Method Name: Quantitative Analysis of Mycotoxins in Cannabis Biomass and Cannabis Derived Products

Purpose:
AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for methods being considered for *Performance Tested Methods* or AOAC *Official Methods of Analysis* and can be used as acceptance criteria for verification at user laboratories.[1]

Approved by:
Final version date:
Effective date:

Intended Use: Testing of cannabis biomass and cannabis derived products.

1. **Applicability**:

Method, or a suite of methods, to identify and quantify Ochratoxin A, Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, and Aflatoxin G_2 in cannabis biomass, hemp and/or cannabis derived products. Ochratoxin A, Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2 are required analytes. The testing of other toxins in addition to the required toxins can be submitted.

2. **Analytical Technique**:

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

3. **Definitions**:

Analytes
Ochratoxin A (CAS 303-47-9)
Aflatoxin B_1 (CAS 1162-65-8)
Aflatoxin B_2 (CAS 7220-81-7)
Aflatoxin G_1 (CAS 1165-39-5)
Aflatoxin G_2 (CAS 7241-98-7)

[1] Refer to Appendix F: *Guidelines for Standard Method Performance Requirements* in the 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012).

Total aflatoxins: sum of Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1 and Aflatoxin G_2

The testing of other toxins in addition to this list may be possible.

Matrices

Cannabis biomass. —Plant material from *Cannabis spp*. and its chemical varieties or chemotypes. For example, flower, trim and fiber. Hemp is included in this definition of cannabis biomass.

Cannabis derived products. — Products or extracts derived from cannabis plant material. Derivative products include but are not limited to:

a. Ingestible / Edibles
b. Inhalation products
c. Concentrates and Extracts
d. Hempseed and hempseed oil

Method developers may choose one or more of the suggested matrices. Method developers must specify the matrix or matrices used.

Quantitative Method of analysis for which response is the amount of the analyte measured either directly (enumeration in a mass or a volume) or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

Limit of Quantitation (LOQ). — The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result (AOAC SMPR guidance 3.5). Accordingly, the LOQ is the lowest concentration or mass that can be reported as a numerical value. Scientific justification for the procedure used to determine LOQ should be provided.

Limit of Detection (LOD). — The limit of detection (LOD) is the smallest measured concentration of an analyte from which it is possible to deduce the presence of the analyte in the test sample with acceptable certainty. There are several scientifically valid ways to determine LOD and any of these could be used as long as a scientific justification is provided for their use.[2]

For examples, see *Guidance for Industry Studies to Evaluate the Metabolism and Residues Kinetics of Veterinary Drugs in Food-Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies*[2]

Recovery. — The fraction or percentage of fortified or incurred analyte that is recovered when the test sample is analyzed using the entire method.

Repeatability. — Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period; expressed as the repeatability standard deviation (SD_r) or % repeatability relative standard deviation (% RSD_r).

[2] Guidance for Industry Studies to Evaluate the Metabolism and Residues Kinetics of Veterinary Drugs in Food-Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies, U.S. Food & Drug Administration, (2015).

Reproducibility. — The standard deviation or relative standard deviation calculated from among-laboratory data; expressed as the reproducibility standard deviation (SD_R) or % reproducibility relative standard deviation (% RSD_R).

Measurement Uncertainty. — Non-negative parameter characterizing the dispersion of the values being attributed to the measured value.[3]

4. Method Performance Requirements:

Table 1: Target levels for analytes to be included in method. Example regulatory limits can be found in the Appendix 1.

Compound	Target level (µg/kg)*	Target LOQ (µg/kg)
Ochratoxin A	20	Less than target level
Aflatoxin B_1	5	Less than target level
Total aflatoxins^	20	Less than target level

* Methods with lower target levels will be accepted. LOQ should be less than the target level a method developer is using for their method.

^ Total aflatoxins: sum of Aflatoxin B_1, Aflatoxin B_2, Aflatoxin

Table 2: Specific method performance requirements

Parameter	Requirement
Limit of quantitation, LOQ (µg/kg)	Less than target level in Table 1
Applicable range, (µg/kg)	Must be stated
Limit of detection, LOD (µg/kg)	Must be determined and method of determination detailed. For example: 3.3 x standard deviation of blank sample (refer to ICH Q2 guidance[4])
Recovery, %	60-120%
Repeatability, RSD_r, %	≤ 22% for 1 to <100 µg/kg ≤ 11% for 100 to 999 µg/kg
Reproducibility, RSD_R, %	≤ 44% for 1-999 µg/kg
Measurement Uncertainty	Must be determined and method of determination detailed.

[3] Guidelines for the Validation of Chemical Methods for the FDA Foods Program, 3rd Edition, U.S. Food & Drug Administration, (2019).

[4] ICH Topic Q2 (R1), Validation of Analytical Procedures: Text and Methodology, International Council for Harmonisation, (1995). Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf

5. **System Suitability Tests and/or Analytical Quality Control**
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

6. **Reference Material(s)**
Refer to Annex F: Development and Use of In-House Reference Materials in Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

ISO 17034:2016 General requirements for the competence of reference material producers; International Organization for Standardization (2016). Available at: https://www.iso.org/obp/ui/#iso:std:iso:17034:en

ISO GUIDE 80:2014 Guidance for the in-house preparation of quality control materials (QCMs); International Organization for Standardization (2014). Available at: https://www.iso.org/obp/ui/#iso:std:iso:guide:80:ed-1:v1:en

7. **Validation Guidance**

All claimed matrices shall be evaluated.

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_k.pdf

Guidelines for the Validation of Chemical Methods for the FDA Foods Program, 3rd Edition, U.S. Food & Drug Administration, (2019). Available at: https://www.fda.gov/media/81810/download

Guidance for Industry Studies to Evaluate the Metabolism and Residues Kinetics of Veterinary Drugs in Food-Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies, U.S. Food & Drug Administration, (2015). Available at: https://www.fda.gov/media/78356/download

ICH Topic Q2 (R1), Validation of Analytical Procedures: Text and Methodology, International Council for Harmonisation, (1995). Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf

Appendix 1: Example regulatory limits based on cannabis, hemp or non-cannabis commodities.

	Example Regulatory Limits* (µg/kg)						
	Ochratoxin A	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins^	Total aflatoxins + Ochratoxin A
Canada		<5				<20	
EU		2				4	
Australia	20					4	
California, USA	≤20	≤2				≤20	
Colorado, USA	< 5	< 5				<20	
Oregon, USA	<20					<20	
Pennsylvania, USA		≤5				≤20	≤20
Florida, USA	≤20	≤20	≤20	≤20	≤20		
Illinois, USA	≤20	≤20	≤20	≤20	≤20		

* based on cannabis, hemp or non-cannabis commodities at the time of publication, XXXXX, 2021.

^ Total aflatoxins: sum of Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1 and Aflatoxin G_2