DRAFT AOAC SPDS Animal-Derived Protein SMPR, v4, 6/30/2016

Identification and Quantitation of Animal-Derived Proteins in Dietary Supplements

Intended Use: Reference method for cGMP compliance.

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 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.

2. **Applicability**:
Method must identify and quantify animal-derived proteins and their corresponding sources in the presence of potential adulterants in ingredients and finished dietary supplements.

3. **Analytical Technique**:
Any analytical technique is acceptable.

4. **Definitions**:

Protein
Naturally occurring and synthetic polypeptides having molecular weights greater than about 10000 daltons (the limit is not precise) (IUPAC Definition)

Limit of Quantitation (LOQ)
The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Limit of Detection (LOD)
The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

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
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).

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

5. **Method Performance Requirements**:
See table 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. **Potential 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

8. **Validation Guidance**:

Data demonstrating method performance for the animal-derived proteins listed in table 3 in the presence of the potential non-protein ingredients including adulterants listed in table 4 is recommended.

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 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

Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL.

9. **Maximum Time-To-Result:** None

Table 1: Method performance requirements (part 1)

Parameters	Acceptable Criteria
Analytical Range (%)	0.1 - 100
LOQ (%)	0.05
LOD (%)	0.025

Table 2: Method performance requirements (part 2)

	Ranges (%)	
	0.1-1	>1
Recovery (%)	90-110	97-103
% RSD_r	≤ 10	≤ 6
% RSD_R	≤ 12	≤8

Table 3 : Recommended Animal-Derived Proteins from these sources

Casein
Egg
Whey
Milk

Table 4: Non-Protein Ingredients Including Adulterants

Melamine
Urea
Free amino acids
Creatine
Caffeine
Taurine
Surfactants
Peptides (less than 10,000 daltons)

DRAFT AOAC SPDS Plant-Derived Protein SMPR, v4, 6/30/2016

Identification and Quantitation of Non-animal-Derived Proteins in Dietary Supplements

Intended Use: Reference method for cGMP compliance.

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 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.

2. **Applicability**:
Method must identify and quantify non-animal -derived proteins and their corresponding sources in the presence of potential adulterants in ingredients and finished dietary supplements.

3. **Analytical Technique**:
Any analytical technique is acceptable.

4. **Definitions**:

Protein
Naturally occurring and synthetic polypeptides having molecular weights greater than about 10000 daltons (the limit is not precise) (IUPAC Definition)

Limit of Quantitation (LOQ)
The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Limit of Detection (LOD)
The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

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
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).

Recovery

The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

5. **Method Performance Requirements**:
See table 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. **Potential 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

8. **Validation Guidance**:

Data demonstrating method performance for the non-animal-derived proteins listed in table 3 in the presence of the potential non-protein ingredients including adulterants listed in table 4 is recommended.

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 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

Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL.

9. **Maximum Time-To-Result:** None

Table 1: Method performance requirements (part 1)

Parameters	Acceptable Criteria
Analytical Range (%)	0.1 - 100
LOQ (%)	0.05
LOD (%)	0.025

Table 2: Method performance requirements (part 2)

	Ranges (%)	
	0.1-1	>1
Recovery (%)	90-110	97-103
% RSD_r	≤ 10	≤ 6
% RSD_R	≤ 12	≤8

Table 3 : Recommended Non-Animal-Derived Proteins from these sources

Algae
Canola (Rapeseed)
Flax
Hemp
Pea
Potato
Pumpkin
Quinoa
Rice
Soy
Wheat

Table 4: Non-Protein Ingredients Including Adulterants

Melamine
Urea
Free amino acids
Creatine
Caffeine
Taurine
Surfactants
Peptides (less than 10,000 daltons)

DRAFT AOAC SPDS Identification of Animal-derived Proteins V 4, 6.30.2016

Identification of Animal-Derived Proteins in Dietary Supplements

Intended Use: Reference method for cGMP compliance.

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 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.

2. **Applicability**:
   Method must identify animal-derived proteins and their corresponding sources in the presence of potential adulterants in ingredients and finished dietary supplements.

3. **Analytical Technique**:
   Any analytical technique is acceptable.

4. **Definitions**:

   Protein
   Naturally occurring and synthetic polypeptides having molecular weights greater than about 10000 daltons (the limit is not precise) (IUPAC Definition)

   Probability of Identification (POI)
   The proportion of positive analytical outcomes for an identification method for a given matrix at a given analyte level or concentration. LPOI is the Laboratory Probability of Identification.

5. **Method Performance Requirements**:
   See table 1.

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. **Potential 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

8. Validation Guidance:

Data demonstrating method performance for the animal-derived proteins listed in table 2 in the presence of the potential non-protein ingredients including adulterants listed in table 3 is recommended.

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 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

Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL.

9. Maximum Time-To-Result: None

Table 2 : Animal-derived Proteins from these sources

Casein
Egg
Whey
Milk

Table 3: Non-Protein Ingredients Including Adulterants

Melamine
Urea
Free amino acids
Creatine
Caffeine
Taurine
Surfactants
Peptides (less than 10,000 daltons)

DRAFT AOAC SPDS Identification of Plant-Derived Proteins V 4, 6.30.2016

Identification of ~~Plant~~Non-animal -Derived Proteins in Dietary Supplements

Intended Use: Reference method for cGMP compliance.

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 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.

2. **Applicability**:
Method must identify ~~plant~~non-animal-derived proteins and their corresponding sources in the presence of potential adulterants in ingredients and finished dietary supplements.

3. **Analytical Technique**:
Any analytical technique is acceptable.

4. **Definitions**:

Protein
Naturally occurring and synthetic polypeptides having molecular weights greater than about 10000 daltons (the limit is not precise) (IUPAC Definition)

Probability of Identification (POI)
The proportion of positive analytical outcomes for an identification method for a given matrix at a given analyte level or concentration. LPOI is the Laboratory Probability of Identification.

5. **Method Performance Requirements**:
See table 1.

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. **Potential 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

8. Validation Guidance:

Data demonstrating method performance for the plant-derived proteins listed in table 3 in the presence of the potential non-protein ingredients including adulterants listed in table 4 is recommended.

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 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

Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL.

9. Maximum Time-To-Result: None

Table 1: Method Performance Table

	Study	Parameter	Parameter Requirements	Target Test Concentration	Minimum Acceptable Results
Single Laboratory Validation	Matrix Study	POI @ low concentration	Minimum of 33 replicates representing all target analytes in Table 2.	0.1 %	90% POI† of the pooled data for all target compounds and matrices.
		POI @ high concentration	Minimum of 5 replicates per matrix type spiked at 10x the designated low level target test concentration.	10%	100% correct analyses are expected(1)
		POI @ zero concentration	Minimum of 5 replicates per matrix type.	0 %	
	Selectivity	False positive rate	Evaluate samples containing non-protein ingredients and adulterants listed in Table 3.	10 %	≤ 5%
Multi- Laboratory Validation	Matrix Study (2)	LPOI	Use Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods.	0.1 %	≥ 0.85†
				10 %.	≥ 0.95†
		$LPOI_{(0)}$		0 %	≤ 0.05†

Notes:
† 95% confidence interval
(1) 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.
(2) Multi-Laboratory Validation Matrix Study (LPOI and LPOI $_{(0)}$) are not required for First Action *Official Methods of Analysis* approval.

Table 2 : Recommended non-animal ~~Plant~~ Derived Proteins from these sources

Algae
Canola (Rapeseed)
Flax
Hemp
Pea
Potato
Pumpkin
Quinoa
Rice
Soy
Wheat

Table 3: Non-Protein Ingredients Including Adulterants

Melamine
Urea
Free amino acids
Creatine
Caffeine
Taurine
Surfactants
~~Hydrolized leather~~
~~Hydrolized proteins~~
Peptides (less than 10,000 daltons)