Method Name: Mycotoxin Screening Technique in Cannabis plant material and cannabis derivatives

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 method 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: Routine testing of cannabis plant material and cannabis derivative products.

1. **Applicability**:

   Detection of the listed mycotoxins, it is recommended that all positive results by confirmed by a separate analytical technique (for detections above the applicable regulatory limit.)

   Method developers may choose to test for all five toxins (analytes, see Section 3) individually, one or more toxins individually (e.g. Aflatoxin B_1 only), Ochratoxin A and the four Aflatoxins as a total parameter, or any other combination thereof. The method scope shall specifically define the individual toxin(s) being tested or specify if the testing for Aflatoxin testing will occur as a Total Aflatoxin parameter (i.e. single result representing the total of the four Aflatoxins).

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)."

Matrices:

Cannabis plant material.—Plant material from Cannabis spp. and its chemical varieties or chemotypes.

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

a. Ingestibles / Edibles
b. Inhalation products
c. Concentrates and Extracts
d. Personal care products

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

Qualitative assay

A method of analysis with two possible outcomes.

Selectivity Study

A study designed to demonstrate that a candidate method does not detect non-target compounds, and at the same time, demonstrate a candidate method's ability to detect the analytes of interest.

Probability of Detection (POD)[2]

Reference: Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods, Official Methods of Analysis of AOAC INTERNATIONAL, 19th edition, 2012.

The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration.

4. Method Performance Requirements:

Table 1: Matrix Dependent Criteria

	Parameter	Parameter Requirements	Target Test Concentration (Ochratoxin A)	Total Aflatoxins (sum B_1, B_2, G_1, G_2)	Aflatoxin B_1	Minimum Acceptable Results
Single Laboratory Validation	POD @ low concentration	Minimum of 33 positive replicates per matrix type, spiked at the applicable regulatory limit.	Low concentration should equal to the applicable regulatory limit	Low concentration should equal to the applicable regulatory limit	Low concentration should equal to the applicable regulatory limit	90% POD§
	POD @ high concentration	Minimum of 5 replicates per matrix type spiked at the	highest concentration of the claimed	highest concentration of the claimed functional	highest concentration of the claimed	100% correct analyses are expected per matrix type‡

[2] Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods, Official Methods of Analysis of AOAC INTERNATIONAL, 19th edition, 2012.

		highest concentration of the claimed functional range of the method.	functional range of the method	range of the method	functional range of the method	
	POD in blank matrix	Minimum of 5 replicates per matrix type.	blank matrix	blank matrix	blank matrix	
Multi -Laboratory Validation	LPOD	Use Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods.	Low concentration should equal to the applicable regulatory limit			≥ 0.85§
			highest concentration of the claimed functional range of the method			≥ 0.95§
	LPOD $_{(0)}$		blank matrix			≤ 0.05§

Note: LPOD and LPOD $_{(0)}$ are not required for Single Laboratory Validations.

‡ 100% correct analyses are expected. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

§ 95% confidence interval

Table 2: Selectivity Study

	Parameter	Parameter Requirements	Final Test Concentration	Minimum Acceptable Results
Single Laboratory Validation	Target	Test each target compound at the final test concentration.	should equal to the applicable regulatory limit	100% positive results**
	Cross Reactivity	Test each cross reactivity panel compound at the final test concentration or at the highest expected matrix concentration in the case of naturally occurring matrix components. A list of potential non-target compounds for immunoassays is provided in Annex I.	highest concentration of the claimed functional range of the method	≥95% negative results

** 100% correct analyses are expected. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

5. System suitability tests and/or analytical quality control:

The controls listed in Table 3 shall be embedded in assays as appropriate. Interference controls should be used for method verification for each new matrix.

Table 3: Controls

Positive Control	This control is designed to demonstrate an appropriate test response. This positive control should be included at a low but easily detectable concentration and should monitor the performance of the entire assay. The purpose of using a low concentration of positive control is to avoid contamination of the test sample and/or instrument.	Single use per sample (or sample set) run	**Success** - Control detected at expected levels. **Failure** - Control not detected or at levels below expected.
Negative Control	This control is designed to demonstrate that the assay itself does not produce a positive detection in the absence of target compounds and any potential interference from a matrix. The purpose of this control is to rule-out contamination in the assay or test.	Single use per sample (or sample set) run	**Success** - No detections made. **Failure** - Detections made.

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

Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at http://www.eoma.aoac.org/app_n.pdf

Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at http://www.eoma.aoac.org/app_h.pdf

Annex I: Cross reactivity panel compounds

A suitable non-target panel shall be selected based on the analytical technique.

Neosolaniol (CAS 36519-25-2)
Zearalenone (CAS 17924-92-4)
Fumonisin B_1 (CAS 116355-83-0)
Deoxynivalenol (CAS 51481-10-8)
Sterigmatocystin (CAS 10048-13-2)