AOAC SMPR® 2020.013

Standard Method Performance Requirements (SMPRs®) for Mycotoxin Screening Technique in Cannabis Plant Material and Cannabis Derivatives

Intended Use: Routine Testing of Cannabis Plant Material and Cannabis Derivative 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 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 [refer to *Appendix F: Guidelines for Standard Method Performance Requirements* (2019) *Official Methods of Analysis of AOAC INTERNATIONAL*, 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA].

2 Applicability

Detection of listed mycotoxins (*see* analytes in *Defintions* section). It is recommended that all positive results be confirmed by a separate analytical technique (for detections above the applicable regulatory limit).

Method developers may choose to test for all five toxins (*see* analytes in *Defintions* section) 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 will occur as a total aflatoxin parameter (i.e., single result representing total of the four aflatoxins).

3 Analytical Technique

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

4 Definitions

Analytes:

Aflatoxin B_1.—CAS No. 1162-65-8.
Aflatoxin B_2.—CAS No. 7220-81-7.
Aflatoxin G_1.—CAS No. 1165-39-5.
Aflatoxin G_2.—CAS No. 7241-98-7.
Ochratoxin A.—CAS No. 303-47-9.

Matrices*:*

Cannabis derivatives.—Products or extracts derived from cannabis plant material. Derivative products include but are not limited to, ingestibles/edibles; inhalation products; concentrates and extracts; and personal care products.

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

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

Probability of detection (POD).—Proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. [*Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods* (2019) *Official Methods of Analysis of AOAC INTERNATIONAL*, 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA].

Qualitative assay.—Method of analysis with two possible outcomes.

Selectivity study.—Study designed to demonstrate that candidate method does not detect nontarget compounds, and, at the same time, demonstrate candidate method's ability to detect analytes of interest.

4 Method Performance Requirements

See Tables 1 and 2.

5 System Suitability Tests and/or Analytical Quality Control

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

6 Reference Material(s)

Annex F: Development and Use of In-House Reference Materials in *Appendix F: Guidelines for Standard Method Performance Requirements* (2019) *Official Methods of Analysis of AOAC INTERNATIONAL*, 21st Ed., 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

ISO Guide 80:2014 *Guidance for the 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

7 Validation Guidance

All claimed matrices shall be evaluated.

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

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

Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods (2019) *Official Methods of Analysis of AOAC INTERNATIONAL*, 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_n.pdf

Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods (2019) *Official Methods of Analysis of AOAC INTERNATIONAL*, 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_h.pdf

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Annex I: Cross-Reactivity Panel Compounds

A suitable nontarget panel shall be selected based on the analytical technique.

Deoxynivalenol.—CAS No. 51481-10-8.
Fumonisin B_1.—CAS No. 116355-83-0.
Neosolaniol.—CAS No. 36519-25-2.
Sterigmatocystin.—CAS No. 10048-13-2.
Zearalenone.—CAS No. 17924-92-4.

Table 1. Matrix-dependent criteria

Type of study	Parameter[a]	Requirements	Target test concn (ochratoxin A)	Total aflatoxins (sum B_1, B_2, G_1, G_2)	Aflatoxin B_1	Minimum acceptable results
Single-lab	POD at low concn	Min. of 33 positive replicates per matrix type, spiked at applicable regulatory limit	Low concn should equal applicable regulatory limit	Low concn should equal applicable regulatory limit	Low concn should equal applicable regulatory limit	90% POD[b]
	POD at high concn	Min. of 5 replicates per matrix type spiked at highest concn of claimed functional range of method	Highest concn of claimed functional range of method	Highest concn of claimed functional range of method	Highest concn of claimed functional range of method	100% correct analyses expected per matrix type[c]
	POD in blank matrix	Min. of 5 replicates per matrix type	Blank matrix	Blank matrix	Blank matrix	
Multi-lab	LPOD	Use *Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods*	Low concn should equal applicable regulatory limit			≥0.85[b]
			Highest concn of claimed functional range of method			≥0.95[b]
	$LPOD_{(0)}$		Blank matrix			≤0.05[b]

[a] LPOD and $LPOD_{(0)}$ not required for single-laboratory validations.
[b] 95% confidence interval.
[c] Some aberrations may be acceptable if aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

Table 2. Selectivity study

Parameter	Requirements	Final test concn	Minimum acceptable results
	Single-laboratory validation		
Target	Test each target compound at final test concn	Should equal applicable regulatory limit	100% positive results[a]
Cross-reactivity	Test each cross-reactivity panel compound at final test concn or at highest expected matrix concn in the case of naturally occurring matrix components. Potential nontarget compounds for immunoassays are provided in Annex I.	Highest concn of claimed functional range of the method	≥95% negative results

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

Table 3. Controls

Positive	Designed to demonstrate appropriate test response. Positive control should be included at low but easily detectable concn and should monitor performance of entire assay. Purpose of using low concn of positive control is to avoid contamination of 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.
Negativel	Designed to demonstrate that assay itself does not produce positive detection in absence of target compounds and any potential interference from matrix. Purpose of this control is to rule out contamination in assay or test.	Single-use per sample (or sample set) run	*Success*.—No detections made. *Failure*.—Detections made.