Quality and reliability in mycotoxin measurement

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Summary

The quality and reliability of the analytical results which should estimate as close as possible the "true value" of any mycotoxin contamination depend of several factors. The availability and reliability of analytical procedures used to estimate that value are more and more considered before any value or maximal limit in any legislation is developed. It is now considered that validated methods and/or standards validated through inter laboratory studies organised by recognised organisations are the most reliable analytical procedures. The quality and reliability of the analytical results depend not only of the performance on the methodology but also on the performance of the laboratory. Analytical laboratories involved in official programmes focused on mycotoxin occurrence may be assumed to be "in control". The current recommendations indicate that such laboratories should be formally accredited, participate in proficiency testing schemes, use internal quality control procedures and use appropriately validated methods of analysis. The way of expressing results by the analysts is very important also. More and more countries or regulation bodies are recommending that the "recovery factor" should be considered and consequently included in the expression of results as "estimated value". Analytical results actually take more and more the form " $a \pm 2u$ " where "a" is the best estimate of the true value and "u" is known as the "standard measurement uncertainty". The measurement uncertainty takes into account both the "trueness" (average departure from a true value) and the "precision" (the degree to which successive results agree with each other).

Key words : mycotoxins, methods, validation, accreditation, recovery factor, uncertainty.

Introduction

To improve the consumer protection against adverse effects due to mycotoxins, both number of countries setting regulations and number of considered compounds are increasing. Consequently, there are a number of actions that may be taken by those responsible for the application of food and feed legislation which can directly affect producers, manufacturers international trade. So, because of any mycotoxin

measurement may have a influence on the judgement that may be made with regard to the compliance of a lot with respect to a legislative specification, it is important that the estimated value found by analytical procedure should be the closest of the “true value”.

Quality of the analytical methodology

The estimate of the value is of course dependent upon the method of analysis used, but is also dependent on the method of sampling used to verify compliance of a lot with the specification. The reliability of analytical data is more and more based on the use of validated methods. Methods of analysis are validated in accordance with international protocols, including those based on performance criteria, such as those accepted by the European Committee for Standardisation (CEN), the International Organisation for Standardisation (ISO) and the International Union of Pure and Applied Chemistry (IUPAC) and the Association of Official Analytical Chemists (AOAC) harmonized protocol. The current requirements for a quantitative method validation follow the standard ISO 5725 or the IUPAC/AOAC international Harmonised Guidelines.

The following criteria are taken account:

- accuracy,
- applicability (matrix and concentration range),
- limit of detection,
- limit of quantification,
- precision ; repeatability intra-laboratory (within laboratory) ; reproducibility inter-laboratory (within and between laboratories) generated from collaborative trial data and where performance criteria for analytical methods have been established ; criteria compliance tests ; rather than measurement uncertainty considerations ;
- recovery,
- selectivity,
- sensitivity,
- linearity.

It is now widely accepted that validated methods and/or standard methods validated through inter laboratory studies or collaborative trials organised by recognised organisations are the most reliable analytical procedures. Table 1 compiles a selection of methods for mycotoxin analysis recently validated through the Harmonized Int'l protocol under the auspices the Measurement and Testing Programme of the European Commission. These methods were also approved as First Action Official Methods of Analysis by AOAC Int'l.

In particular situations where methods of analysis are only validated within a single laboratory (“in-house validation”), the validation protocol should be based on criteria

compliance tests.

Table 1. Selection of full validated methods (ISO 5725) for mycotoxins EN and AOAC (except*)

Mycotoxins	Matrices	LOQ $\mu\text{g/kg}$	Recov %	R _{inter} %
Aflatoxins B&G	Peanut Butter, Pistachios, Figs, Paprika	AFB ₁ : 0.2 $\mu\text{g/kg}$ AFs Total : 0.4 $\mu\text{g/kg}$	82 - 109	9 - 32
Aflatoxins B&G	Baby Food	AFB ₁ : 0.02 $\mu\text{g/kg}$	92 - 101	9 - 32
Aflatoxin M ₁	Whole Liquid Milk	0.005 $\mu\text{g/L}$	70 - 95	21 - 26
Ochratoxin A	Barley Coffee	0.6 $\mu\text{g/kg}$ 0.3 $\mu\text{g/kg}$	65 - 11365 - 97	17 - 3314 - 26
Ochratoxin A*	Baby Food	0.008 $\mu\text{g/kg}$	108	29 - 45
Patulin	Apple Juice Apple Puree	15 $\mu\text{g/kg}$	69 - 10980 - 104	11 - 3329 - 36
Fumonisin	Corn Flour, corn flakes	-	75 - 110	21 - 31

Quality of the analytical laboratory

Quality assurance The estimate of the value is not only dependent upon the method of sampling and the method of analysis used, but also is dependent on the quality and reliability of analytical laboratory. Analytical laboratories involved in mycotoxin analysis may be assumed to be “in control”. The management of the quality assurance system covers some general aspects such as analytical sample tracking (until assay report issue), staff and instrument management, internal inspection, identification of preventives measures to prevent failures and discrepancies, and corrective actions to recover the appropriate quality after failure or discrepancy occurrence inside the system. Some particular technical aspects contribute to prove the laboratory competency such as the implementation of internal control figures by using: certified reference materials, validated methods when available or at least negative and positive control samples and intra-laboratory validated methods, and participation to proficiency testing¹⁾.

Certified reference materials Several organisms around the world such as the Institute for Reference Materials and Measurements (European Commission, E U), or the National Institute of Standard and Technology (Department of Commerce, USA), are currently preparing Certified Reference Materials (CRMs). The certification of the content is obtained through a statistical analysis from an inter laboratories assay. In this specific case any kind of analytical technique is not recommended.

Some CRMs which contain a certified value of a specific mycotoxin in a specific naturally contaminated matrix are already commercially available or are in preparation such as:

- Aflatoxin B₁ in peanut butter
- Aflatoxin B₁ in animal feed
- Aflatoxin M₁ in milk
- Ochratoxin A in wheat
- Vomitoxin (DON) in corn
- Vomitoxin (DON) in wheat
- Zearalenon in corn

Proficiency testing Several organisms around the world are currently organising proficiency tests (PTs) specific for mycotoxins. In PT, data from laboratories are:

- analysed according to appropriate statistical tests
- checked for trueness and inter-laboratory reproducibility

PTs help laboratories to check their competency status, and in the case laboratories do not pass the statistical tests, these labs can carry out corrective actions to improve or recover their analytical competency.

Accreditation Accreditation is a necessity for any analytical laboratory wishing to be recognised as competent. The relevant international standard EN ISO 17025 was established for that purpose.

Accreditation consideration is based on five key aspects: the laboratory should give the proof of the use of:

1. appropriate analytical instrumentation
2. appropriate analytical materials, products and reagents
3. reliable analytical methods
4. laboratory facilities in conformity
5. laboratory staff with competency

To meet these keys aspects it is necessary to implement:

- a global quality approach handled and by a specific responsible manager
- a quality assurance system

Accreditation brings some internal benefits such as improvement of local work organisation, increased motivation of laboratory staff and optimisation of analytical tools, and external benefits such as recognition of competency by an independent body and possibility to get the agreement for official controls or to be designated as reference laboratory.

Expressing results

The way of expressing results by the analysts is of particular importance when

assessing the conformity of a result to a specification. It is essential that interpretation of analytical results is similar. Without it there is no uniform interpretation of any food legislation.

The use of recovery factor The use of recovery information in analytical measurement is a difficult and contentious issue. Some countries or regulation bodies are more and more recommending that the recovery factor should be considered and consequently that the result to be corrected for recovery, as it is already done for some analytes (such as veterinary drug residues).

Measurement of uncertainty It considers the treatment of the measurement uncertainty in the interpretation of a specification. Both aspects are to be considered: how to estimate the measurement uncertainty, and how to take it into account.

Quantitative analytical results are currently only concerned. Qualitative determinations are also important but there are currently no internationally accepted recommendations and approaches for the estimation of the uncertainty of such determinations. However, these are currently being developed.

Analytical results actually are more and more reported under the form " $a \pm 2u$ " where " a " is the best estimate of the true value of the concentration of the analytical result and " $4u$ " is the range within which the true value is estimated, with a given probability (95 %). The value of " u " is known as the "standard measurement uncertainty" and may be estimated and expressed by the analyst. The "measurement uncertainty takes into account both the "trueness" (average departure from a true value) and the "precision" (the degree to which successive results agree with each other). The range within which " a " is likely to fall - that is the uncertainty in " a " - depends on the inherent "trueness" and precision of the analytical method, as well as on the way the method is implemented in the laboratory.

There are many procedures available for estimating the measurement uncertainty of a result. However at that time the approach which is preferred for food analysis (both chemical and microbiological) by the scientific community and recommended by bodies such as the Codex Committee on Methods of Analysis and Sampling is a global approach, based on the standard-deviation of reproducibility S_R on the final result of the analysis ($u = S_R$)

Different possibilities are left open to derive S_R :

- intra-laboratory S_R , estimated by the laboratory itself which will then derive its own measurement uncertainty
- inter-laboratory S_R , from validation data on the method used, derived from an inter-laboratory trial ^{*)}

(*) At the condition that the laboratory demonstrates that it is able to meet this S_R before using it to derive its own measurement uncertainty.

- inter-laboratory S_R derived from a proficiency trial^{*)}
- Horwitz value can be used for most of contaminants but at levels above mcg per kg^{*)}

Conclusion

The analytical “feasibility” for a reliable measurement of toxins and some others contaminants is more and more considered by the regulators before any value or maximal limit in any legislation is settled. Moreover some regulatory recommendations for example made by some committees of Codex Alimentarius (CC) in particular CC on Food Additives and Contaminants (CCFAC) specify the term of “as lowest as reasonably achievable” (ALARA).

It is recommended to define common and uniform criteria for methods of analysis and sampling procedures, as well as their common implementation and interpretation, in order to avoid different judgements as to whether any particular lot is in compliance with its specification. It would also be very useful that future legislations containing maximum levels should always address how analytical results are to be expressed and interpreted. Hopefully, many international organisations such as “Codex Committee on Methods of Analysis and Sampling” (CCMAS) involving IUPAC, AOAC and ISO and European Commission Scientific Cooperation Task 9.1²⁾ are currently working on these aspects and in these directions. Documents such as “International Guidelines on the Use of Recovery Factors when Reporting Analytical Results, and various Guides prepared dealing with Measurement Uncertainty³⁾ are under discussion and preparation.

References

- 1) “Council Directive 93/99/EEC on the Subject of Additional Measures Concerning the Official Control of Foodstuffs”, *O. J.*, 1993, L290.
- 2) Scientific Co-operation Task 9.1 on the “Preparation of a working document in support of the uniform interpretation of legislative standards and the laboratory quality standards prescribed under Directive 93/99/EEC”, downloadable from http://europa.eu.int/comm/food/fs/scoop/9.1_fr_en.pdf
- 3) Thompson, M., Ellison, S.L.R., Fajgelj, A., Paul Willetts, P., Wood, R.: *Pure Appl. Chem.*, **71**, 337-348 (1999)