CERTIFICATE OF ANALYSIS

ERM®-BC600

Fusarium mycotoxins in wheat flour		
Compound [1]	Certified value [2]	Uncertainty [3]
	Mass fraction in µg kg^{-1}	
Deoxynivalenol (DON)	102	11
Nivalenol (NIV)	1000	130
Zearalenone (ZON)	90	8

[1] DON, NIV and ZON as measured by using appropriate sample preparation techniques (e.g. solvent extraction, clean-up, derivatisation), instrumental separation (HPLC, GC) and detection techniques as specified on page 2 and 3 of this certificate, corrected for extraction efficiency/recovery.

[2] Unweighted mean of accepted mean values, independently obtained in different laboratories using various methods. The certified values are traceable to the SI.

[3] Estimated expanded uncertainty U with a coverage factor of $k = 2$, corresponding to a level of confidence of about 95 %, as defined in the Guide to the expression of uncertainty in measurement (GUM), ISO, 1995. Uncertainty contributions arising from characterisation as well as from homogeneity and stability testing were taken into account.

The certified property values will be valid for 12 months beginning with the dispatch of the material from BAM; this validity may be extended as further evidence of stability becomes available.

The minimum sample intake is 5 g.

NOTE

European Reference Material ERM®-BC600 was produced and certified under the responsibility of BAM Federal Institute for Materials Research and Testing according to the principles laid down in the technical guidelines of the European Reference Materials® co-operation agreement between BAM-LGC-IRMM. Information on these guidelines is available on the Internet (http://www.erm-crm.org).

Accepted as an ERM®, Berlin, 10 February 2011

Date of dispatch:

Signed

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DESCRIPTION OF THE SAMPLE

The intended purpose of reference material ERM®-BC600 is i) validation of analytical procedures for the determination of DON, NIV and ZON in wheat, and ii) quality assurance in the analytical laboratory.
The ERM®-BC600 material is a wheat flour sample from a non-commercial source, not intended for human consumption and naturally contaminated with DON, NIV and ZON.
The wheat kernels were ground (< 1 mm) using a centrifugal mill, homogenised by means of drum-hoop mixing followed by a so-called "cross riffling" procedure. A total number of 256 units were bottled in 250 mL amber glass bottles containing (81 ± 1) g sealed with screw caps containing PTFE-inlays, and numbered in the order of leaving the bottling process. The material is stored at BAM at -20 °C until dispatch.
The between-bottle homogeneity was evaluated by analysis of variance (ANOVA) on 10 out of 256 bottles (4 replicate analyses per bottle). Extensive stability tests provided sound evidence for a minimum validity of the certified value as indicated on page 1 of this certificate provided the material is stored according to the instructions given on page 3.

PARTICIPANTS

The following 21 laboratories were involved in the certification study for ERM®-BC600.

Laboratory	City / Country	Compound		
		DON	NIV	ZON
Agentur für Gesundheit und Ernährungssicherheit	Linz, Austria	x	x	x
Bundesanstalt für Materialforschung und -prüfung	Berlin, Germany	x	x	x
Chemisches und Veterinäruntersuchungsamt	Sigmaringen, Germany	x	x	x
Chemisches Untersuchungsinstitut der Stadt Leverkusen	Leverkusen, Germany	x	x	x
Eurofins / Wiertz-Eggert-Jörissen	Hamburg, Germany	x	x	x
Food GmbH	Jena, Germany	x	x	x
Friedrich-Löffler-Institut	Braunschweig, Germany	x		x
General Chemical State Laboratory	Athen, Greece	x		x
Kantonales Labor	Zurich, Switzerland	x	x	
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen	Dresden, Germany	x	x	x
Landwirtschaftliche Untersuchungs- und Forschungsanstalt Nordrhein-Westfalen	Münster, Germany	x		x
Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer	Speyer, Germany	x		x
Max-Rubner Institut	Detmold, Germany	x		x
Niedersächisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit	Stade, Germany	x		x
Peri Medizinische Analytik	Sindelfingen, Germany	x	x	x
Public Analyst's Laboratory	Dublin, Ireland	x		x
R-Biopharm AG	Darmstadt, Germany	x		x
SGS Germany GmbH	Hamburg, Germany	x	x	x
Staatliches Veterinäruntersuchungsamt	Arnsberg, Germany			x
UIS Umweltinstitut synlab GmbH	Stuttgart, Germany	x	x	x
Zentralinstitut für Ernährungs- und Lebensmittelforschung	München, Germany	x		x

ANALYTICAL METHODS AND RESULTS OF THE CERTIFICATION STUDY

The participants of the interlaboratory comparison study (ILC) applied methods of their own choice with own calibration standards of known purities.
Extraction was mostly performed by shaking or ultrasonication using acetonitrile : water mixtures for all three compounds (e.g. acetonitrile : water = 84:16, v:v) or pure water for DON and NIV, respectively.
Sample preparation, generally including dilution of extract, clean-up and derivatisation steps, was handled in different ways. When a clean-up was applied various kinds of solid phase extraction columns (e.g.

MultiSep®, MycoSep®, Bond Elute®) or immunoaffinity columns (IAC) were applied to purify the extracts for DON, NIV and ZON. In some cases (HPLC-MS/MS using internal standards) a clean-up step was omitted. A derivatisation of DON and NIV was necessary either to achieve sufficient sensitivity by using fluorescence detection (FLD) or to increase the volatility to apply GC analysis. For the latter a silylation of DON / NIV was preferably done. The application of a HPLC-FLD method for DON and NIV involves a post-column derivatisation by thermal treatment with sodium hydroxide followed by a chemical reaction to analyse the derived formaldehyde.
For separation of the purified extract mostly liquid chromatography (HPLC) but also gas chromatography (GC) were applied. Different types of detectors (HPLC: UV, FLD, MS and GC: ECD, MS) were used for DON, NIV and ZON depending on sample preparation and separation technique. The tandem MS detection (HPLC-MS/MS) is suited for a sensitive and selective measurement of all three compounds. The HPLC-FLD was an alternative method frequently used for ZON analysis by the participants.

The accepted data sets for DON, NIV and ZON (laboratory means) are shown in the following table.

	Mass fraction in µg kg^{-1}		
Laboratory	**DON**	**NIV**	**ZON**
A	85.5	797.7	95.5
B	78.7	960.3	88.4
C	70.0		82.7
D	71.6	960.3	
E	100.9	885.6	75.2
F	110.0		104.5
G	122.1		91.6
H	126.0	1229.3	92.0
I	110.9	1270.0	102.0
J	109.4		99.8
K	118.1		80.6
L	95.3		79.1
M	124.6	1096.6	109.6
N			75.5
O	105.9	1026.1	88.8
P	122.6		101.3
Q			95.7
R			72.6
S	88.8	848.5	106.7
T	102.7	927.8	100.4
U			73.8
No. of accepted data sets	**17**	**10**	**20**

The laboratory mean values were calculated based on four independently determined values corrected for method recovery determined by each laboratory.

SAFETY INFORMATION

The mycotoxins DON, NIV and ZON should be handled with due caution. Although the mycotoxin content in the sample is at trace levels, any use other than the intended of the content of the bottles is strictly prohibited. Personnel handling the material must adequately be trained and follow regular laboratory safety precautions.

INSTRUCTIONS FOR USE

Before withdrawing a sub-sample the bottle should be allowed to reach room temperature and be mixed thoroughly. Thereafter, the bottle is to be closed tightly. To the best of our knowledge, the stability of the reference material is not affected by short periods of handling at ambient temperature during transport and use. However, BAM cannot be held responsible for any alterations of the material occurring during transportation to, and handling and storage at, the customer's premises, especially of opened samples.

STORAGE

The material has to be stored at a temperature equal to or lower than -20 °C in its original bottle.

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TECHNICAL REPORT

A detailed technical report (pdf file or paper copy) describing the production, general characterisation as well as the analytical procedures applied and the treatment of the analytical data during certification of ERM®-BC600 is available on request from BAM.

Supply of Reference Materials by BAM Federal Institute for Materials Research and Testing

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