye), Leandro Santos (INTI Argentina)

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Table of Contents

1. Scope

This document provides technical guidance and reference data to assist with the establishment of the qualitative identity and quantitative characterization of deoxynivalenol (DON) when present as the primary component in a purified organic solid. In particular it is intended for the characterization of a Primary Reference Material (PRM)[1] to underpin the metrological traceability of routine testing procedures for the detection and quantification of contamination by DON at trace levels in food, feedstuffs and primary produce.

2. Introduction

In collaboration with the National Institute of Metrology (NIM), China, and the National Metrology Institute of South Africa (NMISA), the BIPM initiated in 2016 a Capacity Building and Knowledge Transfer programme for Metrology for Safe Food and Feed in Developing Economies.[2] This project is designed to allow NMIs to work together to strengthen the world-wide mycotoxin metrology infrastructure, to provide knowledge transfer to scientists developing capabilities in this area and to enable NMIs in developing regions to produce calibrants, matrix reference materials and proficiency test samples to support testing and laboratory services for mycotoxin analysis within their countries.

As for all other areas of organic analysis, PRMs consisting of well characterized, high purity compounds are required for each analyte subject to investigation. These materials are the ultimate source of higher-order metrological traceability for the assigned values of derived calibration solutions, matrix reference materials, proficiency test samples and ultimately the results of routine analysis. Access to pure organic compounds and calibration solutions prepared from these materials is an essential element in the measurement infrastructure supporting the delivery of reliable, comparable results. In the case of mycotoxins, purity analysis of source materials involves additional challenges linked to the limited amount of available material and its potential toxicity.

Deoxynivalenol is a member of the trichothecene[RS1] family of sesquiterpenoid natural products.[3] It was the first identified [4] of a family of over one hundred and eighty related structure mycotoxins produced by moulds of the genus *Fusarium* and *Stachybotrys*, particularly *Fusarium graminearum.* Growth of these moulds on corn, wheat, rice and other cereal crops at any time during production, harvesting or storage can result in contamination of derived food and feedstock with DON and related mycotoxins.[5] Exposure to these toxins can arise through either the direct consumption of cereal-based foods or indirectly from foods of animal origin (for example kidney, liver, milk, eggs) produced from animals raised on contaminated feed.[6] Contamination with DON has been detected in a variety of processed food products including breakfast cereals, baby formula and beer and can result in acute transient nausea, vomiting, diarrhoea, abdominal pain, headache, dizziness and fever. The emetic properties of DON are so pronounced that it is commonly known as vomitoxin.[7]

Guidance values have been established by the Commission of the European Communities for

the levels of DON in products intended for animal feed.[8] These are currently 8 and 12 ppm for cereal and maize products and 0.9, 2 and 5 ppm, respectively, for feedstock for pigs, calves, lambs/kids. 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.[9]

A necessary requirement of the BIPM CBKT project was to obtain and characterize a primary reference material for DON that could be used to anchor the metrological traceability[10] of results linked through calibration to this material. This guideline summarizes the characterization and purity assignment studies undertaken to deliver a PRM for DON required for the BIPM MMCBKT programme. It is intended to be of use to National Metrology Institutes and reference measurement service providers needing to characterize their own primary material for DON analysis. 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 content of deoxynivalenol it contained.

Due to its complex structure, purity assignment by qNMR provided in the first instance a value that included a contribution from related structure impurity content in addition to the native DON. This assignment was complicated by the fact that DON spontaneously isomerizes partially into a hemiketal structure in solution.[11] This value was corrected subsequently for the related structure impurity content assigned separately by LC-MS/MS and LC-DAD methods to give the final value for the “true” DON content of the material. Additional analyses for the assessment of other potential impurities were undertaken to support the value assigned using the qNMR and LC data.

3. Nomenclature and Ring numbering

The conventional ring numbering and abbreviations[12,13] established for DON and related compounds are used. DON is a member of the Type-B trichothecenes class of natural products. Its structure with the conventional numbering scheme is shown in Figure 1.

The structures for the related trichothecene mycotoxins are given in Annex 7.1.

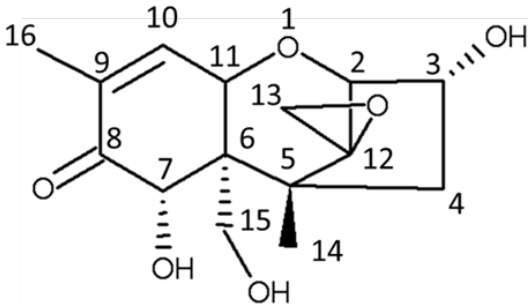


Figure 1: DON structure with numbering scheme.[14]

4. Properties of Deoxynivalenol

4.1 Hazard Identification

The substance poses risks for human health if handled inappropriately. It is toxic by inhalation, in contact with skin and if swallowed (hazard class 6.1, UN3462).

DON is believed to be hepatotoxic, carcinogenic and teratogenic. It is a strong emetic.

DISCLAIMER: The safety recommendations given in this section are based on review of literature reports of best practice but have not been verified by the BIPM.

4.1.1 Protective measures

Avoid inhalation of dust, vapours, mist or gas. Wear a full-face particulate filtering respirator type N100 (US) or type P3 (EN 143) respirator cartridges when working with the solid material. Wear protective gloves, goggles and clothing. Take special care to avoid skin exposure if handling solutions and work in adequately ventilated areas. Wash hands thoroughly after handling.

4.1.2 Emergency procedures

General advice: Immediately call a POISON CENTRE or doctor/physician. Show this safety information to the doctor in attendance. Move out of dangerous area.

If inhaled: Move into fresh air. If not breathing give artificial respiration. Consult a physician.

In case of skin contact: Wash off with soap and plenty of water. Consult a physician.

In case of eye contact: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed: Immediately call a POISON CENTRE or doctor/physician. Never give anything by mouth to an unconscious person. Rinse mouth with water.

4.1.3 Spillage

Contain spillage and then collect by wet-brushing and place in container for disposal. Keep in suitable, closed containers for disposal according to local regulations.

4.2 Physical and Chemical Properties

Common Name:	**Deoxynivalenol**
IUPAC and Chemical Abstracts Names:	(3α,7α)-3,7,15-Trihydroxy-12,13-epoxytrichothec-9-en-8-one
Synonyms:	DON, Vomitoxin, Dehydronivalenol, 4-Deoxynivalenol
CAS Registry Number:	51481-10-8
Molecular Formula:	$C_{15}H_{20}O_6$
Molar Mass:	296.32 g/mol
Monoisotopic mass:	296.1260
Melting point:	152 °C [15] 151-153 °C [16], [17]
Appearance:	Colourless crystals from ethyl acetate[17] or aqueous methanol
Solubility	Soluble in water and polar organic solvents (MeOH, EtOH, $CHCl_3$, ACN)
UV maxima (nm)	ACN: 219 nm (ϵ = 6983 ± 126 $L \cdot mol^{-1} \cdot cm^{-1}$) [18] EtOH: 218 (ϵ = 4500) [17]

Figure 2 shows a number of possible interconversions and transformations between DON and related structure impurities which have been observed to take place in solution.

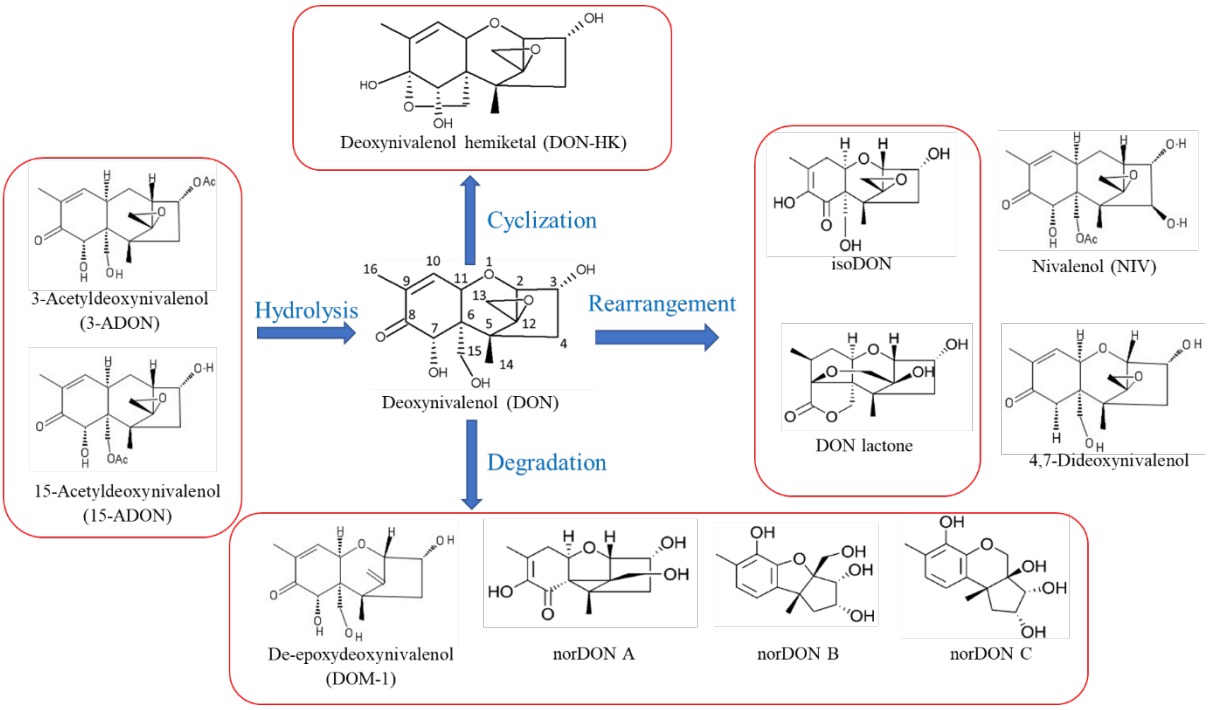


Fig. 2: Structures and relationship of DON precursors and transformation products

4.3 Qualitative identification

4.3.1 **NMR Materials and methods**

Chemicals:

- Deoxynivalenol (DON); BIPM Reference OGO.179a
  Supplier: First Standard, Product No. 1ST7213, Lot ALT601343

NMR Solvents:

- Deuterated methanol (CD_3OD); BIPM Reference OGS.028
- Acetone-d_6; BIPM Reference OGS.029b

Deuterated NMR solvents were purchased commercially and used without further treatment.

4.3.2 **Sample preparation**

For qualitative NMR analyses, sample sizes typically in the range (6 – 7) mg of DON were weighed accurately and made up in 1 mL of deuterated solvent in a glass vial. The sample solution was mixed in a vortex shaker and transferred using a disposable glass pasteur pipette into NMR tubes (HG-Type: high-grade class, 5 mm o.d., with polyethylene caps).

4.3.3 **NMR acquisition parameters**

A JEOL ECS-400 spectrometer operating at 9.4 T (400 MHz for proton) equipped with a direct type automatic tuning (Royal) probe was used for all data acquisition. For qualitative analyses, ^{1}H spectra were acquired for both solvent blank and the DON sample using a pulse-acquire sequence with the parameters listed in Table 1.

Table 1 - Acquisition parameters for qualitative ^{1}H NMR.

Parameter	*Value*
Number of Transients	32
Receiver gain	38
Acquisition time (s)	2.2
Relaxation delay (s)	5.0
Pulse offset (ppm)	5.0
Spectral width (ppm)	15.0
Data points	16384
Temperature (K)	298
Spinning	Off

^{13}C-NMR experiments were conducted using an ordinary power gated sequence (pulse-acquire in ^{13}C channel with proton decoupling both during acquisition and the relaxation delay) using the parameters listed in Table 2.

Table 2 - Acquisition parameters for ^{13}C NMR.

Parameter	*Value*
Number of Transients	10000
Receiver gain	60
Acquisition time (s)	1.04
Relaxation delay (s)	2.0
Pulse offset (ppm)	100
Spectral width (ppm)	250
Data points	32768
Temperature (K)	298
Spinning	Off

4.3.4 **1D ^{1}H- and ^{13}C-NMR spectra**

^{1}H- and ^{13}C-NMR assignments of DON have been reported in solution in solvents including deuterochloroform,[19] methanol-d_4 [20] and acetonitrile-d_3.[21]. ^{1}H NMR Spectra obtained at the BIPM of DON in solution in deuteromethanol and acetone-d_6 using the source material OGO.179 are shown respectively in Figures 3 and 4. In methanol-d_4 the spectral signals of DON were observed to be relatively broad with limited peak resolution and were not optimal for quantitative measurements. In contrast, in solution in acetone-d_6 the individual signals were observed to be both sharper and better resolved, particularly around 5 ppm. It was subsequently demonstrated that DON is also more stable in solution in acetone-d_6 solvent.[22]

The results were consistent with literature NMR assignments.[14,23] Figure 5 shows the basic ^{13}C-NMR spectrum of DON in solution in ACN. Figure 6 is the attached proton test (APT) spectrum obtained using the same solution where inverted signals correspond to CH_2 or quaternary carbons and normal signals to CH or CH_3 carbons.

4.3.5 **2D NMR spectra**

To confirm the identification and stereochemical assignment of the structure, a series of two-dimensional (2D) NMR experiments were undertaken. These included homonuclear (^{1}H-^{1}H) correlated spectroscopy (COSY), heteronuclear single-quantum correlation (^{13}C-^{1}H) (HSQC), ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC), (^{1}H-^{1}H) homonuclear total correlated spectroscopy (TOCSY) and (^{1}H-^{1}H) homonuclear Nuclear Overhauser Effect spectroscopy (NOESY).[24] The corresponding 2D-spectra obtained for the DON material OGO.179 are reproduced in Annex 7.2.

The full peak assignments based on the combined data obtained at the BIPM are compiled in Table 3. They are self-consistent, agree with literature assignments[14] and establish that the NMR properties of the primary component in OGO.179 are fully consistent with the structure of DON.

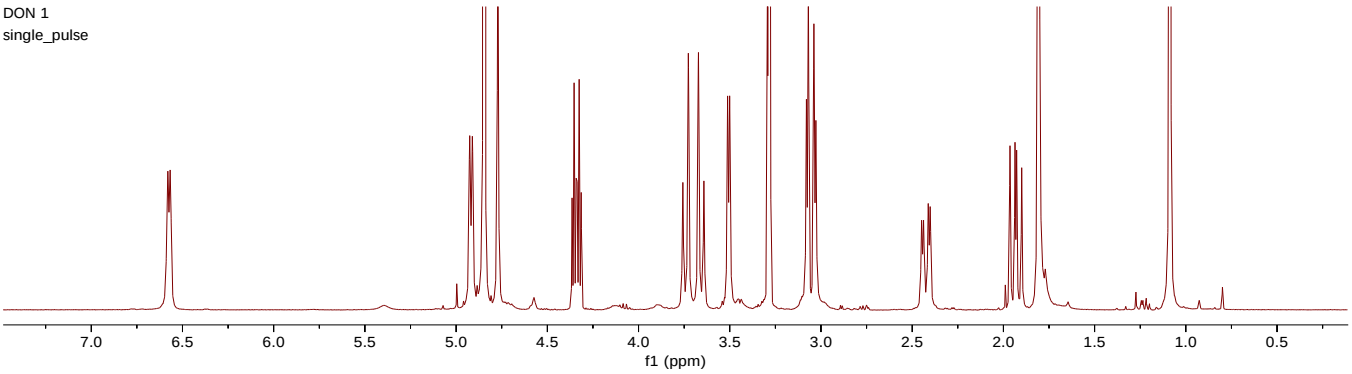


Figure 3 – ^{1}H NMR spectrum of DON in CD_3OD.

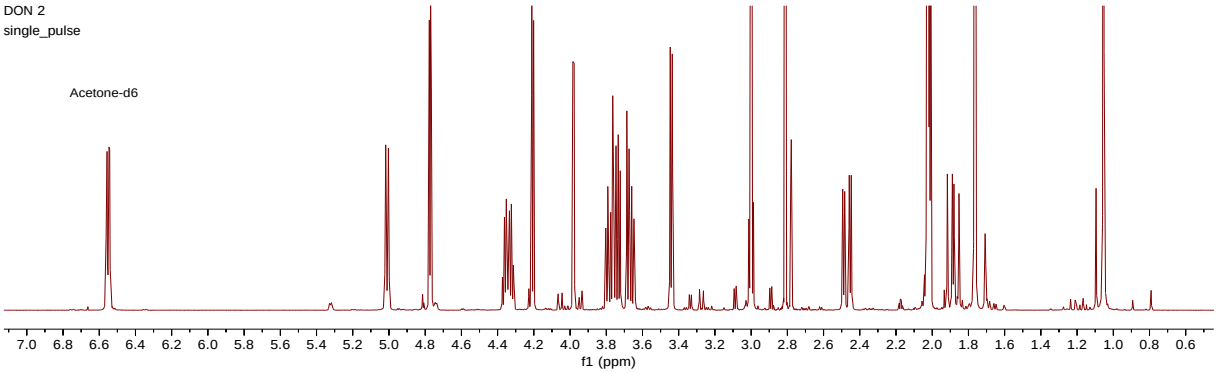


Figure 4 – ^{1}H NMR spectrum of DON in acetone-d_6.

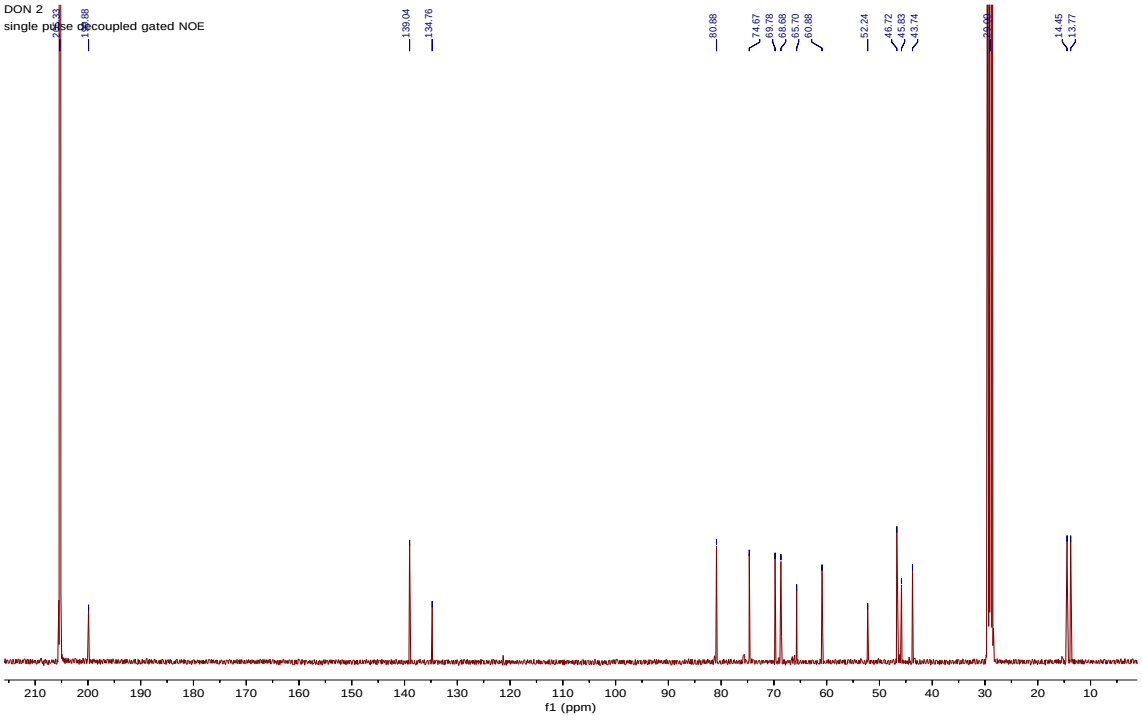


Figure 5 – ^{13}C NMR spectrum of DON in acetone-d_6.

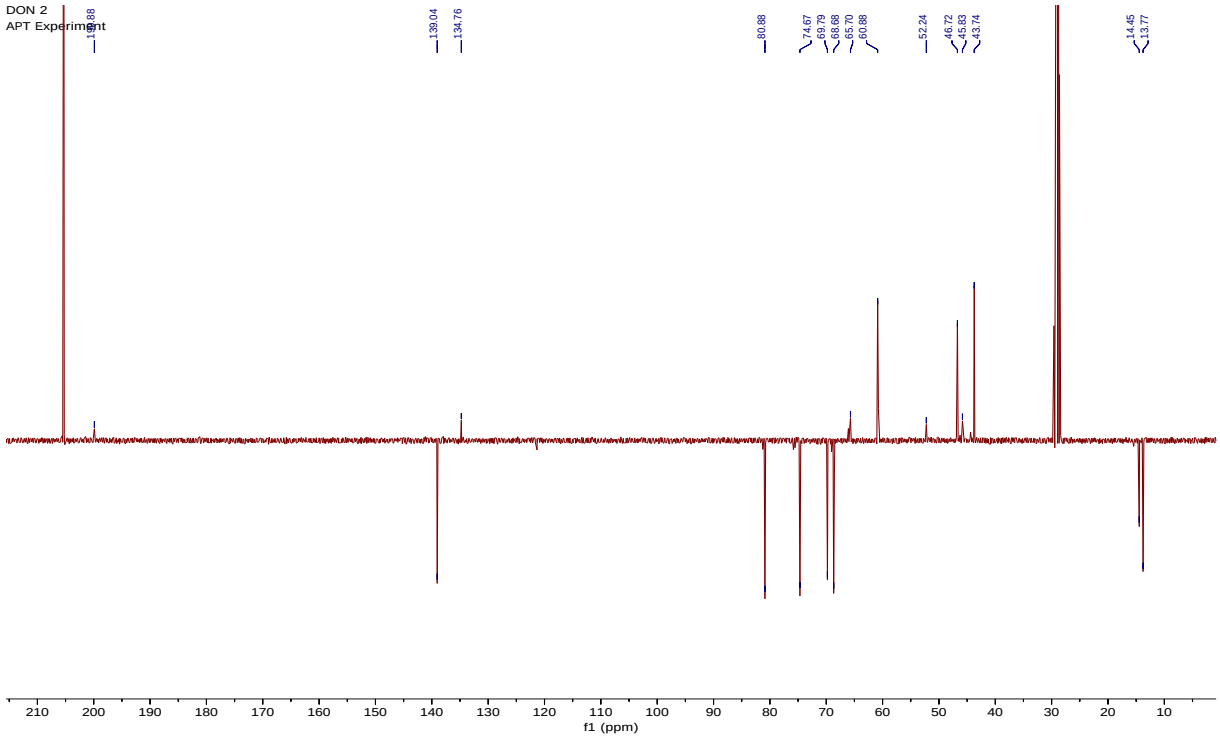


Figure 6 – APT ^{13}C NMR spectrum of DON. Down = C/CH_2; Up = CH/CH_3.

Table 3 – ^{1}H and ^{13}C NMR assignments for DON

Position	^{1}H-NMR (ppm, rel. integral)	^{13}C-NMR (ppm, APT)	COSY (ppm ^{1}H J-coupled)	HMBC (^{13}C connectivity)	NOESY (^{1}H spatially proximate)
2	3.44, 1H	80.9, CH	4.34	C-3, C-4, C-5, C-12	4.34
3	4.34, 1H	68.7, CH	1.88, 2.47, 3.44, 4.24	C-2, C-4, C-5	1.88, 3.44, 4.24
4α	2.47, 1H	43.7, CH_2	1.88, 4.34	C-3, C-5, C-6, C-12	
4β	1.88, 1H		2.47, 4.34	C-2, C-3, C-5, C-6	
5	-	45.8	-	C-3, C-4, C-6, C-14	
6	-	52.2	-	C-4, C-5, C-7, C-10, C-11, C-14	
7	4.77, 1H	74.7, CH	3.77	C-5, C-6, C-11, C-15	
8		199.9	-	C-7, C-9, C-10, C-15	
9		134.8	-	C-10, C-11, C-16	
10	6.55 (1H)	139.0, CH	5.01	C-9, C-11	
11	5.01 (1H)	69.8, CH	6.55	C-6, C-10, C-15	
12		65.7	-	C-2, C-5, C-13, C-14	
13	3.00 (2H)	46.7, CH_2	-	C-5, C-6, C-12	
14	1.05 (3H)	13.8, CH_3	-	C-4, C-5, C-6, C-12	
15	3.67, 3.77 (2H)	60.9, CH_2	3.99	C-5, C-6, C-7, C-11	
16	1.76 (3H)	14.5, CH_3	-	C-8, C-9, C-10	
3-OH	4.24		4.34		
7-OH	4.77		4.77		
15-OH	3.99		3.67, 3.77		

4.3.6 **DON Hemiketal formation in solution**

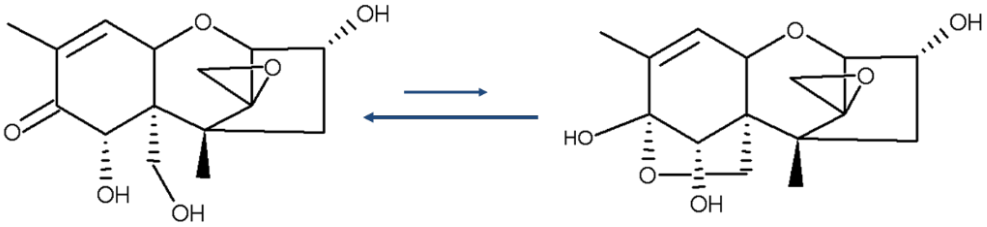


Figure 7: Structures of DON and DON-hemiketal

Once taken up in solution, an equilibrium immediately establishes between DON and the isomeric hemiketal structure (DON-HK) formed by intramolecular cyclization of the 15-OH substituent onto the 8-carbonyl.[19, 21] The structures of the two compounds are shown in Figure 7. Signals due to the hemiketal component were seen regardless of solvent, although because of the close structural similarity the majority of signals that arise from DON-HK are overlaid by corresponding signals from the parent DON. Three signals due to DON-HK that are resolved from the corresponding DON signal are the H-7, H-10 and H-16 protons, of which the DON-HK H-10 signal is the most cleanly resolved from the corresponding signal in native DON and was found to be the most suitable for the quantification of the amount of DON-HK present. Its occurs at δ = 5.4 ppm , upfield from H-10 in DON at δ = 6.5 ppm and well separated from other resonances. The observed ratio of DON: DON-HK based on the relative area ratio of the two signals varies from sample to sample and with solvent and increases with the polarity of the solvent.[19,21] It is also influenced by the presence of trace amounts of water and acid in the solvent, which catalyse the cyclization process. When using acetone-d_6 as solvent the ratio observed at the BIPM was typically in the range 95:5 to 92:8. A quantification of the amounts of each was obtained by a qNMR experiment using conditions that allowed for recovery of each quantitative signal between excitation pulses.

4.3.7 **Residual solvent content by NMR**

The ^{1}H NMR spectrum of the material was examined for signals due to residual solvent.[25] No significant signals arising from the presence of residual solvent were observed in the material.

4.3.8 **UV-Vis spectrophotometry**

Scan and fixed wavelength UV-VIS measurements of solutions of DON in acetonitrile were undertaken in absorbance mode:

Scan mode:

- Deuterium lamp: on
- Tungsten lamp: on
- Scan from 370 nm to 190 nm
- Data interval: 1.00 nm, scan speed: 267 nm/min
- Slit: 2 nm

Fixed wavelength:

- Deuterium lamp: on
- Tungsten lamp: on
- Wavelengths: 218 nm, 228 nm and 274 nm
- Slit: 1 nm
- Response 0.2s

Micro-cuvettes containing a minimum volume of 50 µl of solution were used. The reference cell contained spectroscopic grade acetonitrile. Autozero was performed at the beginning of the method using pure solvent in the sample cuvette. Three measurements were acquired and averaged for each sample replicate. Temperature was controlled and fixed at 20 °C. A representative UV spectrum for a solution with DON content of 6 µg/g in acetonitrile[26] is reproduced in Figure 8.

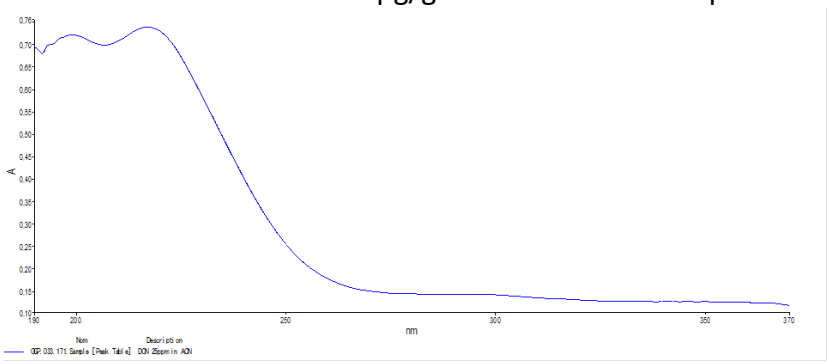


Figure 8: UV-VIS spectrum obtained for DON (ca 6 µg/g) in acetonitrile.

4.3.9 **Mass spectrometry**

Reference MS data for DON are available by searching under the entry for "deoxynivalenol" from open access databases including the European Mass Bank, the Mass Bank of North America and PubChem. From studies undertaken at the BIPM, the MS parameters of DON and a number of related structure materials in a negative electrospray ionization mode were optimized by direct infusion of single LC standards of DON, NIV, 3-ADON, 15-ADON and DOM-1.[a]

Using our spectrometer, an optimized intensity was obtained at ionspray voltage of -4500 V, source temperature of 700 °C with nitrogen as the ion source gas, curtain gas and collision gas. The optimal pressures for Gas 1 and Gas 2 of the ion source were 50 psi and 70 psi. For curtain gas (CUR) the pressure was set at 20 psi. For the collision gas (CAD) the "Mid" setting was used. The declustering potential (DP) was set at -47 V. The entrance potential (EP) was set at -5 V. The collision cell exit potential (CXP) was set at -9 V. Table 4 summarizes the optimized transitions and conditions for MRM detection and quantification of DON and its structurally related impurities under these conditions.

[a] Structures and abbreviations of DON-related tricothecenes are given in Figure 2 and Annex 7.1

Table 4 DON and DON-impurity MRM parameters

Component	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	CE (volt)
DON	295.1	265.1	50	-17
DON	295.1	247.1	50	-18
NIV	311.1	281.1	50	-17
NIV	311.1	191.0	50	-28
3-ADON	337.1	307.1	50	-17
3-ADON	337.1	173.0	50	-16
15-ADON	337.1	150.0	50	-22
15-ADON	337.1	219.1	50	-16
DOM-1	279.1	249.1	50	-17
DOM-1	279.1	231.1	50	-25
norDON	265.1	247.1	50	-22
norDON	265.1	235.1	50	-23

Figure 9 shows the total ion chromatogram (TIC) by LC-MS/MS for a standard mixture of DON, NIV, DOM-1, 3-ADON and 15-ADON at a mass fraction of approximate 270 ng·g^{-1} for each component using these MS/MS parameters. The LC method used is described in Section 5.3.3.

The performance characteristics (linearity, repeatability, limit of detection, intermediate precision, etc.) of the LC-MS/MS method were assessed for use for the quantification of DON and related structure impurities as found in the BIPM OGO.179 material. Calibration curves of NIV, DOM-1 and 3-ADON were established using standard solutions prepared from available pure standards of each material. A commercially supplied standard solution of 15-ADON was also used for this purpose.

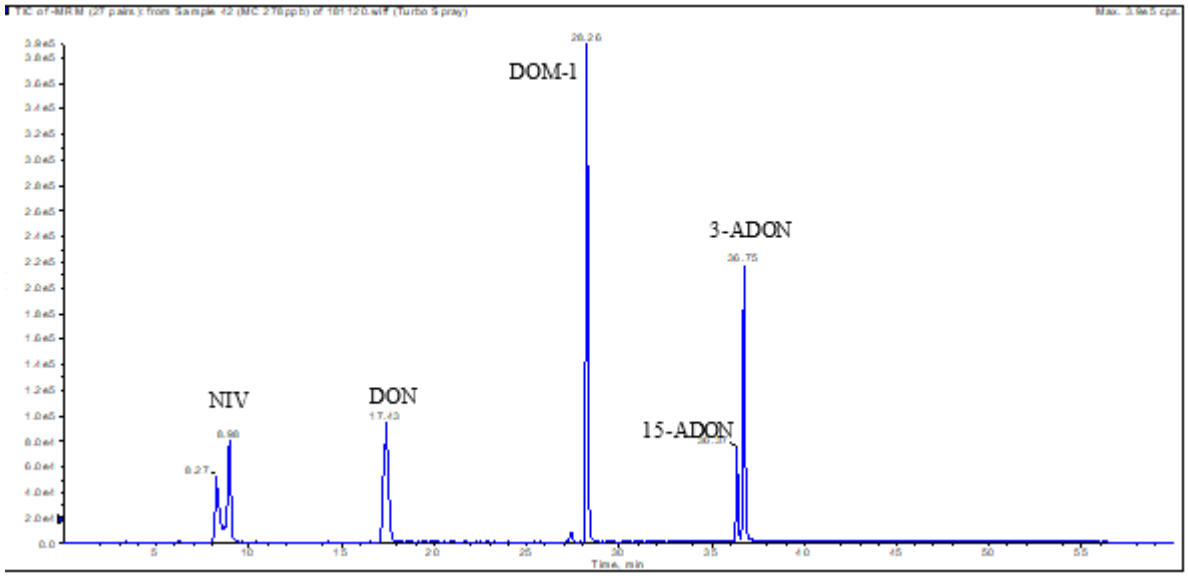


Figure 9 TIC of tricothecene mixture standard solution in acetonitrile (each 270 ng/g) in LC-MRM mode

5. Purity of Deoxynivalenol

5.1 Introduction

The general approach developed during the BIPM MMCBKT programme for the purity assignment of mycotoxins was applied to the DON source material. A quantitative NMR (qNMR) measurement[27,28] was used to assign the combined value for the mass fraction in the material of DON and related impurities containing a signal at the chemical shift of H-10 of DON. This value was corrected for contributions due to the DON-related impurity content quantified separately by LC-UV. The related structure impurities were quantified using the LC-DAD method and the assignments were checked using an LC-MS/MS based on the ionization parameters assigned in Section 4.3.9. This approach has the advantage of requiring a significantly smaller amount of the hard-to-obtain and toxic source material than would be required for a conventional mass balance purity assignment.

The identity of the primary component in the material had been established as DON using the combination of 1D- and 2D-NMR techniques described in Section 4.3.1 – 4.3.5 above.[22, 29] A check for residual solvent impurity content in the material was also obtained by NMR. This identification was supported by determination of the UV-Vis spectrophotometric (Section 4.3.7) and mass spectrometric properties (Section 4.3.8) of the material, which were consistent with reported values.

The initial assignment of the DON content of OGO.179 by qNMR, uncorrected for contributions from related structure impurities, is described in Section 5.2. The development and application of methods for the identification and quantification of the DON-related impurity content of the material by LC-UV(DAD) and LC-MS/MS are described in Section 5.3. These results were used to correct the "raw" qNMR value for contributions due to the DON-related impurity content and gave the final assignment of the "true" DON content of the material.

Supporting analyses undertaken to detect other impurity classes are summarized in Section 5.4 and the combination of the data to give the final purity assignment of the material is described in Section 5.5.

DISCLAIMER: Commercial NMR and LC instruments, software, materials and reagents are identified in this document in order to fully describe some procedures. This does not imply a recommendation or endorsement by the BIPM nor does it imply that any of the instruments, equipment and materials identified are necessarily the best available for the purpose.

5.2 qNMR

5.2.1 Materials

Chemicals

- Deoxynivalenol (DON); BIPM Reference OGO. 179a
- Supplier: First Standard, Product No. 1ST7213, Lot ALT601343
- Dimethylterephthalate (DMTP); BIPM Reference OGE.022b was used as the qNMR internal standard[30]. The mass fraction content of DMTP in the material was assigned at the BIPM by qNMR measurements using CRMs as internal standard as 999.3 ± 0.8 mg/g (k = 2).

NMR Solvents:

- Acetone-d_6; BIPM Reference OGS.029

Deuterated acetone was purchased from a commercial supplier and was used without further treatment. NMR tubes were HG-Type: high-grade class, 8 inch, 5 mm diameter rated for use with 600 MHz spectrometers fitted with PE caps.

5.2.2 qNMR Sample preparation

Gravimetric operations were performed using a Mettler Toledo XP2U ultramicrobalance. Prior to all weighing operations the repeatability of the balance was assessed for suitability to the preparation of qNMR samples by repeat mass determinations of an empty weigh boat. The general recommendations for qNMR sample preparation by Yamazaki *et al* [31] were followed.

In the initial study, using deuterated acetone as solvent, three separate samples were prepared for analysis.[22] The individual sample sizes were in the range 2 mg - 4 mg for both the DON material and for the qNMR internal standard used (DMTP). Each sample was separately weighed into an aluminium weighing boat. In order to avoid contact of the solvent with the metal boat the contents of both were transferred into a common glass vial and each emptied boat was reweighed. The amount of DON and DMTP transferred into the common vial was determined by difference and this value was used for qNMR calculations. 1 mL of deuterated solvent was added to the vial and the sample solution was mixed in a vortex shaker and checked visually for completeness of dissolution. Approximately 800 µL of this solution was transferred into an NMR tube (HG-Type: high-grade class, 8 inch, 5 mm o.d., with PE cap) using a glass pasteur pipette.

5.2.3 Choice of solvent and quantification signals

A number of integration areas within DON were used to give independent quantitative NMR (qNMR) results. Due to the complex structure of the DON molecule and the associated minor components visible in the specimen, it was challenging to select a unique, representative signal. The multiplet resonances at 6.55 (H-10), 5.01 ppm (H-11), 3.45 ppm (H-2) and 2.47 ppm (H-4α) were investigated and qNMR purity assignments were made for each resonance as shown in Figure 10. The signals at 5.01, 3.45 and 2.47 ppm were insufficiently resolved from adjacent signals and no satisfactory baseline could be established for a reliable purity assignment. The qNMR assignments in this report are based on the quantification of the signal from the H-10 proton at 6.55 ppm due to DON and the signal at 5.38 ppm due to DON-HK which is formed from DON in solution but is assumed not to be present in the solid material.

DMTP was used as the internal standard since its singlet resonance from the four equivalent aromatic protons at chemical shift 8.1 ppm occurs in a clean region in the DON spectrum. The 90° pulse calibration was established at 5.83 µs and the longest measured T_1 time constant for the quantified peaks was 5.8 seconds for the DMTP aromatic peak. A relaxation delay of 87 seconds between pulses, corresponding to in excess of fifteen times the longest T_1, was applied for quantification studies. The excitation pulse offset was set at 5.8 ppm and a signal window of 20 ppm was used. The experiments were undertaken using ^{13}C-gated decoupling in either ON or OFF mode.

Use of ^{13}C-decoupling simplifies each resonance by removing contributions from ^{13}C satellite peaks, thereby making it easier to establish a reliable baseline for integration.

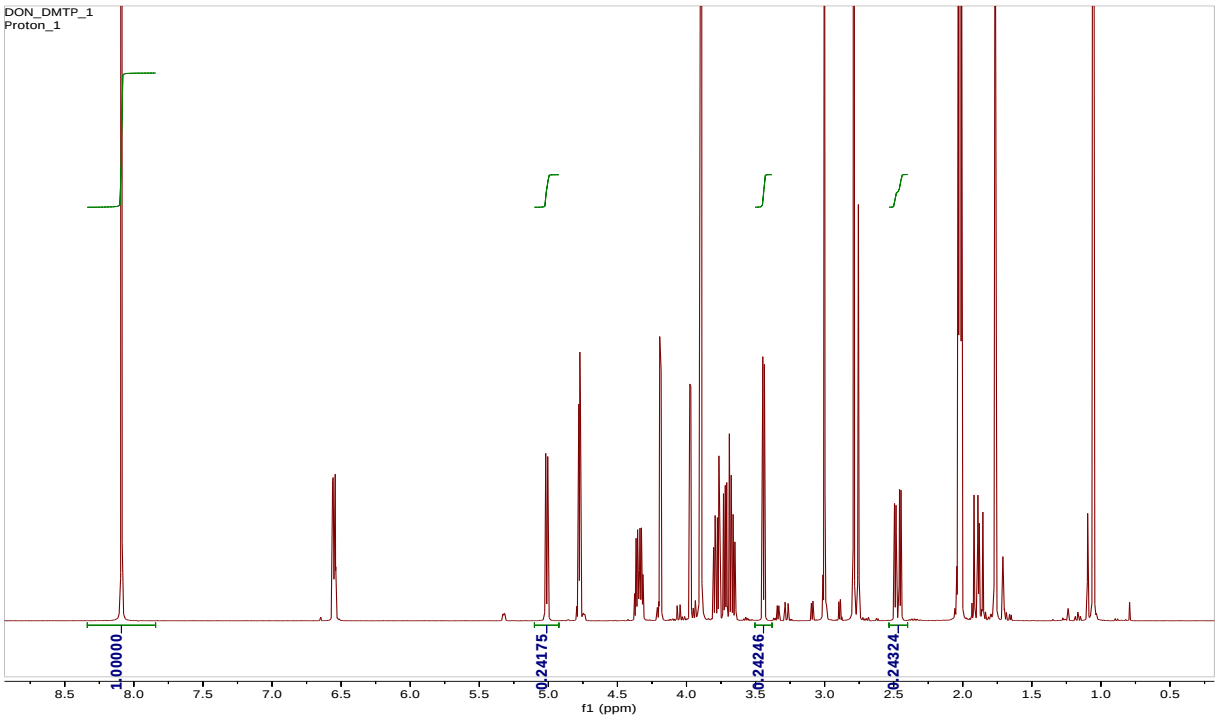


Figure 10 –NMR spectrum of OGO.179a with integrated peaks indicated.
The singlets at 8.1 ppm and 3.8 ppm are from the DMTP internal standard

5.2.4 NMR acquisition parameters

A JEOL ECS-400 spectrometer operating at 9.4 T (400 MHz for proton) equipped with a direct type automatic tuning (Royal) probe operating Delta software was used for all NMR data acquisition.

The general recommendations for optimizing spectrometer performance, determining the relevant NMR experiment parameters and undertaking a qNMR experiment as described in the BIPM Internal Standard Reference Data report for the use of DMTP for qNMR measurements[30] were followed, with the exception that for this assignment the acquisition was carried out with ^{13}C-decoupling activated to eliminate satellite peaks and simplify the integration process. The final qNMR acquisition parameters used for DON are summarized in Table 5.

Parameter	*Value*
DON Sample size (mg)	2.3 – 3.9
DMTP Sample size (mg)	2.0 – 3.1
Number of Transients	64
Receiver gain	40
Acquisition time (s)	4
Relaxation delay (s)	87
Pulse offset (ppm)	5.8
Spectral width (ppm)	20
Data points	39979
Temperature (K)	298
^{13}C-Decoupling	On
Spinning	Off
Integral ratio (DON:DMTP)	0.25 – 0.48

Table 5 - Acquisition parameters for qNMR of DON.

The integration range used start and end points placed fifty Hertz beyond the visible edge of each signal. Results from three independent samples were obtained.

The overlapping signals from the DON-HK and other related structure impurity protons make it a challenge to select the best signal for quantification purposes. The purity assignment was investigated using solutions in acetone-d_6, with and without decoupling of ^{13}C, and against the H-10 and H-2 signals to assess the purity. The results obtained are summarized in Table 6. The values for H-10 in DON and DON-HK obtained using ^{13}C decoupling highlighted below were selected as the best estimates for quantification of DON content.

signal (ppm)	Assignment	Decoupler setting	DON content (mg/g)
6.55	DON H-10	OFF	908.2 ± 2.0
3.44	DON H-2	OFF	902.0 ± 2.1
6.55	DON H-10	ON	920.7 ± 2.4
3.44	DON H-2	ON	918.6 ± 3.4
5.38	DON-HK H-10	ON	57.2 ± 2.1

Table 6: qNMR assignments for DON
Values highlighted in green were used for qNMR assignments

5.2.5 Value assignment and measurement uncertainty

Results from three independent samples, each analysed four times, were obtained. The signals due to DON H-10 (1H, 6.55 ppm) and DON-HK H-10 (1H, 5.38 ppm) were used to quantify the DON and DON-HK present in solution. The combined value for these two assignments was assumed to correspond to the DON content in the solid source material. An example of the measurement uncertainty budget for DON content of three samples analysed in quadruplicate is reproduced in

Table 7. The integral ratio used is the mean of the twelve values. The contributions to the overall standard uncertainty of the assignment are also listed. The standard deviation of the mean of integral ratio, with the ratios for independent sample normalized to take into account their different precise compositions, was taken as the standard uncertainty of the repeatability of the integration ratio. A similar budget was prepared for the DON-HK assignment.

Source of uncertainty	Value	Unit	Type	Standard uncertainty	Sensitivity Coefficient	Relative uncertainty
ANOVA	0.9207	-	A	0.00088	1	8.83E-04
Mass of DON	5.200	mg	B	0.00124	0.17706	2.20E-04
Mass of IS	1.668	mg	B	0.00124	0.55202	6.85E-04
Molar mass of DON	296.3190	g/mol	B	0.01500	0.00311	4.66E-05
Molar mass of IS	194.1860	g/mol	B	0.00900	0.00474	4.27E-05
Natural abundance of DON	0.9999	-	B	0.00004	0.92082	3.72E-05
Natural abundance of IS	0.9999	-	B	0.00004	0.92082	3.72E-05
Mass fraction of IS	0.9993	g/g	B	0.00040	0.92136	3.69E-04
					Combined uncertainty	0.00120
					v_{eff}	10
					k	2
Mass fraction of DON	**0.9207**	**±**	**0.00240**	**g/g**	Expanded uncertainty	0.00240

Table 7: qNMR uncertainty budget for DON

The relative contributions of each component to the combined standard measurement uncertainty are presented in Figure 11*:*

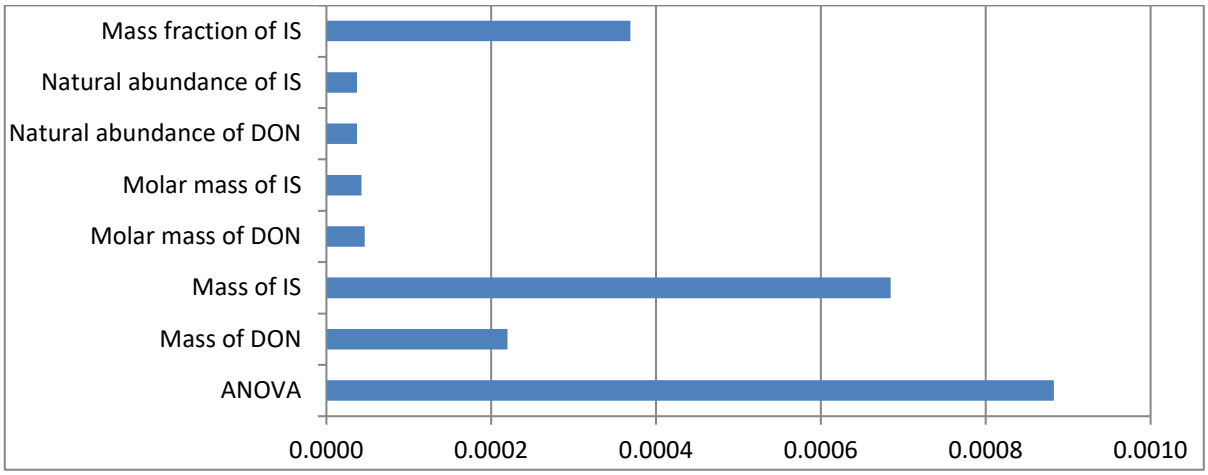


Figure 11 – Relative uncertainty contributions to the overall uncertainty budget for DON purity

Note that at the mass sizes used for the sample preparation the contributions to the overall

uncertainty of the purity assignment from the gravimetric operations and from the purity of the internal standard are similar in magnitude to those due to the precision of the integral ratio determination.

The estimate for the DON plus contributing related structure impurity content of the solid material, determined as the sum of the contributions from DON and DON-HK by qNMR of a solution of the solid material, was 977.9 ± 3.2 mg/g. This value is the sum of the mean of the twelve separate values for DON (920.7 ± 2.4 mg/g) and DON-HK (57.2 ± 2.1 mg). The expanded uncertainty in the combined value is the quadratic combination of the expanded uncertainty of each contributing assignment.

The qNMR assignment process was repeated subsequently by another operator using a single freshly prepared sample, again with acetone-d_6 as solvent.[29] The assignment obtained using this limited qNMR characterization was 980.5 ± 3.6 mg/g, consistent within its assigned uncertainty with the value obtained from the main study.

5.3 LC-DAD and LC-MS/MS

5.3.1 Apparatus

The liquid chromatography (LC) system consisted of an Agilent 1100 series micro vacuum degasser, binary pump, thermostatted standard autosampler, thermostatted column compartment and diode array detector (DAD). An Applied Biosystems 4000 Qtrap hybrid tandem mass spectrometer (MS/MS) was coupled to the LC system employing a Sciex TurboIonSpray (TIS) source and a Valco 10-position valve. A direct flow injection was used for optimization studies.

5.3.2 Materials

Deoxynivalenol (DON). BIPM Reference OGO.179
Nivalenol (NIV), 3-Acetyldeoxynivalenol (3-ADON) De-epoxydeoxynivalenol (DOM-1), Nordeoxynivalenol A (norDON A), 15-Acetyldeoxynivalenol (15-ADON) purchased from First Standard China via NIM (China).

Pure water was obtained from a MilliQ RiOs gradient ultrapure device.

Methanol (MeOH) and Acetonitrile (ACN) were HiPerSolv CHROMANORM from VWR.

5.3.3 HPLC method

As DON is a relatively polar compound, it could be separated from related impurities using a reverse phase column and a mobile phase consisting of organic solvent at a low concentration. If DON is dissolved in pure organic solvent the large polarity difference between solvent and mobile phase can affect chromatographic retention behaviour. Different injection volumes were compared. If dissolved in pure acetonitrile only, a maximum injection volume of 2 µL could be used to obtain normal peak shapes. For a DON solution in acetonitrile/water (50/50, v/v), an injection volume of up to 5 µL could be used. For a DON solution in acetonitrile/water (20/80, v/v) an injection of up to 10 µL still resulted in good separation and sharp, resolved peaks. A DON solution in acetonitrile/water (20/80, v/v) was used in LC-MS/MS method development and 10 µL was injected to obtain better and higher sensitivity signals. Our experience indicates that DON is not very stable in water, undergoing a

number of the transformations listed previously (see Figure 2, p. 6). For the quantification of impurities, pure acetonitrile was used for dilutions of the standard solution and a smaller injection volume of 2 µL was chosen. Figure 12 shows the TIC of the impurity standard solution when analysed in MRM mode.

An LC-DAD-MS/MS method was developed and validated for the detection and quantitative determination of DON and the four DON related structure impurities listed above (DOM-1, 3-ADON, 15-ADON, NIV). The method was validated for the usual performance characteristics (linearity, repeatability, limits of detection, intermediate precision, etc.) and was assessed for the quantification of DON and its main related structure impurities. Calibration curves of DON, DOM-1, 3-ADON, 15-ADON and NIV were constructed using standard solutions prepared from pure materials (DON, NIV, 3- and 15-ADON) or using a commercially purchased standard solution (DOM-1). A multi-component calibrant mixture containing gravimetrically defined amounts of each compound was also prepared. A DON single calibrant was prepared separately to obtain performance characteristics and for use as a calibration standard to quantify related-structure unidentified impurities. Chromatographic separation was performed at 25 °C using a Kinetex EVO C18 100Å, (250 x 4.6 mm, 2.6 µm) column.

The chromatographic conditions used for the separation of the compounds were:

Column:	Phenomenex Kinetex EVO C_{18} 100Å,(250 × 4.6 mm, 2.6 µm)	
Column temperature:	30 °C	
Detector 1:	DAD 219, 229 and 279 nm, ref. wavelength 400 nm.	
Detector 2:	MS/MS (see above)	
Mobile phase:	A) H_2O Milli Q B) Acetonitrile	
Operation mode:	Gradient	
Solvent gradient:	Time (min)	Mobile phase B content (% vol.)
	0	6.5
	17	6.5
	27	20
	35	20
	45	90
	50	90
	51	6.5
	60	6.5
Flow rate:	0.6 mL/min	
Injection volume:	2 µL or 10 µL	

5.3.4 MS/MS parameters

The optimized MRM detection parameters used are described in section 4.3.9

5.3.5 LC-DAD-MS/MS – standard solution

The HPLC-DAD(UV) chromatogram of a mixture in acetonitrile/water (20/80, v/v) at a mass fraction of ca 6 µg/g with detection at 219 nm is shown in Figure 12. Note that 15-ADON was not included in this mixture. The small peak eluting to the left of DOM-1 is an artefact.

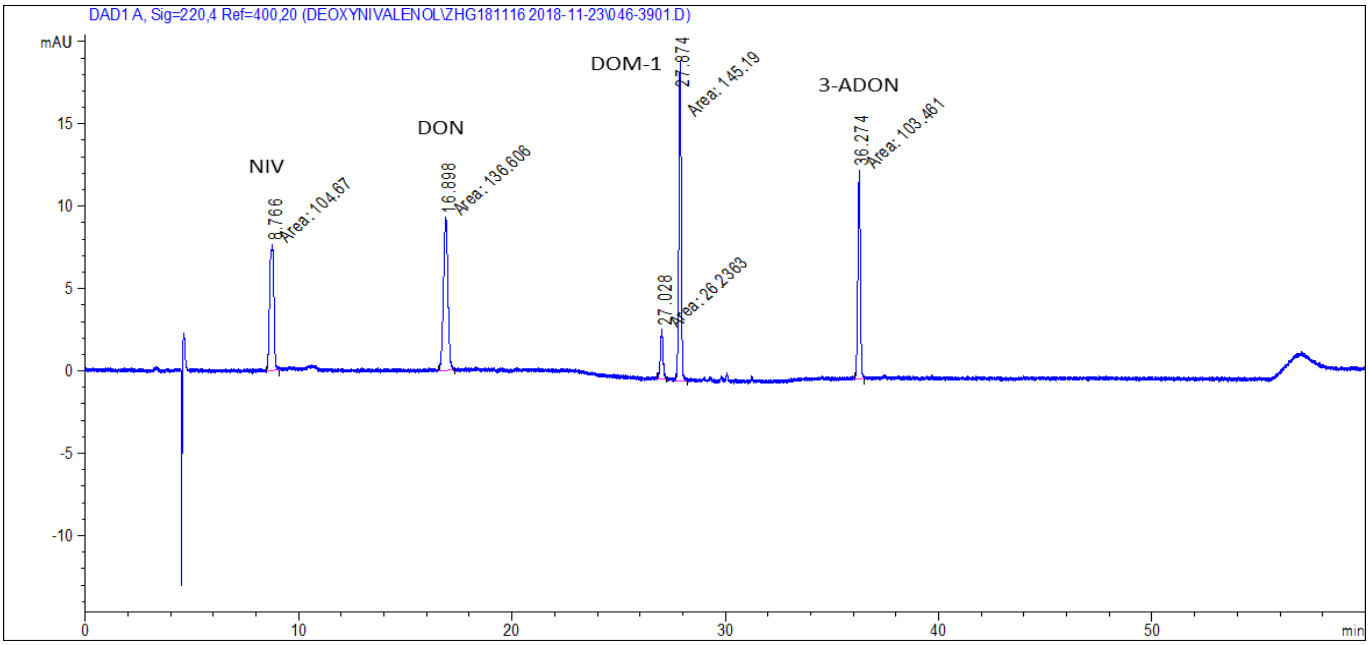


Figure 12 HPLC-DAD chromatogram (λ = 219 nm) of DON standard mixture at 6 µg/g

The LC-MS/MS spectrum of the DON standard mixture in solution at 270 ng/g and including 15-ADON was shown in Figure 9 in Section 4.3.9.

5.3.6 LC-DAD-MS/MS results – OGO.179

Using the HPLC-DAD-MS/MS analysis described in Section 5.3.3 above with UV detection at 219 nm and MS/MS detection using the EMS-IDA-EPI ionization mode, analysis of a solution of the OGO.179 material identified eighteen impurities present in the DON material [RS2] in addition to the primary component.

For identification of impurities a solution containing approximate 1 mg/mL of the DON material in acetonitrile was prepared. To avoid contamination of the sensitive LC-MS/MS instrument by the high content of DON component, after passing through the DAD detector the mobile phase was diverted to the waste position during elution of the major component (DON) to allow for improved detection and analysis of the impurities in the DON material. Figure 13 shows the chromatogram obtained for this solution by UV detection at 219 nm. Figure 14 shows the TIC obtained with the same solution in the EMS-IDA-EPI mode. There are nine components, marked impurity 1, 2, 3 (3a and 3b), 4, 5, 6, 7 (7a and 7b) and 8, visible in the TIC. Based on their EMS and EPI mass and UV absorbance spectra, structures for seven of the impurities (1-5 and 8) were proposed. Impurities 1 and 3a were identified as isomers of NIV. Impurity 2 was identified as an isomer of DON. Impurity 3b was identified as 7-deoxy-DON. Impurity 4 was identified as norDON because it exhibited the reported transitions for authentic material. Impurity 5 and Impurity 8 were identified respectively as DOM-1 and 3-ADON by comparison with authentic materials. Impurity 6 and 7b exhibit transitions for 338.1-308.1 and 339.1-249.1 respectively, however structures consistent with these transitions were not identified. Impurities 1 and 2 were not detectable by DAD but could be detected by MS, indicating that these two impurities lack a UV-active chromophore. The peak at retention time of

32.1 min due to impurity 7a in the UV chromatogram of the DON stock solution was not detected by LC-EMS-IDA-EPI. This unidentified compound was not included in the impurity quantification using the LC-MS/MS method. The sensitivity of MS/MS allowed a further ten minor impurities to be detected by use of the LC-MRM method but their combined mass fraction content was too small to require further study.

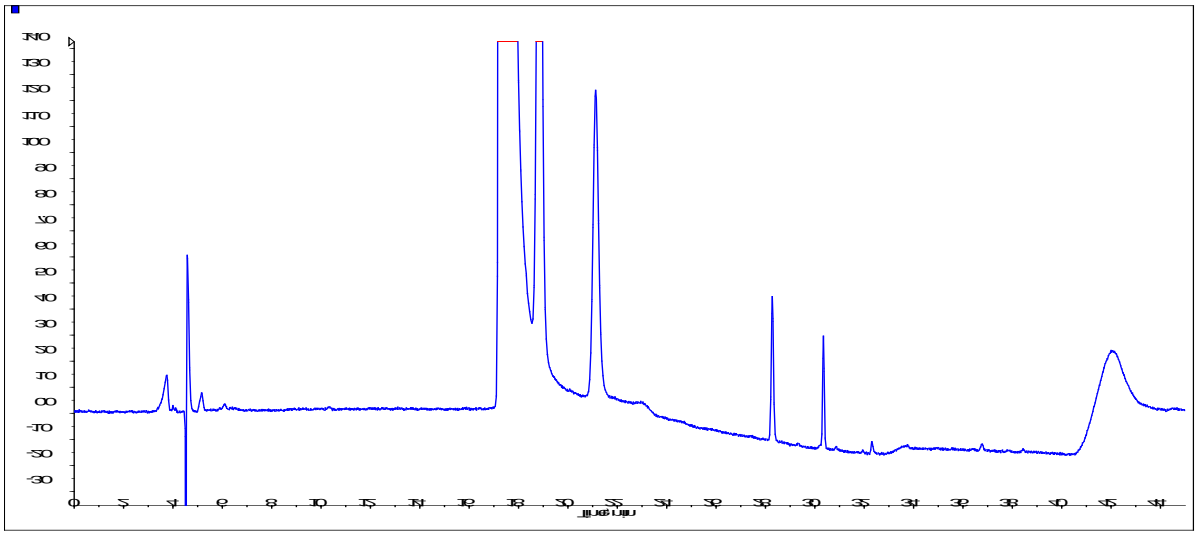


Figure 13 Chromatogram with UV detection at 219 nm of DON stock solution in ACN/H_2O (20/80, v/v)

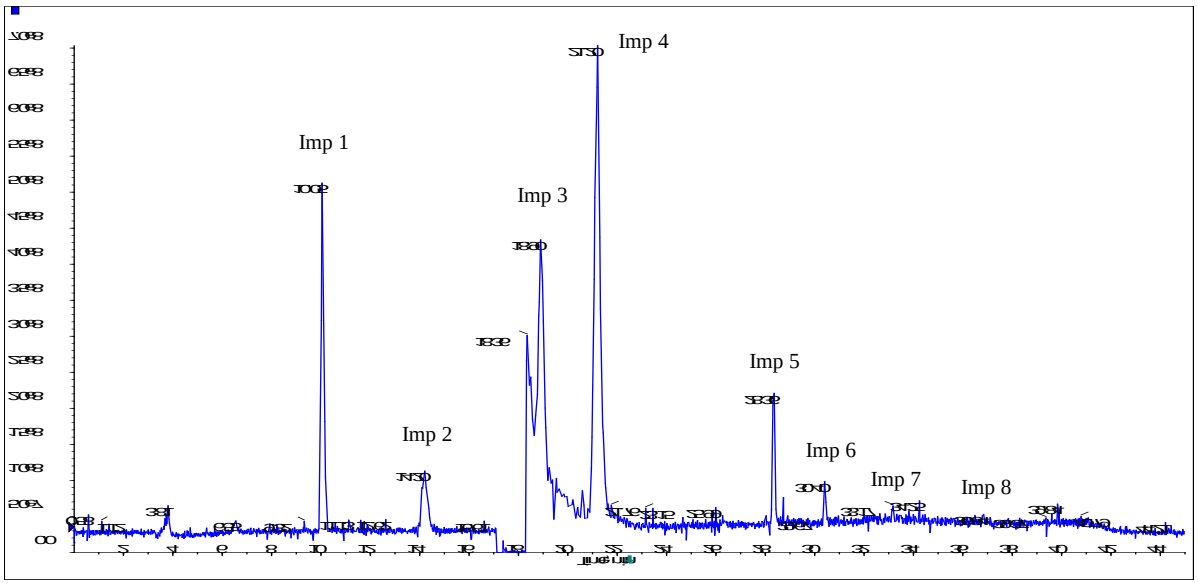


Figure 14 TIC of DON stock solution in ACN/H_2O (20/80, v/v)
(1 mg/mL, 20 °C) in LC-EMS-IDA-EPI mode. Main DON peak diverted to waste.

A summary of parent and daughter ions of all impurities detected in the LC-MS/MS analysis of related structure impurities of the DON material OGO.179 are given in Table 8.

Table 8 Assignment of impurities in OGO.179 solution in ACN by LC-MS/MS (injection volume 2 µL)

Compound	RT (min)	Q1 m/z	Q3 m/z
NIV isomer (Imp 1)	10.1	311.1	281.1/191.0
DON isomer (Imp 2)	14.2	295.1	265.1/247.1
NIV isomer (Imp 3a)	18.9	311.1	281.1/191.0
7-deoxy-DON (Imp 3b)	18.9	279.1	249.1/231.1
norDON A (Imp 4)	21.2	265.1	247.1/235.1
DOM-1 (Imp 5)	28.3	279.1	249.1/231.1
Imp 6	30.4	308.1	290.1
Imp 7a/b	33.2	339.1	249.1
3-ADON (Imp 8)	36.8	337.1	307.1/173.0
Imp 9	24.5	265.1	217.1
Imp 10	25.1	337.1	307.1/173.0
Imp 11	27.5	269.1	137.0
Imp 12	29.3	337.1	307.1/173.0
Imp 13	29.4	265.1	217.1
Imp 14	30.9	293.1	247.1
Imp 15	31.2	339.1	249.1
Imp 16	32.0	337.1	150.0/219.1
15-ADON (Imp 17)	36.4	337.1	150.0/219.1
norDON C (Imp 18)	36.6	265.1	247.1/235.1

The absolute LODs calculated were 0.95 $ng \cdot g^{-1}$ for DON, 1.02 $ng \cdot g^{-1}$ for NIV, 0.50 $ng \cdot g^{-1}$ for DOM-1, 0.77 $ng \cdot g^{-1}$ for 3-ADON and 1.10 $ng \cdot g^{-1}$ for 15-ADON. The quantitative analyses of NIV isomer-1 and NIV isomer-2 (impurities 1 and 3a) used the NIV calibration curves as standard material was not available and they exhibited the same mass transitions. However the result was not used for quantification since the peak intensities were different and there was a major discrepancy with the value assigned by LC-UV. The DON isomer (impurity 2) was quantified using DON standard solutions. 7-deoxy-DON and DOM-1 were quantified using the DOM-1 standard solutions. The quantitative analysis of norDON used the DON, DOM-1 and 3-ADON calibration curves and the mean since no authentic material was available and the mass transitions were different. Impurity 6, 7a and 3-ADON were quantified using the 3-ADON standard solution. The assigned impurity mass fraction values and corresponding expanded measurement uncertainties for the content of each material in OGO.179 are summarized in Table 9. The LC-MS/MS results of NIV isomer-1, NIV isomer-2 and impurity 6 were lower than the values obtained by LC-DAD for these components.

The mass fraction values of each impurity component estimated by LC-DAD and separately by LC-MS/MS are summarized in Table 9. The assigned values for each impurity used for the estimation of the DON content by the mass balance method, with each associated expanded uncertainty, are also shown. The assigned value corresponded to the LC-DAD value for Impurities 1, 6 and 7a. The LC-MS/MS value was used for impurities 7b and minor impurities 9 to 18, all of which were not detected

by LC-DAD. For impurity 2, norDON (Impurity 4), DOM-1 (Impurity 5) and 3-ADON (impurity 8) the mean of the LC-DAD and LC-MS/MS assignments was used.

The main impurity peak 3 was assigned as predominantly NIV-isomer 2 but also containing a small amount of 7-deoxy-DON detected by LC-MS/MS. There was a large deviation between the quantitative LC-UV and LC-MS/MS assignments in this case. The LC-UV value was selected as this lead to a final value for DON content consistent with the independent qNMR result.

Table 9: Related structure impurity mass fractions (in $mg \cdot g^{-1}$) for impurities present in the DON material with associated expanded uncertainties by HPLC-DAD-CAD and LC-MS/MS.

Impurity	Mass fraction (mg/g)			*U* (mg/g) (*k*=2)
	LC-DAD	LC-MS/MS	Assigned	
Imp 1: NIV isomer	1.07	0.16	1.07	0.09
Imp 2: DON isomer	0.62	0.56	0.59	0.11
Imp 3a: NIV isomer	12.52	1.23	12.52	1.12
Imp 3b: 7-deoxy-DON		0.16		
Imp 4: norDON	4.00	3.25	3.63	1.30
Imp 5: DOM-1	0.66	0.69	0.67	0.14
Imp 6: Unknown	0.45	0.04	0.45	0.04
Imp 7a: Unknown (LC-UV)	0.08	/	0.08	0.01
Imp 7b: Unknown (LC-MS)	/	0.03	0.03	0.01
Imp 8: 3-ADON	0.05	0.05	0.05	0.01
Imp 9 - Imp 18: Minor impurities	/	0.03	0.03	0.01
SUM	19.45	6.20	19.11	2.84

5.4 **Water**

Water content measurements by coulometric Karl Fischer titration were carried out on the OGO.179a DON material. The challenges with handling DON in solid form due to its toxicity and fears of contaminating equipment meant that a method based on the use of a heated oven to release water from the material was precluded. A protocol was implemented to avoid exposure to the material in powder form as much as possible.

Individual samples were weighed into a tared GC vial and the vial was sealed as soon as the gravimetric measurement was completed. Solvent (anhydrous acetonitrile, purity > 99.9 %) was added via syringe into the sealed vial immediately prior to the measurement and the vial was weighed. After dissolution of the DON solid, the bulk of the resulting solution was withdrawn by syringe and injected directly into the coulometric titration cell containing the KFT reagent. By measuring the mass changes of the vial and the syringe before and after transfer of the DON solution it was possible to calculate the mass of DON introduced into the titration cell.

"Blank" measurements obtained by injection of solvent only established the level of water introduced in the injection process. A reference material solution with nominal content 103 ± 3 µg/g water in hexane was used to validate the sensitivity of the measurement process.

The injection of five separate samples of DON in solution in acetonitrile, each containing ca 1 mg of solid in ca 200 mg of solvent, gave results that were not statistically different from the results obtained using blank solvent. On the basis of these results it was determined that the material did not contain a quantifiable level (< 0.1 mg/g) of water.

5.5 Purity assignment

The direct qNMR value for the DON mass fraction in the material was estimated as described in 5.2.5 at 977.9 ± 3.0 mg/g. This value was corrected for the total contributions from the related structure impurities present in the material, isoDON, DOM-1 and 3-ADON, 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 content of OGO.179 by qNMR of 976.3 ± 3.1 mg/g.

The mass balance value for the DON content 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.

Secondary component	Mass fraction (mg/g)	Measurement Method	Verification Method
DON related impurities	19.45 ± 2.84	LC-UV	LC-MS/MS
VOCs	-	qNMR	
Water	-	KFT	

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

The two purity estimates are plotted in Figure 15. 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 content of DON in the OGO.179.a 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 analysed at the BIPM and used subsequently in the CCQM-K154.c comparison was 978.6 ± 9.2 mg/g.[32]

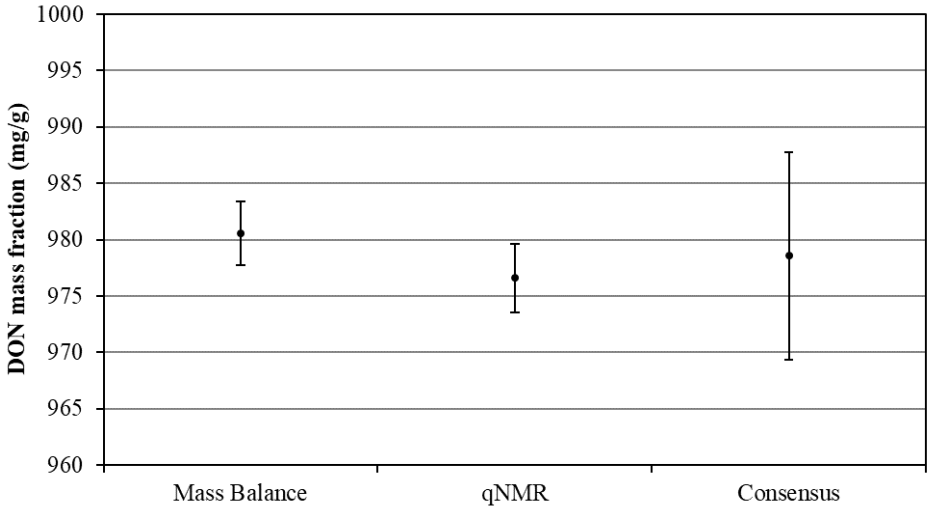


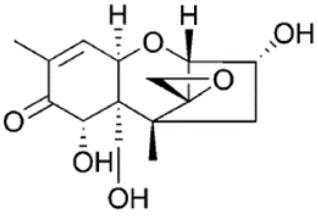
Figure 15 Estimates for DON content in BIPM source material OGO.179

6. Acknowledgements

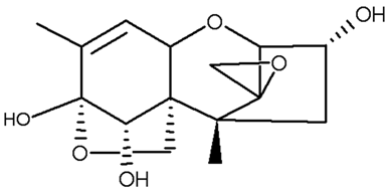
All NMR and LC studies were carried out by the co-authors of this document in the course of secondments at the BIPM. The support of the parent institution for each scientist to the BIPM work programme is gratefully acknowledged. The authors would like to thank the National Key R&D Program of China for funding support (No. 2016YFE026500).

7. Annexes

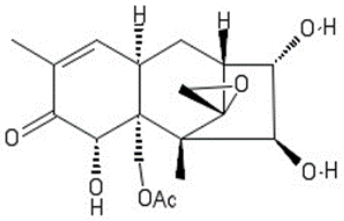
7.1 Chemical structures of deoxynivalenol and related compounds



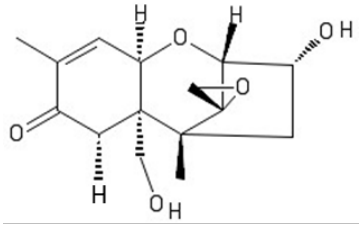
4-Deoxynivalenol (DON)



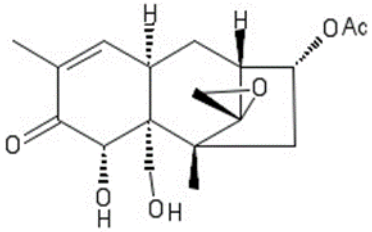
Deoxynivalenol hemiketal (DON-HK)



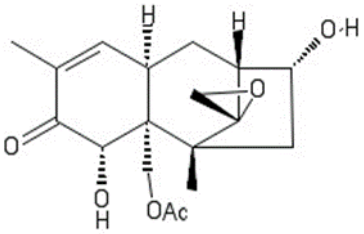
Nivalenol (NIV)



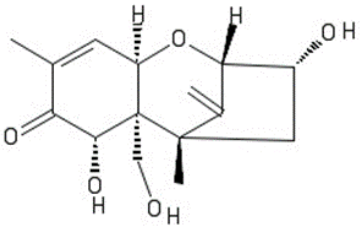
4,7-Dideoxy NIV (= 7-deoxy-DON)



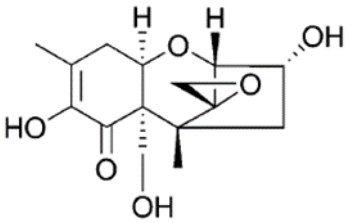
3-Acetyldeoxynivalenol (3-ADON)



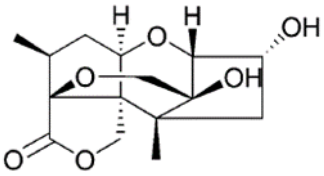
15-Acetyldeoxynivalenol (15-ADON)



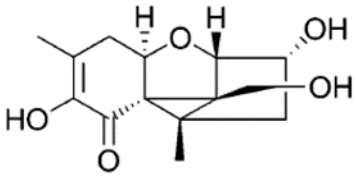
De-epoxy-deoxynivalenol (DOM-1)



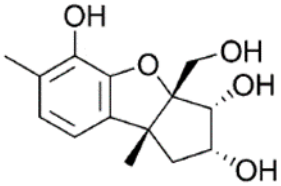
Iso-DON



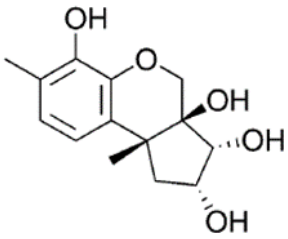
DON lactone



norDON-A



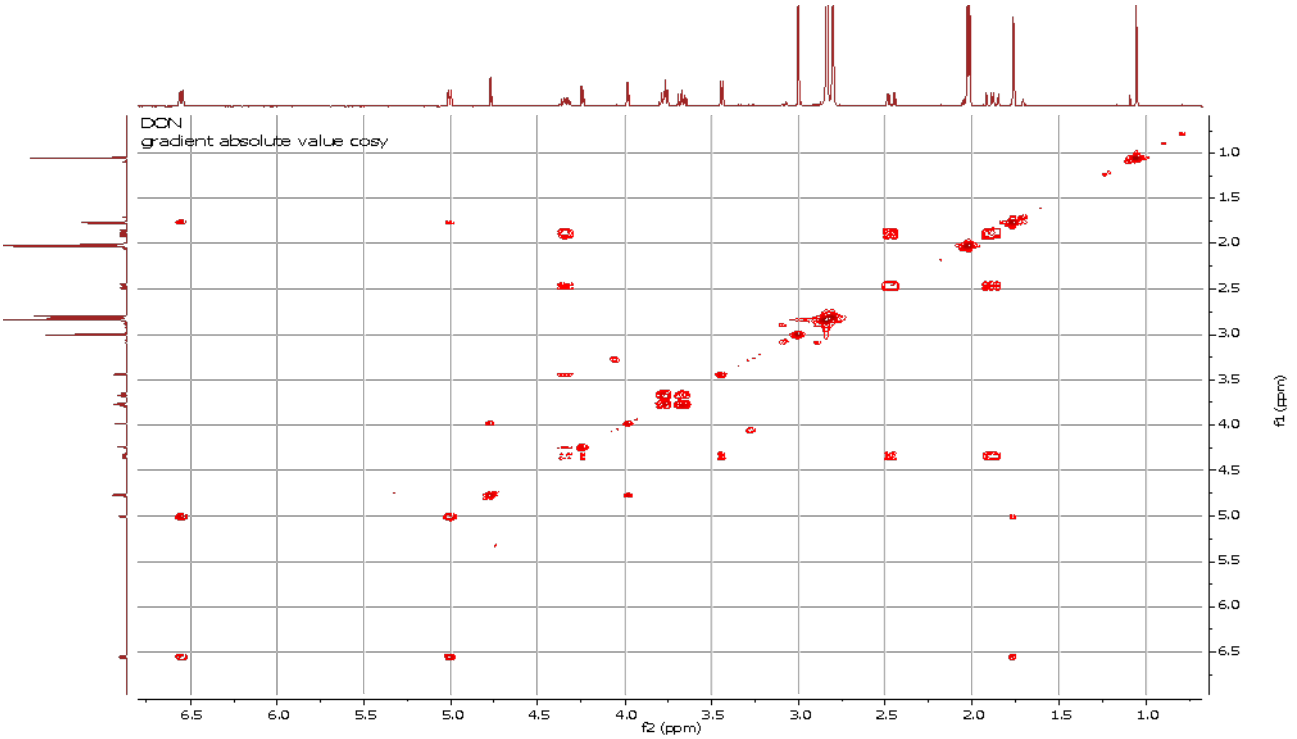
norDON-B



norDON-C

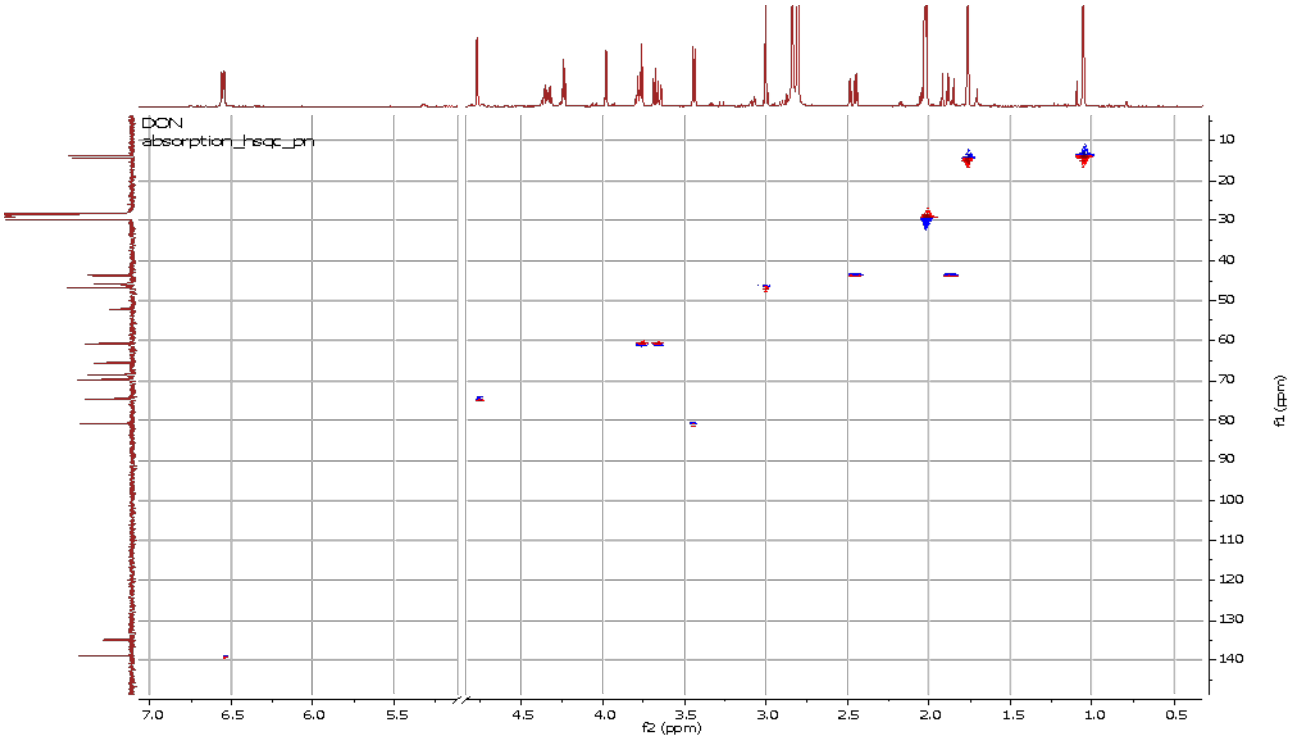
7.2 2D-NMR spectra of DON

7.2.1 COSY



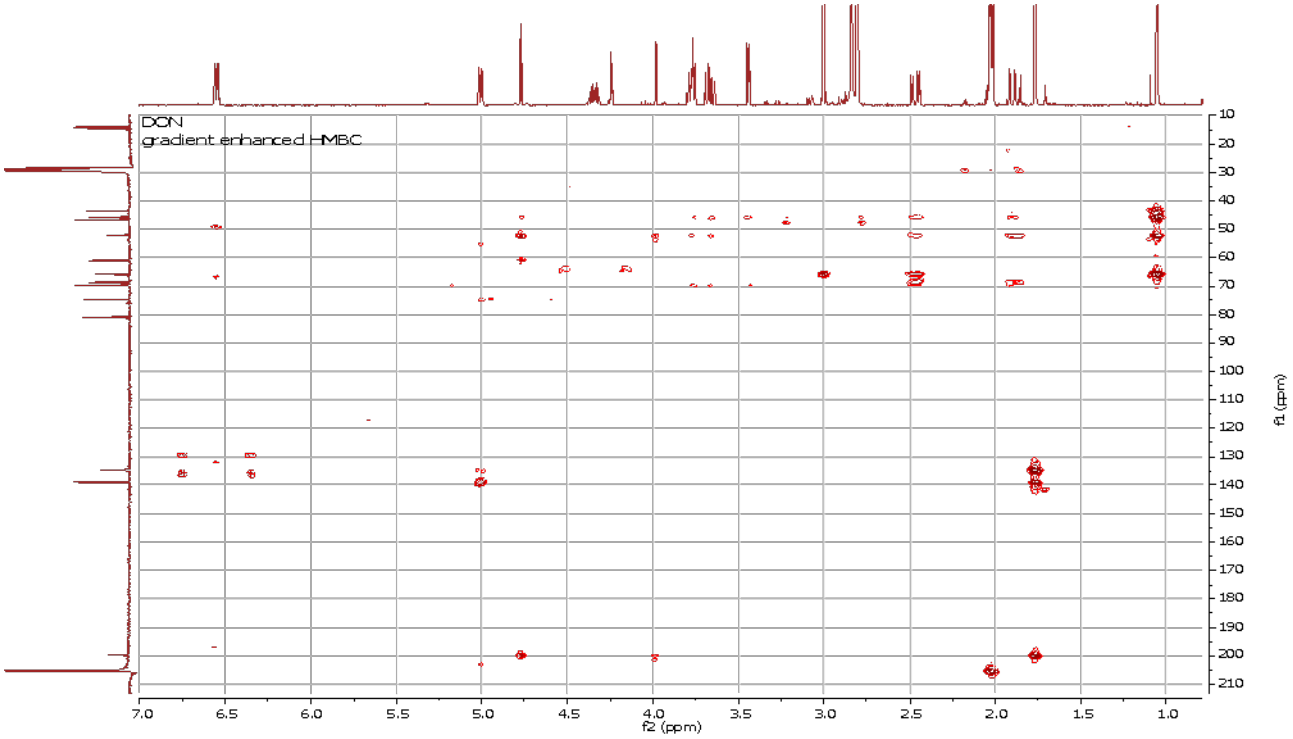
COSY spectrum of DON

7.2.2 HSQC



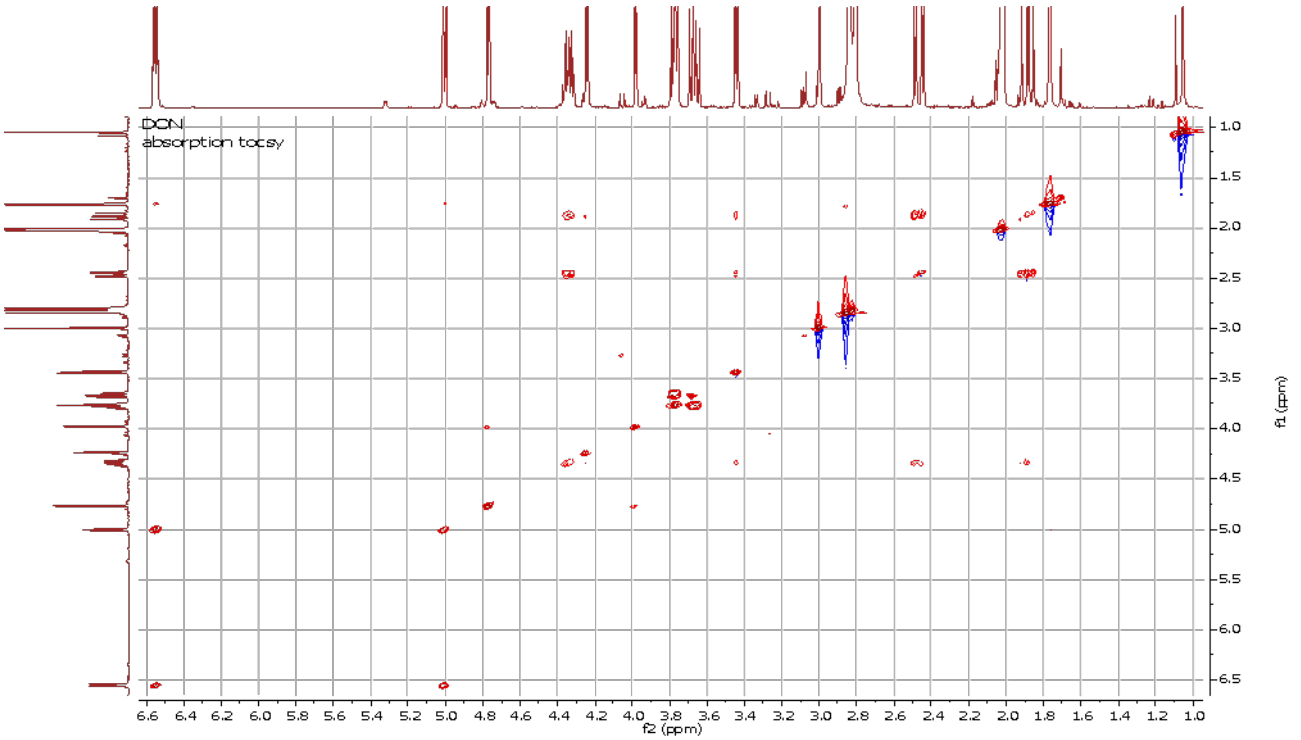
HSQC spectrum of DON

7.2.3 HMBC



HMBC spectrum of DON

7.2.4 TOCSY



TOCSY spectrum of DON

NOESY spectrum of DON in $CDCl_3$

8. References

[1] PRM definition in ISO 1511:2003; Measurement of quantities in biological samples -- Metrological traceability of values assigned to calibrators and control materials

[2] BIPM CBKT programme: Safe Food and Feed in Developing Economies

[3] Cole, R. J., and Cox, R. H. (1981). The Trichothecenes Handbook of toxic fungal metabolites (pp. 152-263). Toronto, Ontario, Canada: Academic Press.

[4] Vesonder, R. F., & Hesseltine, C. W.; *Process Biochem.*, 1980, **60**, 12-44

[5] Alexander, N. J., McCormick, S. P., and Ziegenhorn, S. L.; *Natural Toxins*, 1999, **7**, 265-269

[6] Sobrova P., Adam V, Vasatkova A., Beklova M., Zeman L.; *Interdisciplinary Toxicology.* 2010, **3**, 94–99

[7] https://en.wikipedia.org/wiki/Vomitoxin

[8] Commission Regulation (EC) No. 1881/2006 of 19 December 2006.

[9] JRC-Mycotoxin Factsheet, 2nd Ed. (2012)

[10] De Bièvre, P., Dybkaer, R., Fajgelj, A. and Hibbert, D.; *Pure Appl. Chem*., 2011, **83**, 1873–1935

[11] Jarvis, B. B. et al; *J. Org. Chem*. 1990, **50**, 3660 - 3662

[12] Ellis, W.O. et al; *Crit. Rev. Food Sci. Nutr*., 1991, **30**, 403–439

[13] Hussein, H.S. and Brasel, J.M.; *Toxicology*, 2001, **167**, 101-134

[14] Savard, M. E., Blackwell, B. A., & Greenhalgh, R.; Can. J. Chem., 1987, **65**, 2254-2262

[15] Krska, R. et al; *Anal. Bioanal. Chem.,* 2007, **388**, 1215-1226

[16] CRC Handbook of Chemistry and Physics. 91st ed.: CRC Press Inc., 2010-2011

[17] O'Neil, M.J. (ed.). *The Merck Index - Merck and Co., Inc*., 2006, p 1727

[18] Josephs, R.D. et al; *Toxicol. Let*., 2004, **153**, 123-132.

[19] Shank, R.A.; Ph.D. Thesis - Conformational Analysis of Tricothecenes; University of Lethbridge, 2010

[20] Krska R., Szente E., *J. AOAC Int*. 2004, **87**, 909-919

[21] Bahadoor, A. et al; *J. Agric. Food Chem.* 2022*,* **70***,* 2733-2740

[22] Ilker Un (UME, Turkey), BIPM Internal report CHEM-INT-2018-016

[23] Blunden, G. et al; *Magn. Reson. Chem.*, 1988, **26**, 162-166

[24] Nakanishi, Koji, ed. *1-D and 2-D NMR Spectra by Modern Pulse Techniques.* (1990)

[25] Gottleib, H. Et al; *J. Org. Chem.* 1997, **62**, 7512–7515

[26] Zhen Guo (NIM, China) BIPM Internal report CHEM-INT-2019-032

[27] Holzgrabe, U. (ed); *NMR Spectroscopy in Pharmaceutical Analysis*, Elsevier, 2008

[28] Bharti, S.; Roy, R.; *Trends Anal. Chem*., 2012, **35**, 5-26

[29] Leandro Santos (INTI, Argentina), BIPM Internal report CHEM-INT-2019-010

[30] Rapport BIPM-2019/1 : qNMR Internal Standard Reference Data for Dimethyl Terephthalate

[31] Yamazaki, T. ; Nakamura, S. ; Saito, T.; *Metrologia*, 2017, **54**, 224

[32] Calculated using the NIST Consensus Builder app (https://consensus.nist.gov/app/nicob)