AOAC SMPR® 2021.010

Standard Method Performance Requirements (SMPRs®) for Quantitative Analysis of Mycotoxins in Cannabis Biomass and Cannabis-Derived Products

Intended Use: Testing of Cannabis Biomass and Cannabis-Derived Products

1 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 stakeholders composed of representatives from 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 [refer to Appendix F: *Guidelines for Standard Method Performance Requirements* (2019) 21st Ed., *Official Methods of Analysis of AOAC INTERNATIONAL*, http://www.eoma.aoac.org/app_f.pdf].

2 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, and aflatoxin G_2 are required analytes. Testing of other toxins in addition to the required toxins can be submitted.

3 Analytical Technique

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

4 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.

Total aflatoxins.—Sum of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. Testing 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 ingestible/edibles, inhalation products, concentrates and extracts, and hempseed and hempseed oil.

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

Limit of detection (LOD).—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. 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*, U.S. Food and Drug Administration (2015).

Limit of quantitation (LOQ).—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result [refer to Appendix F: *Guidelines for Standard Method Performance Requirements* (2019) 21st Ed., *Official Methods of Analysis of AOAC INTERNATIONAL*, http://www.eoma.aoac.org/app_f.pdf]. Accordingly, 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.

Measurement uncertainty.—Non-negative parameter characterizing the dispersion of the values being attributed to the measured value [*Guidelines for Validation of Chemical Methods for FDA Foods Program* (2019) 3rd Ed., U.S. Food and Drug Administration].

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

Recovery.—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).

Reproducibility.—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).

5 Method Performance Requirements

See Tables 1 and 2.

6 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.

7 Reference Material(s)

Refer to:

Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements* (2019) 21st Ed., *Official Methods of Analysis of AOAC INTERNATIONAL*, Rockville, MD, USA, http://www.eoma.aoac.org/app_f.pdf

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

Table 1. Target levels for analytes to be included in method; example regulatory limits can be found in Appendix 1

Compound	Target level, µg/kg[a]	Target LOQ, µg/kg
Ochratoxin A	20	Less than target level
Aflatoxin B_1	5	Less than target level
Total aflatoxins[b]	20	Less than target level

[a] Methods with lower target levels will be accepted. LOQ should be less than the target level a method developer is using for their method.
[b] Total aflatoxins = Sum of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2.

Table 2. Specific method performance requirements

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

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

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

8 Validation Guidance

All claimed matrices shall be evaluated.

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2019) 21st Ed., *Official Methods of Analysis of AOAC INTERNATIONAL*, Rockville, MD, USA, http://www.eoma.aoac.org/app_d.pdf

Appendix K: *Guidelines for Dietary Supplements and Botanicals* (2019) 21st Ed., *Official Methods of Analysis of AOAC INTERNATIONAL*, Rockville, MD, USA, http://www.eoma.aoac.org/app_k.pdf

Guidelines for Validation of Chemical Methods for FDA Foods Program (2019) 3rd Ed., U.S. Food and Drug Administration, 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 (2015) U.S. Food and Drug Administration, https://www.fda.gov/media/78356/download

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

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Appendix 1. Example regulatory limits based on cannabis, hemp, or noncannabis commodities

	Example regulatory limits,[a] µg/kg						
	Ochratoxin A	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins[b]	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		

[a] Based on cannabis, hemp, or noncannabis commodities at the time of publication (October 2021).
[b] Total aflatoxins = Sum of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2.