EURL-MP-guidance doc_003 (version 1.1)

Guidance document on performance criteria (draft 17th September, 2021)

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1 Introduction

Performance criteria for methods of analysis used for official control for mycotoxins in food are included in Commission Regulation (EC) No 401/2006 (Annex II, section 4). In 2018/2019 the criteria of this existing regulation were reviewed by the EURL-MP. It was concluded that an update/revision was needed. In the existing situation different performance criteria are specified for the different mycotoxins, and, in addition, for the different levels of the same mycotoxins. Part of the precision criteria are Horwitz-based, others have been derived from, in many cases rather old, collaborative studies or expert judgments. With current methods, there is no longer any clear scientific rationale for these mycotoxin/concentration dependent criteria. Furthermore, certain performance parameters like intermediate precision and LOQ requirements are missing in the existing legislation. These observations were communicated with the Commission, who then requested the EURL-MP to propose a revision of this part of the regulation.

For mycotoxins and plant toxins in feed, some requirements are laid down in Commission Regulation (EC) No 152/2009 (currently under revision). In addition, for mycotoxins in feed, performance criteria have been provided in CEN/TS 17455:2020.

For plant toxins in food, no regulation with method performance criteria yet exists. It is the opinion of the EURL-MP that method performance characteristics are, and therefore also the criteria should be, similar to those for mycotoxins. The Commission informed the EURL-MP that for plant toxins establishment of a separate regulation is foreseen. In this new regulation, the same performance criteria as drafted for mycotoxins are recommended to be included, in order to have harmonisation within the EURL-MP/NRL/OL community.

The current version of this document aims to provide the background and rationale for updated harmonised criteria for mycotoxins and plant toxins in food and feed. In addition, where considered necessary and in response to questions received from the NRLs, explanations and guidance are given, both on performance criteria that are embedded in legislation and other performance criteria related to validation and quality control which are not explicitly included in the legislation. NRLs are encouraged to provide feedback that will be taken into account in EURL-MP workshops and future updates of this document.

2 Definitions

Below definitions of typical method performance parameters / validation parameters are given. Not all are explicitly included in legislation, but they are included here when discussed in this guidance document.

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2.1 Should and shall

In legislation 'should' and 'shall' are used to indicate where, with common sense, exceptions/flexibility may be acceptable, and where not. Incidental exceptions can be accepted because it is recognised that with the numerous mycotoxins, plant toxins, food, feed, and concentration combinations, especially with multi-toxin methods, it may be very difficult to fully meet all criteria for every individual toxin/matrix/concentration combination.

SHOULD means a recommendation that may be ignored but only in particular circumstances (based on valid reasons) and the full implications of ignoring the recommendation shall be understood and carefully assessed before choosing a different course of action.

SHOULD NOT means not recommended, although it may be acceptable in particular circumstances, but the full implications of ignoring the recommendation shall be understood and carefully assessed.

SHALL means an absolute requirement (the action is mandatory).

SHALL NOT means an absolute no.

2.2 Matrix effects

Matrix effects are relevant for methods based on LC with MS detection and all GC-based methods.

In LC-MS(/MS): suppression (or enhancement) of analyte response when spiked to a blank sample extract compared to analyte response in solvent at the same concentration. It can occur during ionisation of the analyte in the ion source in the presence of co-extracted matrix and can affect quantification.

In GC-MS(/MS): enhancement (or suppression) of analyte response when spiked to a blank sample extract compared to analyte response in solvent at the same concentration. In GC it usually originates from active sites in the liner of the injector, and can affect quantification.

Note: matrix effects should not be confused with matrix interference, which is a detector response from another, (partially) co-eluting, compound that contributes to the analyte response.

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The matrix effect (ME) is typically expressed as a percentage and can be calculated as follows:

$$ME = \frac{A_{extract}}{A_{solvent}} \times 100 \qquad \textbf{(Eq. 1)}$$

where:

$A_{extract}$ = the peak area of the toxin spiked to a blank sample extract

$A_{solvent}$ = peak area of the toxin in solvent standard at the same concentration

ME <100% corresponds with a signal suppression of [ME-100%]. ME >100% corresponds with a signal enhancement of [ME-100%]. Example for LC-MS/MS: ME = 65% means that 35% ion suppression occurs.

2.3 Linearity

Direct proportionality of response obtained during measurement with the concentration of the analyte.

2.4 Recovery

Several definitions exist for recovery (see Appendix 1), which may also be differently interpreted. Factors that complicate the interpretation are whether or not matrix effects (see 2.2) and/or losses during sample preparation are accounted for, which depends on the approach used for calibration/quantification (see also 3.2, Table 1).

For mycotoxins and plant toxins in food and feed the following definition applies:

$$\text{Rec (\%)} = x/x_{ref} \times 100 \qquad \textbf{(Eq. 2)}$$

where:

x = measured concentration (for spiked samples corrected for background concentration if applicable)

x_{ref} = reference concentration (the concentration of a Certified Reference Material (CRM), Proficiency Test material, or spiked sample)

Note: it is recognized that, depending on the method and way of quantification, the recovery as defined above may correspond to different other descriptions or definitions:

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a) *Recovery* or *Recovery Factor as defined by IUPAC* [1], also referred to as *'extraction recovery' or 'absolute recovery'*. This is the yield of an analyte from the extraction/cleanup stage. When there are no matrix effects (see 2.2.), it can be determined using quantification method 1 from 3.2 Table 1.
   In case matrix-effects are significant, then matrix-effects need to be corrected for in order to obtain the absolute recovery. This can be done by using quantification method 2, 4a, or 5a from 3.2 Table 1.
b) *Apparent recovery* as defined by IUPAC [1]. This is basically any other recovery than described under a). It is the recovery observed with or without correcting for extraction/cleanup losses and/or matrix-effects, using the various options for quantification given in 3.2 Table 1.
   Example 1, method using quantification procedure 1 from Table 1 in 3.2: when losing 20% of analyte during sample preparation, and in the LC-MS/MS analysis 20% ion suppression occurs, then the apparent recovery is ~60%.
   Example 2, method using quantification procedure 3, 4b or 5b from Table 1 in 3.2: an apparent recovery of around 100% may be obtained even when the extraction yield would be 30% and matrix-effects (signal suppression) are significant.
c) *Trueness,* defined as the closeness of agreement between the average value obtained from a series of test results and an accepted reference value (ISO 5725-1), is normally expressed in terms of bias. Bias is the (relative) difference between the measured and the true concentration. The true concentration ideally is a CRM value, if not available the assigned value from a proficiency test or collaborative study, and if also not available, a spiked concentration. Trueness and recovery are sometimes used interchangeably, but are not the same because trueness is expressed as bias. Relative bias and recovery are correlated: bias (%) = recovery (%) – 100%.

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2.5 Precision

2.5.1 Reproducibility relative standard deviation, RSD_R

Relative standard deviation (%) calculated from results generated under reproducibility conditions involving multiple laboratories (and inherently different operators and instruments), analysing the same sample material.

Note: the RSD_R can be derived from collaborative trials, where multiple laboratories use the same method. The RSD_R can also be derived from proficiency tests where laboratories may use different methods (in that case the RSD_R typically is the robust standard deviation of the participants' results).

2.5.2 Repeatability relative standard deviation, RSD_r

Relative standard deviation (%) calculated from results generated under repeatability conditions (repeatability precision): using the same method on the same sample material in one laboratory by the same operator, with the same instrument, within a short interval of time (1 day or 1 sequence).

2.5.3 Within-laboratory reproducibility relative standard deviation, RSD_{wR}

Relative standard deviation (%) calculated from results generated under within-laboratory reproducibility conditions (intermediate precision, elsewhere also abbreviated as RSD_{Ri}): using the same method on the same sample material in one laboratory but on different days (preferably a longer time interval), and may include other conditions involving different operators and/or different (equivalent) instruments.

Note*: this parameter was not included in the existing Commission Regulation (EC) No 401/2006, but is a more relevant and realistic in-house precision parameter compared to RSD_r.*

2.6 Limit of detection (LOD) and limit of quantification (LOQ)

2.6.1 Introductory remarks

Various definitions and methods for determination of LOD and LOQ exist, which may result in different outcomes. Example definitions for LOD include 'analyte content which can be distinguished from the blank with an error probability of (1-ß)' [4], and 'minimum amount or concentration of the analyte in a sample which can be detected reliably, but not necessarily

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quantified' [5]. Examples for LOQ include 'lowest level at which the performance is acceptable for a typical application' [3], 'analyte content which can be determined with a certain level of precision' [4], and 'lowest concentration or amount of the analyte in a test sample which can be quantitatively determined with an acceptable level of precision and accuracy' [5].

The determination of LOD is often based on statistical approaches or signal-to-noise (S/N) approaches. Statistical approaches involve replicate analyses of pseudo-blanks and procedural calibration at equidistant concentrations in the range close to the (anticipated) LOQ [3,4]. Especially in LC-MS/MS-based methods the LOD may be matrix dependent as a result of ion suppression. As a consequence, estimation of the LOD for a wide variety of matrices can be a heavy burden for the laboratory. Estimation of the LOD based on S/N (LOD = concentration corresponding to S/N=3) is relatively straightforward. Visual inspection of chromatograms of a single measurement at low level can give an indication of the lower limit at which the analyte can be detected. However, determination of LOD through S/N is not unambiguous, especially in the case of MS detection, because noise depends on acquisition rate (duty cycle), smoothing, method of S/N calculation (manual or software-based, and in the latter case, the software algorithm).

For the determination of the LOQ, also different options have been described:

- based on the LOD obtained using statistical approaches: LOQ = 3*LOD or 3.3*LOD

- based on S/N approaches: LOQ = concentration corresponding to S/N ≈ 5-10

- based on determination of recovery and precision, iteratively at decreasing levels until the requirements are no longer met, or a fit-for-purpose LOQ has been reached. In the latter case, the LOQ may not be the lowest possible level, but the lowest tested level.

***Note**: in MS-based methods, in all approaches, at least two (product)ions are needed for identification and the one with the lowest sensitivity/selectivity will determine the LOD/LOQ.*

2.6.2 Official control

For official control purposes (checking compliance against maximum levels/guidance values/indicative levels) we define the LOQ as the lowest successfully validated level: the lowest tested concentration of analyte in a sample material, for which it has been demonstrated that the criteria for recovery, precision, and identification [2] are met. This definition and the way of determination of the LOQ is less ambiguous then the various options mentioned in 2.6.1. It should be noted that with this definition, there is no relationship between LOQ and LOD. Based on the LOQ requirements (see 3.5), the lowest validation level is chosen. This level may be well above the LOD and the technically feasible LOQ of the method.

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For official control purposes determination of the limit of detection (LOD) is not required. The rationale for this is that the maximum levels/guidance values/indicative levels for mycotoxins and plant toxins are often well above the method LOD, so their determination has no added value for this purpose, while unnecessarily adding to the validation workload.

2.6.3 Generation of monitoring data for risk assessment

For risk assessment, often the data generated during official control are submitted and used, supplemented with data from dedicated monitoring surveys (other methods/samples). For this reason, for LOQ the same definition as described under 2.6.2 applies.

For monitoring purposes, the required LOQs are often (much) lower than strictly necessary for official control. When the method is to be used both official control and monitoring, then the lowest level included in the validation needs to be adjusted accordingly.

Unless the LOQ is low enough to provide quantifiable results in (virtually) all samples, or the LOQ is below the level in the food/feed matrix considered to be relevant for risk assessment (to be provided by the risk assessor, e.g. EFSA or competent authority), the determination and reporting of the method LOD is required. The determination of the LOD should be done following the statistical approach according to [3,4].

Note*: the consequence of the need of two ions for identification in MS-based methods is that the absence of an analyte can often be demonstrated at a lower level than its presence. Example: the detectability of a toxin using ion-1 is 1 µg/kg and using ion-2 is 5 µg/kg. Not detecting ion-1 means the toxin is <1 µg/kg. Detecting ion-1 at 2 µg/kg means a potential presence of the analyte, but it cannot be reported as being present because the 2nd ion is not yet detectable, and hence, does not comply with the identification criteria. For risk assessment all three situations: <1 µg/kg, potentially present between 1-5 µg/kg, and present (identified) ≥5 µg/kg may be relevant to know. This is an issue that is not yet addressed in reporting formats of EFSA.*

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3 Performance criteria

Below the performance criteria are provided, with the background, explanations, and clarifications to aid in the uniform interpretation of the criteria in the legislation. Not all performance criteria are embedded in legislation, but they are included here as additional information and guidance to the NRLs.

3.1 Matrix effects

Matrix effects in LC-MS(/MS) or GC(-MS/MS) (see 2.2) should be investigated during validation to assess to what extent they occur. In case of strong matrix effects (>50% suppression/enhancement), it is recommended to try to reduce them (e.g. by dilution or applying a cleanup step). However, no criterion applies since matrix-effects are covered by the recovery criteria. When the recovery requirements (see 3.3) are not met due to matrix-effects, a calibration/ quantification procedure shall be used that corrects for matrix-effects, i.e. options 2-5 in Table 1, 3.2.

3.2 Calibration/quantification procedures

For quantification, multi-level calibration (3-5 concentrations, covering the lowest and highest level to be quantified) is preferred. An appropriate calibration function shall be used (e.g. linear, with or without weighing; quadratic). The deviation of the back-calculated concentrations of the calibration standards from the true concentrations using the calibration equation used should not be more than ±20%. This criterion is preferred over reliance on correlation coefficients.

There are different options for quantification/calibration. Calibration standards can be prepared in solvent/eluent, in sample extracts, in sample material, and with or without the use of isotopically labelled internal standards. Depending on the procedure used, the result obtained is corrected for matrix-effects, and in some cases also for losses during sample preparation. An overview is given in Table 1.

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Table 1. Overview calibration/quantification procedures

Quantification method	Calibration procedure	corrects for	
		sample prep. losses	matrix effects
1. solvent standard	calibration standards prepared in solvent	no	no
2. matrix-matched standards	calibration standards prepared in extract of blank sample of the same matrix	no	yes
3. procedural calibration	calibration standards prepared in sub-portions of blank sample of the same matrix, *added before extraction*	yes	yes
4. isotope dilution (all responses are normalised to that of the corresponding isotope labelled internal standard)	**4a.** isotope analogue added to the calibration standards, and to the final extract of each sample[a)]	no	yes
	4b. isotope analogue added to the calibration standards, and *to each sample before extraction*	yes	yes
5. standard addition method	**5a**. standards added to aliquots of the extract of each sample	no	yes
	5b. standards added to sub-portions of each sample *before extraction*	yes	yes

[a)] another option is to add the isotope analogue after extraction, to an aliquot of the extract, before clean-up. In this case, the method corrects for part of the sample preparation losses: it does not correct for low extraction yields, but it does correct for losses during the clean-up step.

3.3 Recovery requirements

3.3.1 Recovery

For mycotoxins and plant toxins in food and feed the average recovery should be between 70 and 120%.

Here the average recovery is the average value from replicates (typically ≥5) obtained during validation when determining the precision parameters RSD_r and RSD_{wR}. The criterion applies

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to all concentrations and all individual toxins, with the exception of ergot alkaloids. For ergot alkaloids the criterion applies to the sum of each epimer-pair.

In exceptional cases, average recoveries outside this range can be acceptable but shall lie within 50-130%, and only when the precision criteria for RSD_r and, if available, RSD_{wR} are met. It should be documented why an average recovery of 70-120% could not be achieved, why the deviation is considered acceptable, and what the implications for quantitative measurement are.

3.3.2 Requirements for extraction/cleanup yields

The yield of the sample preparation steps (extraction, cleanup) should be investigated during method development or validation to gain insight in extraction efficiency and losses during cleanup. Although the aim should always be to use methods with high yields, no criteria for the yields apply since losses during sample preparation are covered by the recovery criteria.

As described in 2.4 and 3.2, there are certain calibration/quantification procedures that correct for incomplete extraction and losses during clean-up (see Table 1, procedure 3, 4b and 5b). This way it is possible to obtain good recoveries (Eq. 2) while in fact e.g. only 30% of the toxin is extracted. This is considered acceptable as long as the recovery and precision criteria are met, the required LOQ can be achieved, and good accuracy can be demonstrated through proficiency test data or CRMs.

3.4 Precision

The criteria for precision apply to all concentrations.

In case the maximum level applies to a sum of toxins, then the criteria for precision apply to both the sum and the individual toxins. For ergot alkaloids, the criteria for individual toxins applies to the sum of each epimer pair.

3.4.1 Reproducibility relative standard deviation, RSD_R

As indicated in the definition, this is not a parameter for individual laboratories / single lab validations, but a parameter for interlaboratory validation. The criterion for RSD_R is based on what is currently feasible.

Insight in the current situation regarding variability of analysis between laboratories, can be obtained from validation data from collaborative trials (same method), and the (robust) relative standards deviation as observed in proficiency testing (various fit-for-purpose methods). In CEN/Technical Specification for mycotoxins in feed (CEN/TS 17455:2020, Stroka/JRC [5]), it was concluded that a RSD_R of 22% is generally feasible for collaborative studies, irrespective

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of the concentration, matrix and toxin. An inventory by the EURL-MP of robust RSD_R data from a large number of proficiency tests (>750 mycotoxin/matrix/ concentration combinations, 2013-2018) showed that the median and 75th percentile of the collected RSD_R's were 22% and 26%, respectively. The similarity of the RSD_R's observed in collaborative trials and PTs (although not always calculated using the same statistics) was also noticed by Thompson et al [6]. Based on these available data, it is concluded that a RSD_R of 25% is an appropriate benchmark both as criterion for acceptability of methods validated through collaborative trials, and for use as target relative standard deviation in PTs.

For the revision of Commission Regulation (EC) No 401/2006 the proposed criterion is: RSD_R should be ≤25%. The word 'should' is used here because in some cases the RSD_R in currently used CEN methods is higher than 25%.

The criterion of 25% for the RSD_R also sets the target for the criteria for RSD_{wR} and RSD_r, as these are expected to be lower than the RSD_R.

***Note**-1: the value of 25% was chosen over the 22% from CEN/TS 17455:2020: i) in order to take into account that different laboratories will use different methods, ii) because of the trend towards increased use of LC-MS-based multi-toxin methods. The 25% criterion is identical to the value used in the field of pesticides in food and feed [7], often analysed by LC-MS/MS, which also covers a wide range of organic molecules in a wide variety of matrices and concentrations.*

***Note**-2: it is recognised that the 25% criterion may be challenging for certain 'new' toxins for which there currently might be a lack of experience. However, for NRLs and OLs there is usually sufficient time between first announcement of upcoming legislation and actual enforcement of maximum levels, to implement, validate and familiarise themselves with fit-for-purpose methods. Another reason to set the RSD_R to a value not higher than 25% is that higher values may restrict the possibilities to take legislative action. This is because for enforcement of maximum levels the expanded measurement uncertainty (MU) needs to be subtracted from the analysis result to ascertain an exceedance of the maximum level beyond reasonable doubt [1]. Taking into account that a food or feed product may be tested at different laboratories in the EU member states, the estimated expanded MU in case of a RSD_R of 25% is at least 2*RSD_R = 50%. This means that in this case enforcement action can only be taken (i.e. the analysis result exceeds the ML beyond reasonable doubt) when the analysis result is twice the ML. Example:*

Commented [MH1]: For discussion: Exception: when the ARfD or TDI is exceeded at a level between ML and 2*ML, then the product might be considered potentially hazardous and this would justify taking action when the ML as such is exceeded.

[1] Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins. https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_alfatoxins_guidance-2010_en.pdf

ML = 100 µg/kg, RSD_R = 25% => expanded MU = 50%, the toxin concentration exceeds the ML when >200 µg/kg (200 - 50% = 100 µg/kg).

3.4.2 Repeatability relative standard deviation, RSD_r

Repeatability assessment is typically carried out during initial validation by 5-6 replicate analyses of the matrix for each concentration tested. The RSD_r shall be ≤20%.

3.4.3 Within-laboratory reproducibility relative standard deviation, RSD_{wR}

The within-laboratory reproducibility is determined by analysing samples in different batches, on different days.

The RSD_{wR} can be determined by analysing (C)RMs or spiked samples (e.g. at LOQ and/or 5-10 times LOQ, or ML) either during initial validation or during routine analysis together with the samples.

For laboratories that (foresee to) apply the method less than six times per year, the RSD_{wR} can be assessed during initial validation. The validation design should be such that data are generated on at least three different days/batches. It should be noted that the RSD_{wR} obtained this way may give an underestimation compared to an RSD_{wR} derived from routine analysis (see below).

For laboratories that (foresee to) apply the method on a more routine basis and therefore generate sufficient data through concurrent analysis of quality control samples, the RSD_{wR} is preferably determined based on at least six batches.

The RSD_{wR} shall be ≤20%.

Note: *in theory, the RSD_{wR} is expected to lie between the RSD_r and RSD_R. In the existing 401/2006 legislation, for certain mycotoxins a relationship $RSD_r \leq 0.66\ RSD_R$ has been suggested, corresponding to 16.5% based on the 25% criterion for RSD_R indicated above. The RSD_{wR} then would lie in between, e.g. 21%. At the moment, for mycotoxins and plant toxin in food/feed there is insufficient experimental evidence to support such relationships. Starting from an RSD_R of 25%, an RSD_{wR} of 20% was considered appropriate and set as criterion. Since the RSD_r requirements in the existing 401/2006 were in the 15%-40% range, it was decided to set the new requirement not below 20%, despite the resulting equivalence to the RSD_{wR} criterion..*

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3.5 Limit of Quantification, LOQ for official analysis

The criteria in Commission Regulation (EC) No 401/2006 and its revision apply to official control, i.e. analysis for enforcement purposes.

When a specific requirement for the LOQ of a toxin has been included in the legislation, the method shall have an LOQ at or below this value. In all other cases, the following applies:

LOQ shall be ≤ 0.5*ML, and should preferably be lower (≤0.2*ML).

In case the maximum level applies to a sum of toxins, then the LOQ of the individual toxins shall be ≤ 0.5*ML/n, with n being the number of toxins included in the ML definition. This does not apply when specific requirements for the LOQs of the individual toxins have been included in legislation and when there is a need to set very strict maximum levels for public health reasons, then it is acceptable to deviate from the general rule and to set the LOQ as low as reasonably possible (LOQ however not exceeding the ML established).

From a scientific perspective it does not make sense to sum individual LOQs into a sum-LOQ as parameter to compare against a sum-ML. Therefore LOQs are determined and reported for individual toxins only.

3.6 Recovery correction

Recovery correction, if applicable, is done for each of the individual toxins before summation of the concentrations. For ergot alkaloids, the correction can also be done based on the recovery obtained for each of the epimer pairs.

3.7 Reporting of sum-toxin results

In case the maximum level has been set for the sum of toxins (e.g. aflatoxins, T2/HT2-toxin, fumonisins, ergot alkaloids), the analytical results of all individual toxins should be reported. For ergot alkaloids it is also allowed to report the sum of each of the six epimer pairs instead of the 12 individual epimers.

For compliance verification with the sum-ML, a lower-bound approach is applied which means that results for individual toxins that are <LOQ will be replaced by zero for the calculation of the sum.

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References

[1] Burns DT, Danzer K, Townshend K. 2002. Use of the terms "recovery" and "apparent recovery" in analytical procedures (IUPAC Recommendations 2002), Pure Appl. Chem., 2002, 74(11), 2201-2205.

[2] SANTE/12089/2016. Guidance document on identification of mycotoxins in food and feed. https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_guid-doc-ident-mycotoxins.pdf

[3] Magnusson B and Örnemark U (eds.). 2014. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). Available from www.eurachem.org.

[4] Wenzl T, Haedrich J, Schaechtele A, Robouch P, Stroka J. 2016. Guidance document on the estimation of LOD and LOQ for measurements in the field of contaminants in feed and food; EUR 28099, Publications Office of the European Union, Luxembourg, 2016, ISBN 978-92-79-61768-3; doi:10.2787/8931.

https://publications.jrc.ec.europa.eu/repository/bitstream/JRC102946/eur%2028099%20en_lod%20loq%20guidance%20document.pdf.

[5] CEN/TS 17455:2020. 2020. Animal feeding stuffs - Methods of sampling and analysis - Performance criteria for single laboratory validated and ring-trial validated methods of analysis for the determination of mycotoxins.

[6] Thompson M, Sykes M, Wood R. 2019. Comparisons between reproducibility standard deviations (SD_R) derived from proficiency tests and from collaborative trials: mycotoxins in food. Accreditation and Quality Assurance. https://doi.org/10.1007/s00769-019-01413-8

[7] Pesticides EURLs, EUPT General Protocol. http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=821&LabID=100&Lang=EN

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Appendix 1. Definitions for recovery.

1. IUPAC definition

Source: IUPAC Recommendations 2002, Pure Appl. Chem., 2002, 74(11), 2201-2205

RECOVERY or RECOVERY FACTOR: yield of a preconcentration or extraction stage of an analytical process for an analyte divided by amount of analyte in the original sample.

APPARENT RECOVERY: observed value, x(obs), derived from an analytical procedure by means of a calibration graph, divided by reference value, x(ref).

2. EU report 2004

Source: Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU food and feed legislation, with particular reference to community legislation concerning contaminants in food and undesirable substances in feed.

https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_analysis-report_2004_en.pdf

"Recovery" is the amount of material extracted for analysis as a fraction of the amount present. In most analytical work, not all the material is recovered (i.e. the "recovery" is less than 100%).

3. CEN TC327/WG5 (natural toxins in feed)

Source: CEN/TS 17455:2020, Animal feeding stuffs - Methods of sampling and analysis - Performance criteria for single laboratory validated and ring-trial validated methods of analysis for the determination of mycotoxins.

Recovery: percentage of the true concentration of a substance recovered during the analytical procedure (details see next page)

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CEN/TS 17455:2020

3.10
recovery

R_{rec}

percentage of the true concentration of a substance recovered during the analytical procedure

Note 1 to entry: An ideal approach to demonstrate that recovery is consistent over the whole working range is to conduct a series at least 4 fortification experiments over the whole working range. Linearity shall be demonstrated by a "lack of fit" test. The slope of the curve for recovery determination (× 100 for expression in %), provided the fortified increment mass fraction (c_+) is plotted as *X*-axis against the measured mass fraction (c_f- c_0) on the *Y*-axis.

Note 2 to entry: Practical information for considerations in setting up recovery experiments are given in [25].

Note 3 to entry: The relative recovery (R_{rec}) can be expressed as:

$$R_{rec}(\%) = \frac{x}{x_{ref}} \times 100 \quad (9)$$

where:

x is the measured concentration (mass fraction);

x_{ref} is the reference concentration (mass fraction)

Note 4 to entry: For fortified (spiked) samples, the relative recovery (R_{rec}) is the difference observed after analysis of the test material and fortified (spiked) to the test material and can be expressed as:

$$R_{rec}(\%) = \frac{x_f - x_0}{x_{spike}} \times 100 \quad (10)$$

where:

x_f is the measured concentration (mass fraction) in the spiked or fortified sample;

x_0 is the measured concentration (mass fraction) in the unfortified sample;

x_{spike} is the spiked/added concentration (mass fraction).

Note 5 to entry: Bias and recovery are correlated as follows: Recovery (%) + bias (%) = 100 %.

Note 6 to entry: The amount added for recovery estimation should be a substantial fraction of, or more than, the amount present in the unfortified material. Ideally, the unfortified material should contain no measurable level of the analyte under test.

Note 7 to entry: A true or assigned value is known only in cases of fortified materials, certified reference materials, or by analysis by another (presumably unbiased) method. The concentration (mass fraction) in the unfortified material is obtained by direct analysis or by the method of standard additions. In other cases, there is no direct measure of bias, and consensus values derived from the collaborative study itself often can be used for the reference point.

[SOURCE: EuraChem Guide, see [19], modified]