AOAC Agricultural Materials Task Force
Sub-Committee on Feed Additives and Contaminants

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Project:

A method for the determination of multiple mycotoxins in feeds and raw grains intended for feeds

Project Lead:

Method Needs Statement and Validation Criteria

1. Method Needs Statement

Mycotoxins are naturally occurring, secondary metabolites of moulds and fungi which may invade cereal grains. Hence feeds, and grains intended for feeds, may be contaminated with mycotoxins. Feeds contaminated with mycotoxins may result in decreased weight gain, general poor performance, or serious mycotoxicosis in poultry, swine and livestock.

Guidelines for the maximum levels of aflatoxins (AFs), deoxynivalenol (DON), T-2 toxin (T-2), ochratoxin A (OTA), zearalenone (ZON) and total fumonisins (FBs) in various feeds and feedstuffs exist in many countries (see attachment A).

Traditionally, laboratories determine the levels of mycotoxins in grains and feedstuffs by sequentially analysing samples for each individual or group of mycotoxins. For example, validated methods exist for the determination of aflatoxins B1, B2, G1 and G2; other methods exist for the determination of DON and T-2, fumonisins B1 and B2, and individual methods exist for the determination of OTA and ZON. Employing a sequential process for the determination of all of these mycotoxins is time-consuming and expensive.

Therefore, the need for a cost effective, validated method capable of determining all or at least many of these mycotoxins in grains and feedstuffs is evident.

2. Performance Characteristics

The following performance characteristics must be demonstrated by the method.

2.1 Selectivity (Specificity)

The method must be capable of detecting all or most of the following: aflatoxin B1, total aflatoxins (B1+B2+G1+G2), DON, T-2, OTA, ZON, and total FBs (FB1+FB2+FB3). The method must be capable of distinguishing these mycotoxins from each other as well as from other substances within grains and feedstuffs.

2.2 Target Quantitation Levels:

The method must be capable of quantifying the specified mycotoxins in grains and feeds at the levels indicated in Table 1.

International guidelines for mycotoxins vary dramatically from country to country. Within countries, guidelines vary for different grains and feedstuffs, based upon their intended use. Therefore, the ideal method would have a Limit of Quantitation (LOQ), for each mycotoxin, equal to or better than the lowest known global guideline. This is considered an unattainable requirement. Therefore, the project team concluded that candidate methods should attain, as a minimum requirement, a "target quantitation level" for each mycotoxin. The "target quantitation level" for each mycotoxin was chosen by concensus by the project team, ensuring that each "target quantitation level" was equal to or lower than the median value of all known global guidelines (Attachment A).

Candidate methods will be evaluated against the "target quantitation level" as a minimum requirement, and their demonstrated ability to achieve lower LOQs will be considered a competitive advantage.

2.3 Operational range:

The method must be capable of detecting and quantifying the specified mycotoxins over the ranges indicated in Table 1.

2.4 Accuracy:

The method must demonstrate accuracy as specified in Table 1. This accuracy requirement must be met by measuring naturally incurred or fortified mycotoxins in grains and feedstuffs, at levels 2x the target quantitation level.

The Commission of the European Communities, in a draft Commission directive (SANCO-0023-2004 rev 1) recommends recoveries of 70 - 110% for DON methods (>100 ng/g), 60 – 120% for T-2 methods (<250 ng/g), 70 – 120% for ZON methods, and 70 – 110% for individual FB methods (>500 ng/g).

The AOAC's Single Lab Validation document recommends general recovery limits of 70 –125% at levels of 10 ng/g, and 75 – 120% at 1000 ng/g. It notes, however, that "These limits may be modified as needed in view of the variability of individual results or which set of regulatory requirements are referenced". The document also points out Codex limits (for veterinary drug residues) of 60 –120% at 1 to 10ng/g, 70 – 110% at 10 to 100ng/g, and 80 –110% at >100 ng/g. The Project Team has selected the Codex accuracy requirements.

AOAC's Single Lab Validation document recommends that accuracy be measured at "1x or 2x the expected concentration". For the purposes of this document, the "target quantitation level"

may be considered the "expected concentration". The project team has chosen to require accuracy measurements to be made at "2x the target quantitation level".

2.5 Repeatability

The method must demonstrate a repeatability coefficient of variation of less than 25% for each mycotoxin. The repeatability shall be measured by multiple analyses of naturally incurred or fortified mycotoxins in grains and feeds, at 2x the target quantitation level.

2.6 Reproducibility

The method must demonstrate a reproducibility coefficient of variation of less than 40% for each mycotoxin. The reproducibility shall be measured by multiple analyses of naturally incurred or fortified mycotoxins in grains and feeds, at 2x the target quantitation level.

3. Special consideration criteria

In addition to LOQs below that of the "target quantitation level", as described in Sec 2.2, candidate methods will also be evaluated against subjective criteria including method simplicity, method costs, use of commercially available consumables and common laboratory instrumentation, and existence of in-house, single-laboratory validation. The project team will judge these criteria and will recommend method(s) for formal validation. The sub-committee as a whole will confirm the project team's recommendation.

4. Method validation protocol

A validation protocol specific to the proposed method of analysis will be developed by the project team, through consultation with the method's author or sponsor, and approved by the sub-committee as a whole.

5. Prospective technologies

At this time, three technologies that may be able to address the method needs have been identified. They are 1) a suite or battery of immunoassays, 2) hplc/ms, and 3) hplc with a photochemical reactor, post column derivatisation, UV and fluorescence detection. Both 2) and 3) would likely involve the clean-up of crude sample extracts with a commercially available clean-up column. Each technology has its own advantages and disadvantages.

Table 1. Required Method Performance Characteristics:

Table 1a. Fusarium Toxins

	DON	T-2	OTA	ZON
Target Quantitation Level (ng/g)	1000	100	100	500
Operational Range (ng/g)	100 - 10,000	10 - 1000	10 - 1000	50 - 5,000
Accuracy	80 - 110%	70 - 110%	70 - 110%	80 – 110%

Table 1b. Aflatoxins

	AFB1	Total AFs
Target Quantitation Level (ng/g)	5	10
Operational Range (ng/g)	0.5 – 50	1 – 100
Accuracy	60 – 120%	60 – 120%

Table 1c. Fumonisins

	Total FBs
Target Quantitation Level (ng/g)	1000
Operational Range (ng/g)	100 – 10,000
Accuracy	80 – 110%