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# The use of mycotoxin methodology in practice: a need for harmonization

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## Abstract

*Background* In the EU, sampling and analysis for the official control of the levels of mycotoxins in foodstuffs should be performed in accordance with the methods and criteria set out in Commission Regulation 401/2006. For each mycotoxin, the values of recovery, repeatability and reproducibility of the analytical method selected by each laboratory must fall within the range of acceptability as prescribed in the Regulation. *Aims* Carry out a survey on current practices concerning the use and application of mycotoxin test methods for what are considered to be the most current commercially significant mycotoxins. *Materials and Methods* Nineteen control, commercial and research laboratories from 12 countries (United Kingdom, Italy, Belgium, Spain, Germany, The Netherlands, Bulgaria, Hungary, Greece, Turkey, New Zealand and China) participated in a survey of current practices concerning the use and application of methods for the determination of the principal mycotoxins found in foods and subject to regulatory control: [aflatoxins (AFs: $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$), aflatoxin $M_1$ ($AFM_1$) fumonisins (FBs: $FB_1$, $FB_2$), ochratoxin A (OTA), deoxynivalenol (DON), patulin (PAT), zearalenone (ZEA), and T-2 and HT-2 toxins]. *Results and Discussion* Fourteen of the laboratories surveyed were accredited to ISO 17025:2005 and the accreditation paralleled participation in proficiency testing schemes such as FAPAS®. Most of the laboratories declared that they received laboratory samples weighing between 0.004–1 kg. The number and types of food matrices analysed for each mycotoxin or group of mycotoxins varied consistently between mycotoxins, laboratories and countries. In general the highest number of food matrices capable of being assessed for a particular mycotoxin was for OTA followed - in decreasing order - by AFs, DON, ZEA, T-2/HT-2 toxins, FBs, $AFM_1$ and PAT. Analysis for OTA, AFs, PAT, ZEA, DON, FBs, T-2/HT-2 toxins and $AFM_1$, were performed in 95%, 84%, 74%, 74%, 63%, 58%, 58% and 53% of the laboratories, respectively. Most laboratories stated that they used HPLC coupled with either a fluorometer, ultraviolet or mass spectrometric (MS) detectors for detection and quantification of mycotoxins. Only one laboratory used GC/MS for analysis of T-2 and HT-2 toxins whereas two laboratories used TLC based methods for the determination of all mycotoxins except fumonisins. The use of LC-MS methodology by eight laboratories is remarkable because LC/MS is not an official method for mycotoxins within a CEN or AOAC context. Some mycotoxins are not amenable to all detection techniques reported above. ELISA kits were used in three laboratories for the analysis of AFs, OTA, ZEA, DON, FBs and/or T-2/HT-2 toxins. Several other test kits were used in one laboratory for the determination of OTA (six different test kits)

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and DON (eight different test kits). Six different definitions of limit of detection (LOD) and nine different definitions of limit of quantification (LOQ) have been reported by participating laboratories with the signal/noise ratio being the most popular (used by 40% of laboratories). In some cases the values of LOD, LOQ and measurement uncertainty for the same mycotoxin varied from laboratory to laboratory. In particular, a large variability of measurement uncertainty was noted that was probably due to non-harmonized interpretation of the term. *Conclusion* This survey suggests that the primary issues needing to be harmonized are: accreditation needed, appropriate size of laboratory sample, guidelines on the most convenient analytical method for each combination of mycotoxins/matrix, use of method validated through a collaborative study, participation in proficiency testing, use of reference/certified materials/standard solutions, use of the same definition/calculation for LOD, LOQ, recovery and measurement uncertainty.

## Introduction

The mycotoxins currently regulated within EU by Commission Regulations 1881/2006 and 1126/2007 are aflatoxins (AFs: $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$), aflatoxin $M_1$ ($AFM_1$) fumonisins ($FB_1$, $FB_2$), ochratoxin A (OTA), deoxynivalenol (DON), patulin (PAT), zearalenone (ZEA), and the sum of T-2 and HT-2 toxin. Although no maximum level was established for the sum of T-2 and HT-2 toxin it will be allocated one in the near future as soon as sufficient and reliable data on the natural occurrence of these toxins in cereals and cereal-based foods will be available.

In the EU, sampling and analysis for the official control of the levels of mycotoxins in foodstuffs should be performed in accordance with the methods and criteria set out in Commission Regulation 401/2006. Control laboratories are not forced to use official methods as published by AOAC International or the European Standardization Organization (CEN). However, for each mycotoxin, the values of recovery, repeatability and reproducibility of the analytical method selected by each laboratory must fall within the range of acceptability as prescribed in the Regulation.

In this paper, we report the results of a survey, involving 19 laboratories, on current practices concerning the use and application of mycotoxin test methods (both rapid and reference) for what are considered to be the most current commercially significant mycotoxins. The participating laboratories were mainly partners in the MoniQA project, and members of the working group 'Mycotoxins and Phycotoxins'. Working group members with limited or no experience in this field, or not involved in routine analyses, were invited to send the questionnaire to the appropriate laboratories in their countries, some of whom also replied. Various aspects of laboratory practices, quality assurance and control, analytical methods used and relevant method performances were included in a questionnaire, which was divided in two parts. The first part contained questions on laboratory accreditation, participation in proficiency testing and laboratory practices such as weight and homogenization of laboratory sample, recovery experiments, use of validated methods, certified reference materials and reference calibrants. In the second part, the laboratories were requested to report the food commodities usually tested for the presence of particular mycotoxins ($AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$, $AFM_1$, $FB_1$, $FB_2$, OTA, DON, PAT, ZEA, T-2 and HT-2 toxins), the types of method used, method performance characteristics [% recovery, limits of detection and quantification (LOD, LOQ)], measurement uncertainty and relevant costs for each of the mycotoxins considered in the questionnaire

The list of 19 laboratories from 12 countries that participated in the survey is reported in Table 1. Of these, five were control laboratories 10, research laboratories and four, commercial laboratories.

## Laboratory practices, accreditation and proficiency testing

The proportions (%) of laboratories which meet each quality control parameters are reported in Table 2. With the exception of the use of reference materials (47%), the majority of laboratories fulfilled the control parameters identified.

The number of accredited laboratories for each mycotoxin in 10 countries are also reported (Table 3). As might be expected a higher number of accredited laboratories was reported for aflatoxins followed by other regulated mycotoxins. The number of accredited laboratories seems also to


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**Table 1** Participating laboratories

| Name | Short name | Country | Typology of laboratory | Contact person |
|---|---|---|---|---|
| Campden & Chorleywood Food Research Association | CCFRA | United Kingdom | Commercial | Anton J. Alldrick |
| Joint Research Center | JRC IRMM | Belgium | Research | Joerg Stroka |
| Centre d'Economie Rurale | CER | Belgium | Research | Nathalie Gillard |
| Department of Food Science University of Naples Federico II | DSA | Italy | Research | Alberto Ritieni |
| Gaiker Centro Tecnologico | GAIKER | Spain | Research | Ainhoa Bilbao |
| Eurofins Analytik GmbH | EUROFINS | Germany | Commercial | Scarlett Biselli |
| Chinese Cereals and Oils Association | CCOA | Rep. of China | Research | Yan Meirong; Ju Xingrong |
| Budapest University of Technology and Economics | BUTE | Hungary | Research | Sándor Tömösközi |
| Institute of Environmental Science and Research Limited | ESR | New Zealand | Research | Susan Paulin |
| TUBITAK Marmara Research Center | TUBITAK | Turkey | Research | Hayrettin Özer; Nihat Ozcan |
| Ministry of Agriculture Ankara Province Control Laboratory | APCL | Turkey | Control | Umran Uygun; Hamit Koksel |
| Sichuan University, West China School of Public Health | SCU | Rep. of China | Research | Lishi Zhang |
| ARPA Emilia Romagna | ARPA | Italy | Control | Cecilia Bergamini |
| General Chemical State Laboratory | GCSL | Greece | Control | Jhon Gardikis |
| Chemical laboratories | CHELAB | Italy | Commercial | Italo Commissati |
| Food and Consumer Product Safety Authority NRL for mycotoxins and pesticides in food | VWA | the Netherlands | Control | Martien Spanier |
| University of Food Technologies | UFT | Bulgaria | Research | Angel Angelov |
| Laboratorio Agroalimentario de Valencia | LAV | Spain | Commercial | Garcia Joscua |
| Agencia de Salud Pública de Barcelona | ASPB | Spain | Control | Ainhoa Bilbao |

**Table 2** Laboratory practices

| | % of laboratories that responded YES | % of laboratories that responded NO | % no response |
|---|---|---|---|
| Accreditation 17025:2005 | 74 | 21 | 5 |
| Laboratory participation in proficiency testing | 74 | 21 | 5 |
| Routine recovery experiments | 85 | 5 | 10 |
| Use of certified reference standard solutions | 74 | 21 | 5 |
| Use of reference materials | 47 | 37 | 16 |
| Use of collaboratively validated methods | 69 | 26 | 5 |
| Controlled sample storage conditions | 85 | 5 | 10 |
| Sample homogeneization | 64 | 26 | 10 |

be correlated with the occurrence of each mycotoxin in a particular commodity. For example there are few accredited laboratories for FBs and PAT, mycotoxins that occur in a limited number of food commodities, i.e. maize and derived products and fruits and derived products, respectively.

The participating laboratories were also asked to report the weight of laboratory sample normally received for each of the mycotoxins. The laboratory sample is also known as aggregate sample which is the final result of the sampling plan used to collect it. This sample is then delivered to the laboratory for chemical analysis. In the European Union, for regulatory control purposes, this weight is regulated by EC 401/2006 (European Commission, 2006) and varies between 1 and 10 kg depending of the matrix, size of the lot, etc. The EU legislation also states that 'in case the portion to be sampled is so small that it is impossible to obtain an aggregate sample of 1 kg, the aggregate sample weight might be $< 1$ kg'.

The legislation contemplates that the analysis of some mycotoxins requires a larger sample amount as they are distributed less homogeneously in the affected commodity (e.g. aflatoxins in nuts versus *Fusarium* toxins in grains). As shown in Table 4 only three laboratories receive laboratory samples with a weight according to EC 401/2006 whereas the other laboratories declared the weight of laboratory samples in the range of 3–1000 g. In the same table the time from receipt of sample to obtain results is also reported for each laboratory. A large variability of this parameter has been observed ranging for 1.5 h to 45 days, however, most of the laboratories analyse the sample within a reasonable time ($\leq$ 5 days).

Another important step is the homogenization of the laboratory sample before taking the test portion to be submitted to chemical analysis. The homogenization of laboratory sample is performed in 13 laboratories mainly by grinding or slurrying the sample. No sample homogenization/grinding was performed by five laboratories probably because the weight of the laboratory sample was equal to the weight of the test portion size to be submitted to the

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**Table 3** Number of accredited laboratories for each mycotoxin in each country

| Mycotoxin | Germany | Italy | the Netherlands | Turkey | Hungary | Belgium | Greece | Bulgaria | China | UK |
|---|---|---|---|---|---|---|---|---|---|---|
| Aflatoxins | > 100 | 56 | 15 | 25 | NR | 3 | 6 | 8 | > 100 | 18–45* |
| Aflatoxin $M_1$ | NR | 45 | 5 | 8 | NR | NR | 3 | NR | 1 | 18–45* |
| Ochratoxin A | > 100 | 53 | 7 | 15 | 7 | 3 | 7 | NR | | 18–45* |
| Patulin | > 100 | 7 | 3 | 4 | 3 | 2 | NR | 8 | | 18–45* |
| Deoxynivalenol | NR | 9 | 5 | 1 | 4 | 4 | 1 | NR | 4 | 18–45* |
| Zearalenone | NR | 20 | 4 | 1 | 5 | 2 | 1 | NR | | 18–45* |
| Fumonisins | > 100 | 6 | 3 | 1 | NR | 1 | 1 | NR | 5 | 18–45* |
| T-2 and HT-2 toxins | NR | 2 | 1 | NR | 2 | NR | NR | NR | 22 | 18–45* |

*The number of accredited laboratories varies depending of particular commodities.
NR, not reported.

**Table 4** Laboratory sample weight and time necessary to perform the analysis

| Laboratory | Weight of laboratory sample (g) | Time from receipt of sample to obtain results |
|---|---|---|
| 1 | 100–500 | 5 days |
| 2 | 25–50 | 6–24 hours |
| 3 | 500 | 1–5 days |
| 4 | > 250 | 3–5 hours |
| 5 | 15–50 | 1–15 days |
| 6 | 10–75 | 2–72 hours |
| 7 | NR | 3–24 hours |
| 8 | NR | NR |
| 9 | > 500 | 1–5 days |
| 10 | 10–50 | 1.5–3 hours |
| 11 | NR | 1 day |
| 12 | 4–20 | 7 days |
| 13 | EC 401/2006 | 7–45 days |
| 14 | EC 401/2006 | 2–5 days |
| 15 | 2.5–20 | 48–60 hours |
| 16 | EC 401/2006 | 3 days |
| 17 | 50–1000 | 8 hours |
| 18 | 300 | Depending of the number of samples |
| 19 | 1000 | 5 days |

NR, not reported.

**Table 5** Matrices analysed for mycotoxins

| Matrices | Mycotoxins |
|---|---|
| Food, luxury foods and semi-manufactures, (ground) nuts, nut products, copra, peanut butter, (dried) figs, dried fruit, spices, paprika powder, herbs/feed, feed raw materials, cereals, cereal products, maize, corn and corn products, wheat | Aflatoxins ($AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$) |
| Food, luxury foods and semi-manufactures, spices, baby food/feed, feed raw materials, mixed feed raw materials and mixed feed, cereals, cereal products | Aflatoxin $B_1$ |
| Milk, milk powder | Aflatoxin $M_1$ |
| Food, bread pastry, bakery raw materials, raisins, wine, oils, grains, green and roasted coffee, coffee powder, spices, fat, baby food, cereals/feed, feed raw materials, compound feed, seed, cereals, meat and derived products | Ochratoxin A |
| Food, bread pastry, bakery raw materials, oils, spices, fat/feed, feed raw materials compound feed, cereals, seed | Zearalenone |
| Food, bread pastry, bakery raw materials, oils, spices, fat/feed, feed raw materials, compound feed, cereals, seed, wheat, maize | Deoxynivalenol |
| Maize, cereals, maize derived products including baby foods, feed, feed raw materials, compound feed | Fumonisins ($FB_1$, $FB_2$) |
| Apples and apple products, fruit juice and fruit puree | Patulin |
| Cereals and derived products including baby foods, feed, soy oils (maize, oats) | T-2 and HT-2 toxin |

analysis. Adequate storage (e.g. congelation or refrigeration) of the laboratory sample before analysis depend on the kind of food/feed sample and is necessary to avoid possible further mycotoxins accumulation due to mould activity. Sixteen laboratories store the sample properly, whereas no storage precautions are taken by one laboratory (laboratory 13) which was the laboratory with the longest time from receipt of sample to obtain results (up to 45 days).

To check the accuracy of the analytical methods, 16 laboratories reported that they perform routine recovery experiments. The use of certified reference standard solutions (mycotoxin solutions with a certificate of analysis) and certified reference materials also belongs to good quality control practices. Internal or certified reference materials were used by nine laboratories, whereas six laboratories did not use them. Certified standard solutions were routinely used by 14 laboratories whereas three laboratories did not, one laboratory purchased solid materials and quantified the concentration in standard solution by using standard AOAC procedures. The origin of calibrant solutions was reported by few laboratories since it was not specifically asked in the questionnaire.


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However, certified reference standard solutions for all mycotoxins considered in this survey are commercially available.

## Food matrices, methods and costs

The matrices analysed for mycotoxins reported by participating laboratories were in line with those reported in the EU Regulation No. 1881/2006, No. 1126/2007 (2). The list of principal food matrices analysed for mycotoxins is reported in Table 5. The number of food matrices analysed for each mycotoxin or group of mycotoxins varied consistently between mycotoxins, laboratories and countries. In general the highest number of food matrices were reported for OTA followed in decreasing order by AFs, DON, ZEA, T-2/HT-2 toxins, FBs, $AFM_1$ and PAT.

The use of HPLC-based methods coupled with either ultraviolet (UV or diode array detector), fluorometer (FLD) or mass spectrometry (MS) detector was reported in all participating laboratories although laboratory seven and laboratory 12 use HPLC methodology only for the determination of fumonisins. It is noteworthy that these two laboratories reported using TLC or ELISA methods for the determination of AFs, $AFM_1$, OTA, DON, ZEA, T-2 and HT-2 toxins. The type of detector coupled to HPLC apparatus is mainly related to the specific chemical characteristics of the mycotoxin determined. For example a UV detector is used for the determination of DON and PAT whereas a FLD detector is used for the determination of AFs, OTA, ZEA and FBs. A pre- or post-column derivatization with appropriate reagent is necessary to obtain fluorescent derivative compounds of FBs that do not have native fluorescence and do not absorb UV light. A pre- or post-column derivatization is also necessary to increase the fluorescence of $AFB_1$ and $AFG_1$ and obtain the requested limits of detection.

Eight laboratories use the mass spectrometer detector coupled to HPLC. LC-MS/MS is used both for single or simultaneous determination of several mycotoxins. The choice is probably based on the combination of matrix–mycotoxin(s) because some matrices can be contaminated by a single mycotoxin whereas other matrices can be contaminated by several mycotoxins. For example maize can be contaminated by AFs, FBs, OTA, DON, T-2 and HT-2, ZEA, whereas milk of animal origin can only be contaminated by $AFM_1$. When LC-MS/MS is used for simultaneous analysis of several mycotoxins, the sample extract is injected into the apparatus after a minimal or no cleanup procedure. This approach makes the method quite fast but has a number of disadvantages such as high limit of detection (LOD)/quantification and a significant matrix effect. Moreover the extraction efficiency could not be adequate for all tested mycotoxins due to their different chemical structures that require a specific extraction solvent mixture for each mycotoxin or group of mycotoxins. In addition, it is not clear whether this concerns merely the recovery of the extraction step (and performance of a matrix-matched calibration) or whether these values add up extraction losses and signal suppression. However, the MS approach remains a powerful tool for screening purposes. The mass spectrometry detector can be considered a universal detector although it is very expensive as compared with traditional HPLC detectors. Within the eight laboratories that use LC-MS/MS, only three laboratories use this technique for the determination of the mycotoxins considered in this questionnaire whereas the other five laboratories use either HPLC (coupled either to UV or FLD detector) or LC-MS/MS depending of the combination matrix–mycotoxin(s) analysed.

The LC-MS/MS methods used by laboratories participating in this survey can be classified as 'traditional' or 'rapid' depending of the sample preparation protocol used before LC-MS/MS determination. For example laboratory 16 basically extracts the slurry and injects the extract into the LC-MS/MS apparatus, therefore this method can be considered 'rapid'. On the other hand for the analysis of PAT in fruit juices, $AFM_1$ in milk, AFs+OTA in spices and baby food, this laboratory purifies the sample extract by solid phase extraction or immunoaffinity column before LC-MS/MS determination. In these cases the methods are not rapid and have therefore to be classified as 'traditional'.

The use of LC-MS methodology by eight laboratories is remarkable because LC/MS is not an official method for mycotoxins in CEN and AOAC context. Owing to the fact that there have not been full and satisfying interlaboratory validation studies of methods employing this technique, it cannot be formally used in regulatory analysis.

The use of gaschromatography (GC-MS) has been reported by one laboratory for the analysis of T-2 and HT-2 toxins. These toxins are not fluorescent and have a very low molar extinction coefficient that does not permit an acceptable LOD by using a UV detector coupled to HPLC.

The use of TLC based methods, alone or in addition to other methods, has been reported by three laboratories for the analysis of AFs, $AFM_1$, OTA, DON, ZEA, T-2 and HT-2 toxins.

Rapid test kits, alone or in addition to other methods, are used in four laboratories. Laboratory 1, in addition to HPLC-based methods, uses test kits for the determination of OTA (Veratox, Ridascreen, Ochrascan, Ochracard, OTA-Flow through test, ROSA OTA) and DON (Veratox 5/5DON, Ridascreen Fast DON, Rida quick DON, Ridascreen

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DON Express, AgraQuant DON, Reveal for DON, ROSA DON P/N, ROSA DON-Q).

ELISA kits are used by laboratory 7 for the determination of AFs, OTA, DON, ZEA, FBs, T-2 and HT-2 toxins; by laboratory 8 for the determination of OTA, DON, ZEA, T-2 and HT-2 toxins; by laboratory 12 for the determination of ZEA, T-2 and HT-2 toxins.

Seventeen out of 19 participating laboratories reported the cost per analysis applied. For chromatographic methods the costs are quite variable ranging from 50 to 365 euro with a mean of about 100 euro. Much less expensive are the costs reported for rapid test kits that range between 5 and 27 euro with a mean of about 13 euro per analysis.

## Method performance and definitions of terms

A summary of the methods used by participant laboratories and relevant performance characteristics in terms of % recovery, LOD, LOQ and percentage measurement uncertainty is reported in Table 6. In the same table, the number of laboratories is also reported, that use a specific method for each of the tested mycotoxins. Wide ranges (up to 1000 times) of LOD and LOQ for the same mycotoxin have been reported by laboratories that use HPLC-based methods. A possible explanation of these results could be the use of different extraction and cleanup protocols selected by each laboratory and the use of different definitions of LOD and

**Table 6** Comparison of method performance characteristics reported by participant laboratories for each mycotoxin

| Mycotoxin | Method | No. of laboratories | % recovery | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % uncertainty |
|---|---|---|---|---|---|---|
| Aflatoxins ($B_1$,$G_1$,$B_2$,$G_2$) | HPLC-FLD | 11 | 70–110 | 0.01–0.5 | 0.02–0.5 | 5–30 |
| | LC-MS/MS | 4 | 57–120 | 0.01–20 | 0.05–50 | 10–50 |
| | TLC | 2 | > 75 | 2.5–5 | 2.5–5 | NR |
| | ELISA | 1 | 80–110 | 0:01 | 0:01 | 10 |
| Aflatoxins $M_1$ | HPLC-FLD | 5 | 40–101 | 0.0005–0.5 | 0.0015–0.015 | 7–54 |
| | LC-MS/MS | 3 | 82–98 | 0.001–0.05 | 0.01–0.1 | 11–50 |
| | TLC | 2 | 92–105 | 5 | 0.5 | NR |
| Ochratoxin A | HPLC-FLD | 13 | 50–100 | 0.008–5 | 0.02–50 | 4–60 |
| | LC-MS/MS | 2 | 53–105 | 0.01–8 | 0.05–16 | 17–50 |
| | TLC | 2 | NR | 10 | 10 | 20 |
| | ELISA | 1 | 70–100 | 1 | 10 | 15 |
| | Test kits* | 1 | na | 0.63–4 | 0.1–2 | n.a. |
| Zearalenone | HPLC-FLD | 6 | 70–120 | 0.007– < 100 | 3–20 | 4–30 |
| | LC-MS/MS | 6 | 45–120 | 1–40 | 2–80 | 8–50 |
| | ELISA | 3 | 70–110 | 0.1– < 100 | 0.25–1 | 15–60 |
| | TLC | 1 | NR | > 40 | NR | 20 |
| Deoxynivalenol | LC-MS/MS | 7 | 38–120 | 0.5–83 | 1.25–50 | 5–50 |
| | HPLC-UV | 3 | 83–110 | 7–100 | 22–200 | 5–30 |
| | TLC | 1 | 80–100 | 100 | NR | NR |
| | ELISA | 1 | 85–110 | 1 | 10 | 15 |
| | Test kits** | 1 | < 90 | 100 | 200–250 | 25 |
| T-2 and HT-2 toxin | LC-MS/MS | 7 | 40–120 | 0.02–50 | 3.3–200 | 7–50 |
| | ELISA | 3 | NR | 1 | 10 | 20–30 |
| | GC-MS | 1 | 95–105 | 5 | 10–15 | 20–30 |
| | TLC | 1 | NR | NR | NR | 20 |
| Patulin | HPLC-UV | 9 | 68–99 | 0.2–100 | 1.2–1000 | 4–29 |
| | LC-MS/MS | 5 | 55–110 | 1–10 | 2–50 | 10–50 |
| Fumonisins ($B_1$, $B_2$) | HPLC-FLD | 5 | 70–110 | 5–100 | 20–74 | 20–31 |
| | LC-MS/MS | 6 | 36–106 | 1–100 | 5–200 | 8–55 |
| | ELISA | 1 | 80–100 | 8 $ng\,ml^{-1}$ | NR | NR |

*Veratox for OTA, Ridascreen OTA, Ridascreen OTA 30/15, Ochrascan, Ochracard, OTA-flow through test and ROSA OTA. The reported values of LOD and LOQ are not applicable for qualitative test such as Ochrascan, OTA-flow through test, Ochracard.

**Veratox 5/5 DON, Ridascreen fast DON, Ridascreen DON express, Rida quick DON, Agraquant DON, Reveal for DON, ROSA DON P/N and ROSA DON-Q. The reported values of LOD and LOQ are not applicable for qualitative test such as Rida quick DON, Ridascreen DON express, Reveal for DON and ROSA DON P/N. The reported value of uncertainty is applicable only for Veratox 5/5 DON, Ridascreen fast DON and AgraQuant DON.

NR, not reported; na, not applicable.


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**Table 7** Definitions of limit of detection (LOD) reported by participant laboratories

| Definition | Number of laboratories |
|---|---|
| Signal/noise ratio (0.6–10) | 8 |
| Noise of blank + 3 SD | 2 |
| The level below which the analyte cannot be measured | 1 |
| Confidence interval of calibration curve | 1 |
| The smallest amount detected to which is associated a deviation > 40% | 1 |
| ISO 5725:1987 | 1 |
| $(3 \times S)/b \times f$(conc.) | 1 |

**Table 8** Definitions of limit of quantification (LOQ) reported by participant laboratories

| Definition | Number of laboratories |
|---|---|
| Signal/noise ratio (6–500) | 6 |
| Noise of blank + 10 SD | 1 |
| 3 × LOD | 1 |
| Confidence interval of calibration curve | 1 |
| The smallest amount detected to which is associated a deviation > 35% | 1 |
| The lowest level measured with acceptable precision and accuracy | 2 |
| The lowest unambiguously determinable quantity of analyte | 1 |
| The lowest level measured with an accuracy of 20% | 1 |
| ISO 5725:1987 | 1 |

LOQ. In addition, definition of LOD by S/N may be especially problematic for LC-MS/MS in the selected reaction monitoring mode, as the baseline may equal zero in some cases. On the other hand, the wide ranges of uncertainty values could be attributed to the non-harmonized use of the term measurement uncertainty.

The definitions of LOD and LOQ reported by participant laboratories are classified in Tables 7 and 8, respectively. As shown in Table 7, seven different definitions of LOD were reported with those based the signal/noise ratio being the most popular. Nine different definitions of LOQ were reported, again with those based on the signal/noise ratio being the most popular.

Detailed information on performance characteristics for each mycotoxin are reported below.

## Aflatoxins

In Table 9, the methods used for the determination of aflatoxins are reported. Relevant performance characteristics are included, as reported by the 16 laboratories that reported a capability to determine these mycotoxins.

HPLC-FLD, LC-MS/MS, TLC and ELISA were used in 11, 4, 2 and 1 laboratories, respectively. For HPLC-FLD methods recovery ranged from 70% to 110%, LOD from 0.01 to 0.5 μg kg$^{-1}$, LOQ from 0.02 to 2.5 μg kg$^{-1}$ and the measurement uncertainty from 5% to 30%.

**Table 9** Aflatoxins ($B_1$, $B_2$, $G_1$, $G_2$): test method used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % recovery | LOD (μg kg$^{-1}$) | LOQ (μg kg$^{-1}$) | % uncertainty |
|---|---|---|---|---|---|
| 1 | HPLC-FLD | 70–110 | 1 | 1 | NR |
| 2 | HPLC-FLD | 75–95 | 0.2 | 0.5 | 30 |
| 3 | LC-MS/MS | 82–108 | 0.1–2 | 0.2–5 | 18–39 |
| 5 | HPLC-FLD | 72 | 0.01 | 0.4 | 10 |
| 6 | HPLC-FLD | 70–120 | 0.03–0.05 | 0.1 | 5–30 |
| | LC-MS/MS | 80–120 | NR | 0.01–1.5 | 10–30 |
| 7 | TLC | NR | 2.5–5 | 2.5–5 | NR |
| | ELISA | 80–110 | 0.01 | 0.1 | 10 |
| 9 | HPLC-FLD | 70–90 | 0.04 | 0.1 | 5–9 |
| 10 | HPLC-FLD | 80–100 | 0.04–0.18 | 0.04–0.2 | 10 |
| 11 | HPLC | 73–97 | 0.01–0.2 | 0.02–0.3 | 19–26 |
| 12 | TLC | > 75 | NR | 5 | NR |
| 13 | HPLC-FLD | 84–88 | 0.05–0.5 | NR | ± 0.02–0.22 μg kg$^{-1}$ |
| 15 | LC-MS/MS | 57–94 | 0.05–20 | 0.1–50 | 8–10 |
| 16 | LC-MS/MS | 90–105 | 0.01–2 | 0.02–4 | 50 |
| 17 | HPLC-FLD | > 80 | 0.3 | 0.1 | NR |
| 18 | HPLC-FLD | NR | NR | 1–2.5 | NR |
| 19 | HPLC-FLD | 82 | NR | 0.1–0.5 | 27 |

NR, not reported; HPLC-FLD, high-performance liquid chromatography with fluorescence detection; LC-MS/MS, liquid chromatography with tandem mass spectrometry detection; TLC, thin layer chromatography; ELISA, enzyme-linked immunosorbent assay.

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**Table 10** Aflatoxin $M_1$: test method(s) used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % recovery | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % uncertainty |
|---|---|---|---|---|---|
| 5 | HPLC-FLD | 89 | 0.5 | 0.008 | 10 |
| 6 | HPLC-FLD | 40–100 | 0.003 | 0.01 | 10–25 |
| 7 | TLC | NR | 5 | NR | NR |
| 10 | HPLC-FLD | 98 | 0.0005–0.005 | 0.0015–0.015 | 9 |
| 12 | TLC | 92–105 | NR | 0.5 | NR |
| 13 | LC-MS/MS | 98 | 0.008 | NR | $\pm 0.003\,\mu g\,kg^{-1}$ |
| 14 | HPLC-FLD | 88–101 | 0.003 | 0.009 | 7–54 depending of the level |
| 15 | LC-MS/MS | 82 | 0.001–0.05 | 0.01–0.1 | 11 |
| 16 | LC-MS/MS | 95 | 0.005 | 0.01 | 50 |
| 19 | LC-MS/MS | 78 | NR | 0.1 | 35 |

NR, not reported.

For LC-MS/MS methods recovery ranged from 57% to 120%, LOD from 0.01 to $2\,\mu g\,kg^{-1}$, LOQ from 0.01 to $5\,\mu g\,kg^{-1}$ and measurement uncertainty from 10% to 50%.

In the case of TLC methods recoveries were $> 75\%$, while LOD and LOQ ranged from 2.5 to $5\,\mu g\,kg^{-1}$ (no data were given for the measurement uncertainty).

In terms of ELISA-based test kits recoveries ranged from 80% to 110%, while the LOD was reported as $0.01\,\mu g\,kg^{-1}$, the LOQ as $0.1\,\mu g\,kg^{-1}$ and the measurement uncertainty as 10%. In this case the measurement uncertainty was probably confused with the within-laboratory repeatability of results.

The data reported suggests that sometimes the use of sophisticated and expensive methods such as LC-MS/MS may lead to LOQ values (up to $50\,\mu g\,kg^{-1}$) significantly higher than those for HPLC-FLD methods and comparable to those obtained with veteran methods based on TLC.

## Aflatoxin $M_1$

Ten laboratories reported that they undertook analyses for $AFM_1$ (Table 10).

HPLC-FLD, LC-MS/MS and TLC were used in five, three and two laboratories, respectively.

For HPLC-FLD methods recovery ranged from 40% to 101%, LOD from 0.0005 to $0.5\,\mu g\,kg^{-1}$, LOQ from 0.0015 to $0.015\,\mu g\,kg^{-1}$ and the measurement uncertainty from 7% to 54%.

In the case of LC-MS/MS methods recovery ranged from 82% to 98%, LOD from 0.001 to $0.05\,\mu g\,kg^{-1}$, LOQ from 0.01 to $0.1\,\mu g\,kg^{-1}$ and measurement uncertainty from 11% to 50%.

Information regarding TLC methods indicated recovery ranging from 92% to 105%. Of the two laboratories that used TLC, one only reported the LOD ($5\,\mu g\,kg^{-1}$) whereas the other laboratory only reported the LOQ ($0.5\,\mu g\,kg^{-1}$) and no measurement uncertainty values were reported. The LOD value of $5\,\mu g\,kg^{-1}$ is very high and is inadequate to fulfil the legal limits in force in the EU ($0.05\,\mu g\,kg^{-1}$) and in the United States ($0.5\,\mu g\,kg^{-1}$).

## Ochratoxin A

Eighteen laboratories indicted that they undertook analyses for OTA (Table 11). HPLC-FLD, LC-MS/MS and TLC were used in 13, 3 and 2 laboratories, respectively. One laboratory uses both TLC and an ELISA test while another laboratory in addition to HPLC-FLD also used rapid methods (Veratox® for OTA, Ridascreen® OTA, Ridascreen® OTA 30/15, Ochrascan, Ochracard, OTA-flow through test and ROSA® OTA).

For HPLC-FLD methods recovery ranged from 50% to 100%, LOD from 0.008 to $5\,\mu g\,kg^{-1}$, LOQ from 0.02 to $50\,\mu g\,kg^{-1}$ and measurement uncertainty from 4% to 60%.

Respondents using LC-MS/MS methods reported recovery ranging from 53% to 105%, LOD from 0.01 to $8\,\mu g\,kg^{-1}$, LOQ from 0.05 to $16\,\mu g\,kg^{-1}$ and measurement uncertainty from 17% to 50%.

In the case of TLC methods no data were reported for recovery, the values for both LOD and LOQ were $10\,\mu g\,kg^{-1}$ and the measurement uncertainty was 20%. This LOD-LOQ value is much higher than the EU legislation limits for most food commodities which range from 0.5 to $5\,\mu g\,kg^{-1}$.

Data in respect of ELISA test kit used indicated that, recovery ranged from 70% to 100%, with a LOD of $1\,\mu g\,kg^{-1}$, a LOQ of $10\,\mu g\,kg^{-1}$ and a measurement uncertainty of 15%.

## Zearalenone

Fourteen laboratories reported that they analysed for this mycotoxin (Table 12). HPLC-FLD, LC-MS/MS, ELISA and TLC were used in six, six, three and one laboratories, respectively. One laboratory used HPLC-FLD, ELISA and TLC methods.


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**Table 11** Ochratoxin A: test method(s) used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % recovery | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % uncertainty |
|---|---|---|---|---|---|
| 1 | HPLC-FLD | 60–100 | 1 | 1 | NR |
| | Veratox for OTA | NR | 1 | 2 | NR |
| | Ridascreen OTA | NR | 0.63 | 0.1 | NR |
| | Ridascreen OTA 30/15 | NR | 1.25 | 0.1 | NR |
| | Ochrascan | NR | 2 | NR | NR |
| | Ochracard | NR | 3 | NR | NR |
| | OTA-flow through test | NR | 4 | NR | NR |
| | ROSA OTA | NR | $< 1$ | 0.7 | NR |
| 2 | HPLC-FLD | 80–90 | 0.8 | 2.8 | 20–30 depending of the matrix |
| 3 | LC-MS/MS | 90–100 | 0.2–3 | 0.2–10 | 27–33 depending of the matrix |
| 4 | HPLC-FLD | 68–97 | 0.1–5 | 0.5–10 | 8–14 depending of the matrix |
| 5 | HPLC-FLD | 75 | 0.02 | 0.2 | 10 |
| 6 | HPLC-FLD | 50–100 | 0.008–0.1 | 0.02–0.3 | 10–25 depending of the matrix and levels |
| 7 | TLC | NR | 10 | 10 | 20 |
| | ELISA | 70–100 | 1 | 10 | 15 |
| 8 | HPLC-FLD | NR | 0.02–1 | NR | 20–60 depending of the matrix and levels |
| 9 | HPLC-FLD | $> 90$ | 0.1–0.2 | $< 0.5$ | 4–10 depending of the matrix and levels |
| 10 | HPLC-FLD | 93–95 | 0.21–0.35 | 0.23–0.45 | 9–14 depending of the matrix |
| 11 | HPLC | 86–99 | 0.07–0.1 | 0.2–0.3 | 18–20 |
| 12 | TLC | NR | 10 | NR | NR |
| 13 | HPLC-FLD | 75–98 | 0.1–0.8 | NR | $\pm$0.1–0.3 $\mu g\,kg^{-1}$ |
| 14 | HPLC-FLD | 81–97 | 0.3–0.7 | 0.9–2.1 | 6–37 depending of the matrix and levels |
| 15 | LC-MS/MS | 53–93 | 0.01–0.5 | 0.05–1 | 17–19 |
| 16 | LC-MS/MS | 75–105 | 0.03–8 | 0.06–16 | 50 |
| 18 | HPLC-FLD | NR | NR | 0.01–50 | NR |
| 19 | HPLC-FLD | 77 | NR | 002–0.3 | 40 |

NR, not reported.

**Table 12** Zearalenone: test method(s) used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % recovery | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % uncertainty |
|---|---|---|---|---|---|
| 1 | LC-MS/MS | 60–120 | 2 | 2 | NR |
| 2 | HPLC-FLD | 85–105 | 3–5 | 5–20 | 20–30 |
| 3 | LC-MS/MS | 82–95 | 13–50 | 50–100 | 25–39 depending of the matrix |
| 4 | LC-MS/MS | 92–94 | 1.2 | 2.5 | 10–12 |
| 6 | HPLC-FLD | 70–120 | 1 | 10 | 10–15 |
| 7 | ELISA | 70–110 | NR | 1 | 15 |
| 8 | HPLC-FLD | NR | $0.007 < 100$ | NR | 20 |
| | ELISA | NR | $> 5 - < 100$ | NR | 30–60 |
| | TLC | NR | $> 40$ | NR | 20 |
| 10 | HPLC-FLD | 100 | 4 | 14 | 16 |
| 12 | ELISA | NR | NR | 0.25 | NR |
| 13 | HPLC-FLD | 110 | 30 | NR | $\pm$13 $\mu g\,kg^{-1}$ |
| 14 | HPLC-FLD | 80–90 | 1 | 3 | 4 |
| 15 | LC-MS/MS | 45–92 | 5 | 10 | 8–22 depending of the matrix |
| 16 | LC-MS/MS | 80–100 | 10–40 | 20–80 | 50 |
| 19 | LC-MS/MS | 100 | NR | 20 | 29 |

NR, not reported.

For HPLC-FLD methods recovery ranged from 70% to 120%, LOD from 0.007 to $< 100\,\mu g\,kg^{-1}$, LOQ from 3 to 20 $\mu g\,kg^{-1}$ and measurement uncertainty from 4% to 30%.

In the case of LC-MS/MS methods recovery ranged from 45% to 120%, LOD from 1.2 to 50 $\mu g\,kg^{-1}$, LOQ from 2 to 100 $\mu g\,kg^{-1}$ and measurement uncertainty from 8% to 50%.

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**Table 13** Deoxynivalenol: test method(s) used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % Recovery | LOD ($\mu g/kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % Uncertainty |
|---|---|---|---|---|---|
| 1 | LC-MS/MS | 60–120 | 10 | 10 | NR |
| | Veratox 5/5 | < 90 | 250 | 100 | 25 |
| | Ridascreen Fast DON | < 90 | 250 | 100 | 25 |
| | Rida Quick DON | < 90 | NR | NR | NR |
| | Ridascreen DON express | < 90 | NR | NR | NR |
| | AgraQuant DON | < 90 | 250 | 100 | 25 |
| | Reveal for DON | < 90 | NR | NR | NR |
| | ROSA DON P/N | < 90 | NR | NR | NR |
| | ROSA DON-Q | < 90 | 200 | 100 | NR |
| 2 | HPLC-UV | 90–110 | 50–100 | 100–200 | 20–30 |
| 4 | LC-MS/MS | 83–94 | 0.5 | 1.25 | 5–7 |
| 6 | LC-MS/MS | 70–120 | 5 | 20 | 10–25 |
| 7 | ELISA | 85–110 | 1 | 10 | 15 |
| | TLC | 80–100 | 100 | NR | NR |
| 10 | HPLC-UV | 83 | 7 | 22 | 14 |
| 12 | NR | NR | 0.1 | NR | NR |
| 13 | LC-MS/MS | 96 | 83 | NR | $\pm 36\,\mu g\,kg^{-1}$ |
| 14 | HPLC-UV | 100–102 | 30 | 90 | 5 |
| 15 | LC-MS/MS | 38–59 | 20 | 50 | 13–38 depending of the matrix |
| 16 | LC-MS/MS | 90–100 | 10–20 | 20–40 | 50 |
| 19 | LC-MS/MS | 100 | NR | 200 | 23 |

NR, not reported.

For ELISA kit, recovery ranged from 70% to 110%, LOD from 0.1 to $< 100\,\mu g\,kg^{-1}$, LOQ from 0.25 to $1\,\mu g\,kg^{-1}$ and measurement uncertainty from 15% to 60%.

Users of the TLC method did not provide information concerning recovery and LOQ. Data for the LOD were $> 40\,\mu g\,kg^{-1}$ and measurement uncertainty was 20%.

## Deoxynivalenol

Twelve laboratories reported that they undertook analyses for this mycotoxin (Table 13.)

LC-MS/MS, HPLC-UV, and TLC were used in seven, three and one laboratories, respectively. One laboratory used both LC-MS/MS and rapid test kits (Veratox® 5/5, (Neogen Corporation, Lansing, MI, USA) Ridascreen® fast DON, Ridascreen® DON express, Rida® quick DON, (R-Biofarm Group, Glasgow, UK) Agraquant® DON, (Romer Labs Inc., Union, MO, USA) Reveal for DON, ROSA® DON P/N and ROSA® DON-Q (Charm Science, Lawrence, MA, USA)). Another laboratory used both TLC and an ELISA test.

For LC-MS/MS methods, recovery ranged from 38% to 120%, LOD from 0.5 to $83\,\mu g\,kg^{-1}$, LOQ from 1.25 to $200\,\mu g\,kg^{-1}$ and measurement uncertainty from 5% to 50%.

In terms of HPLC-UV methods, recovery ranged from 83% to 110%, LOD from 7 to $100\,\mu g\,kg^{-1}$, LOQ from 22 to $200\,\mu g\,kg^{-1}$ and measurement uncertainty from 5% to 30%.

Information concerning the TLC method, suggested recovery ranged from 80% to 100%, with a LOD of $100\,\mu g\,kg^{-1}$ (no details of LOQ or measurement uncertainty were reported).

Responses for the ELISA test, indicated recovery ranged from 85% to 110%, with a LOD of $1\,\mu g\,kg^{-1}$, LOQ of $10\,\mu g\,kg^{-1}$ and measurement uncertainty of 15%.

## T-2 and HT-2 toxins

Eleven laboratories stated that they performed analyses for T-2 and HT-2 toxins (Table 14).

LC-MS/MS, ELISA, TLC and GC-MS were used in seven, three, one and one laboratories, respectively. One laboratory used both TLC and an ELISA test.

For LC-MS/MS methods, recoveries ranged from 40% to 120%, LOD from 0.02 to $150\,\mu g\,kg^{-1}$, LOQ from 0.6 to $300\,\mu g\,kg^{-1}$ and measurement uncertainty from 7% to 50%.

For ELISA test kits no recovery data were reported. However, values for LOD ($1\,\mu g\,kg^{-1}$), LOQ ($10\,\mu g\,kg^{-1}$) and measurement uncertainty (20% to 30%) were reported.

The laboratory using the GC-MS method reported recovery ranging from 95% to 105%, with a LOD of $5\,\mu g\,kg^{-1}$.


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**Table 14** T-2 and HT-2 toxin(s): test method(s) used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % Recovery | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % Uncertainty |
|---|---|---|---|---|---|
| 1 | LC-MS/MS | 60–120 | 10 | 10 | NR |
| 2 | GC/MS | 95–105 | 5 | 10–15 | 20–30 |
| 3 | LC-MS/MS | 84–109 | 0.1–150 | 0.6–300 | 22–41 depending of the matrix |
| 4 | LC-MS/MS | 86–110 | 1–2.5 | 3.3–5 | 7–10 |
| 6 | LC-MS/MS | 70–120 | 0.02 | 10 | 10–15 |
| 7 | ELISA | NR | 1 | 10 | 20 |
| 8 | TLC | NR | NR | NR | 20 |
| | ELISA | NR | NR | NR | 30 |
| 12 | ELISA | NR | 1 | NR | NR |
| 15 | LC-MS/MS | 49–80 | 20 | 50 | 12–14 |
| 16 | LC-MS/MS | 40–100 | 25–50 | 50–100 | 50 |
| 19 | LC-MS/MS | 100 | NR | 200 | 21–25 |

NR, not reported.

**Table 15** PATULIN: test method(s) used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % Recovery | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % Uncertainty |
|---|---|---|---|---|---|
| 1 | LC-MS/MS | 60–110 | 2 | 2 | NR |
| 2 | HPLC-UV | 70–80 | 3 | 10 | 20 |
| 4 | HPLC-UV | 85 | 100 | 1000 | 11 |
| 6 | LC-MS/MS | 70–100 | 2–5 | 10 | 10–20 |
| 8 | HPLC-UV | NR | 0.2–20 | NR | NR |
| 9 | HPLC-UV | $>85$ | 10 | 23 | 4 |
| 10 | HPLC-UV | 84 | 10 | 11 | 8 |
| 11 | HPLC-UV | 75 | 6 | 17 | 25 |
| 13 | LC-MS/MS | 88 | 2 | NR | $\pm 0.9\,\mu g\,kg^{-1}$ |
| 14 | HPLC-UV | 97–99 | 0.4 | 1.2 | 7 |
| 15 | LC-MS/MS | 55 | 10 | 50 | 12 |
| 16 | LC-MS/MS | 73–98 | 1 | 2 | 50 |
| 17 | HPLC-UV | 87–98 | 2–10 | 3 | NR |
| 19 | HPLC-UV | 68 | NR | 10 | 29 |

NR, not reported.

LOQ ranging from 10 to 15 $\mu g\,kg^{-1}$ and the measurement uncertainty from 20% to 30%.

In the case of the TLC method, no data for recovery, LOD and LOQ were reported, whereas the measurement uncertainty was declared as 20%.

## Patulin

Fourteen laboratories advised that they analysed for PAT (Table 15). HPLC-UV and LC-MS/MS are used in nine and five laboratories, respectively.

For HPLC-UV methods, recovery ranged from 68% to 99%, LOD from 0.2 to 100 $\mu g\,kg^{-1}$, LOQ from 1.2 to 1000 $\mu g\,kg^{-1}$ and measurement uncertainty from 4% to 29%.

In contrast, for LC-MS/MS methods, recovery ranged from 55% to 110%, LOD from 1 to 10 $\mu g\,kg^{-1}$, LOQ from 2 to 50 $\mu g\,kg^{-1}$ and measurement uncertainty from 10% to 50%.

## Fumonisins $B_1$ and $B_2$

Eleven laboratories declared that they analysed for FBs (Table 16).

HPLC-FLD, LC-MS/MS and ELISA are used in five, six and one laboratories, respectively. One laboratory used both HPLC and an ELISA test.

For the HPLC-FLD methods, recovery ranged from 70% to 110%, LOD from 5 to 100 $\mu g\,kg^{-1}$, LOQ from 20 to 50 $\mu g\,kg^{-1}$ and measurement uncertainty from 10% to 31%.

Comparative for LC-MS/MS methods were, recovery from 36% to 106%, LOD from 1 to 100 $\mu g\,kg^{-1}$, LOQ from

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**Table 16** FUMONISINS B1 and B2: test method(s) used in the participant laboratories and relevant performance characteristics

| Laboratory | Method | % Recovery | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) | % Uncertainty |
|---|---|---|---|---|---|
| 2 | HPLC-FLD | 75–110 | 10–20 | 30–50 | 20–30 |
| 3 | LC-MS/MS | 98–106 | 1–20 | 5–40 | 24–55 |
| 4 | LC-MS/MS | 68–91 | 8–15 | 25–40 | 8 |
| 6 | HPLC-FLD | 80–110 | 5 | 20 | 10–20 |
| 7 | HPLC | 70–100 | 28–37 | NR | NR |
| | ELISA | 80–100 | $8\,ng\,ml^{-1}$ | NR | NR |
| 10 | HPLC-FLD | 94 | 56 | 74 | 31 |
| 12 | HPLC | NR | 50–100 | NR | NR |
| 13 | LC-MS/MS | 79–87 | 11–32 | NR | $\pm$ 5–14 $\mu g\,kg^{-1}$ |
| 15 | LC-MS/MS | 36–80 | 4–5 | 10 | 13–20 |
| 16 | LC-MS/MS | 95 | 10–100 | 20–200 | 50 |
| 19 | LC-MS/MS | 100 | NR | 60–200 | 20–24 |

NR, not reported.

5 to 200 $\mu g\,kg^{-1}$ and measurement uncertainty from 8% to 55%.

In the case of the ELISA test, no LOQ and measurement uncertainty data were reported. However, a LOD value of $8\,ng\,ml^{-1}$ was reported, which makes it difficult to compare with other data.

## Conclusions

Taken together, the results of this survey highlight a number of primary issues that need to be harmonized in the field of analytical methods for mycotoxins.

These are:

- laboratories have to be accredited;
- guidelines on the most 'convenient' analytical method for each combination of mycotoxins/matrix;
- use of methods validated through collaborative studies (if available);
- participation in proficiency testing;
- use of reference/certified materials/standard solutions;
- use of a common definition/calculation for LOD, LOQ, repeatability and measurement uncertainty;
- use of methods with LOD and LOQ values lower than the maximum permitted legal limits;
- appropriate laboratory sample and test portion sizes.

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