- 1 DRAFT AOAC SPDS Animal-Derived Protein SMPR, v4, 6/30/2016
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- Identification and Quantitation of Animal-Derived Proteins in Dietary Supplements
- Intended Use: Reference method for cGMP compliance.

7 1. Purpose: AOAC SMPRs describe the minimum recommended performance characteristics 8 to be used during the evaluation of a method. The evaluation may be an on-site 9 verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are 10 written and adopted by AOAC Stakeholder Panels composed of representatives from the 11 industry, regulatory organizations, contract laboratories, test kit manufacturers, and 12 academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their 13 evaluation of validation study data for method being considered for Performance Tested 14 Methods or AOAC Official Methods of Analysis, and can be used as acceptance criteria for 15 verification at user laboratories.

## 17 **2.** Applicability:

18 Method must identify and quantify animal-derived proteins and their corresponding sources 19 in the presence of potential adulterants in ingredients and finished dietary supplements.

## 21 **3.** Analytical Technique:

Any analytical technique is acceptable.

#### 24 **4.** Definitions:

### Protein

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

### 30 Limit of Quantitation (LOQ)

The minimum concentration or mass of analyte in a given matrix that can be reported as aquantitative result.

### 34 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.

### 37 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<sub>r</sub>); or % repeatability relative standard deviation
 (%RSD<sub>r</sub>).

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## 43 **Reproducibility**

- The standard deviation or relative standard deviation calculated from among-laboratory
   data. Expressed as the reproducibility standard deviation (SD<sub>R</sub>); or % reproducibility relative
   standard deviation (% RSD<sub>R</sub>).
- 47

## 48 Recovery

49 The fraction or percentage of spiked analyte that is recovered when the test sample is

- 50 analyzed using the entire method.
- 51

52 53 54	5.	Method Performance Requirements: See table 1 and 2.
55 56 57 58	6.	<b>System suitability tests and/or analytical quality control:</b> Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.
59 60	7.	Potential Reference Material(s):
61 62 63 64 65 66		Refer to Annex F: <i>Development and Use of In-House Reference Materials</i> in <u>Appendix F</u> : <i>Guidelines for Standard Method Performance Requirements</i> , 19 <sup>th</sup> Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: <u>http://www.eoma.aoac.org/app_f.pdf</u>
67 68	8.	Validation Guidance:
69 70 71 72		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.
73 74 75 76		<u>Appendix D</u> : Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19 <sup>th</sup> Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf
77 78 79 80		<u>Appendix F</u> : Guidelines for Standard Method Performance Requirements; 19 <sup>th</sup> Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf
81 82		Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL.
83 84 85	9.	Maximum Time-To-Result: None
86 87		Table 1: Method performance requirements (part 1)

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

- 92 93 94 Table 2: Method performance requirements (part 2)
- 95

	0.1-1	>1
Recovery (%)	90-110	97-103
% RSD <sub>r</sub>	≤ 10	≤ 6
% RSD <sub>R</sub>	≤ 12	≤8

Ranges (%)

96 97

#### 98 Table 3 : Recommended Animal-Derived Proteins from these sources

99 100 Casein

101 Egg

102 Whey

103 Milk

104

- 105
- 106 Table 4: Non-Protein Ingredients Including Adulterants
- 107
- 108 Melamine 109 Urea

110

Free amino acids 111 Creatine

112 Caffeine

113 Taurine

114 Surfactants

Peptides (less than 10,000 daltons) 115

- 1 DRAFT AOAC SPDS Plant-Derived Protein SMPR, v4, 6/30/2016
- 2 3

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- Identification and Quantitation of Non-animal-Derived Proteins in Dietary Supplements
- Intended Use: Reference method for cGMP compliance.
- 7 1. Purpose: AOAC SMPRs describe the minimum recommended performance characteristics 8 to be used during the evaluation of a method. The evaluation may be an on-site 9 verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are 10 written and adopted by AOAC Stakeholder Panels composed of representatives from the 11 industry, regulatory organizations, contract laboratories, test kit manufacturers, and 12 academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their 13 evaluation of validation study data for method being considered for Performance Tested 14 Methods or AOAC Official Methods of Analysis, and can be used as acceptance criteria for 15 verification at user laboratories.

## 17 **2.** Applicability:

- 18Method must identify and quantify non-animal -derived proteins and their corresponding19sources in the presence of potential adulterants in ingredients and finished dietary
- 20 supplements.
- 21

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- 22 **3.** Analytical Technique:
  - Any analytical technique is acceptable.
- 25 **4.** Definitions:
- 26 27
  - Protein
  - Naturally occurring and synthetic polypeptides having molecular weights greater than about 10000 daltons (the limit is not precise) (IUPAC Definition)

## 31 Limit of Quantitation (LOQ)

- The minimum concentration or mass of analyte in a given matrix that can be reported as a
   quantitative result.
- 35 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.

## 38 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<sub>r</sub>); or % repeatability relative standard deviation
 (%RSD<sub>r</sub>).

## 44 **Reproducibility**

- The standard deviation or relative standard deviation calculated from among-laboratory
  data. Expressed as the reproducibility standard deviation (SD<sub>R</sub>); or % reproducibility relative
  standard deviation (% RSD<sub>R</sub>).
- 48 49 **Rec** 
  - 9 Recovery

50 51 52		The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.
52 53 54	5.	Method Performance Requirements: See table 1 and 2.
55		
56	6.	System suitability tests and/or analytical quality control:
57		Suitable methods will include blank check samples, and check standards at the lowest point
58		and midrange point of the analytical range.
59		
60	7.	Potential Reference Material(s):
61		
62		Refer to Annex F: Development and Use of In-House Reference Materials in Appendix F:
63		Guidelines for Standard Method Performance Requirements, 19" Edition of the AOAC
64		INTERNATIONAL Official Methods of Analysis (2012). Available at:
65		http://www.eoma.aoac.org/app_f.pdf
66 67		
07 68	0	Validation Guidance:
60 60	0.	valuation Guidance.
70		Data demonstrating method performance for the non-animal-derived proteins
71		listed in table 3 in the presence of the potential non-protein ingredients
72		including adulterants listed in table 4 is recommended
73		
74		Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a
75		Method of Analysis; 19 <sup>th</sup> Edition of the AOAC INTERNATIONAL Official Methods of Analysis
76		(2012). Available at: http://www.eoma.aoac.org/app_d.pdf
77		
78		Appendix F: Guidelines for Standard Method Performance Requirements; 19 <sup>th</sup> Edition of the
79		AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at:
80		http://www.eoma.aoac.org/app_f.pdf
81		
82		Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis
83		(2016) 20th Ed., AOAC INTERNATIONAL.
84	9.	Maximum Time-To-Result: None
85		
86		
87		Table 1: Method performance requirements (part 1)

# Table 1: Method performance requirements (part 1)

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

- 90
- 91
- 92
- 93
- 94 95

## Table 2: Method performance requirements (part 2)

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## Ranges (%)

	0.1-1	>1
Recovery (%)	90-110	97-103
% RSD <sub>r</sub>	≤ 10	≤ 6
% RSD <sub>R</sub>	≤ 12	≤8

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98

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

- 100 101 Alga
- 101 Algae102 Canola (Rapeseed)
- 102 Curre 103 Flax
- 104 Hemp
- 105 Pea
- 106 Potato
- 107 Pumpkin
- 108 Quinoa
- 109 Rice
- 110 Soy
- 111 Wheat
- 112
- 113

## 114 Table 4: Non-Protein Ingredients Including Adulterants

- 115116 Melamine
- 117 Urea
- 118 Free amino acids
- 119 Creatine
- 120 Caffeine
- 121 Taurine
- 122 Surfactants
- 123 Peptides (less than 10,000 daltons)
- 124

1 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.

7 1. Purpose: AOAC SMPRs describe the minimum recommended performance characteristics 8 to be used during the evaluation of a method. The evaluation may be an on-site 9 verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are 10 written and adopted by AOAC Stakeholder Panels composed of representatives from the 11 industry, regulatory organizations, contract laboratories, test kit manufacturers, and 12 academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their 13 evaluation of validation study data for method being considered for Performance Tested 14 Methods or AOAC Official Methods of Analysis, and can be used as acceptance criteria for 15 verification at user laboratories.

### 17 **2.** Applicability:

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18 Method must identify animal-derived proteins and their corresponding sources in the 19 presence of potential adulterants in ingredients and finished dietary supplements.

## 21 **3.** Analytical Technique:

Any analytical technique is acceptable.

#### 24 **4. Definitions**:

25		
26		Protein
27		Naturally occurring and synthetic polypeptides having molecular weights greater than about
28		10000 daltons (the limit is not precise) (IUPAC Definition)
29		
30		Probability of Identification (POI)
31		The proportion of positive analytical outcomes for an identification method for a given
32		matrix at a given analyte level or concentration. LPOI is the Laboratory Probability of
33		Identification.
34		
35	5.	Method Performance Requirements:
36		See table 1.
37		
38	6.	System suitability tests and/or analytical quality control:
39		Suitable methods will include blank check samples, and check standards at the lowest point
40		and midrange point of the analytical range.
41		
42	7.	Potential Reference Material(s):
43		
44		Refer to Annex F: Development and Use of In-House Reference Materials in Appendix F:
45		Guidelines for Standard Method Performance Requirements, 19 <sup>th</sup> Edition of the AOAC
46		INTERNATIONAL Official Methods of Analysis (2012). Available at:
47		http://www.eoma.aoac.org/app_f.pdf
48		

49

#### 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; 19<sup>th</sup> 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; 19<sup>th</sup> 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

- 75 Table 2 : Animal-derived Proteins from these sources
- 76
- 77 Casein
- 78 Egg
- 79 Whey
- 80 Milk
- 81
- 82

## 83 Table 3: Non-Protein Ingredients Including Adulterants

- 84
- 85 Melamine
- 86 Urea
- 87 Free amino acids
- 88 Creatine
- 89 Caffeine
- 90 Taurine
- 91 Surfactants
- 92 Peptides (less than 10,000 daltons)
- 93
- 94

1	DRAFT AOAC SPDS Identification of Plant-Derived Proteins V 4	, 6.30.2016
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1 2		DRAFT AOAC SPDS Identification of Plant-Derived Proteins V 4, 6.30.2016					
- 3 1	Identification of PlantNon-animal -Derived Proteins in Dietary Supplements						
5		Int	ended Use: Reference method for cGMP compliance.				
7 8 9 10 11 12 13 14 15 16		1.	<b>Purpose:</b> 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 <i>Performance Tested Methods</i> or AOAC <i>Official Methods of Analysis</i> , and can be used as acceptance criteria for verification at user laboratories.				
17		2.	Applicability:				
18	1		Method must identify <del>plant</del> non-animal-derived proteins and their corresponding sources in				
19	I		the presence of potential adulterants in ingredients and finished dietary supplements				
20							
20		R	Analytical Technique <sup>,</sup>				
$\frac{21}{22}$		9.	Any analytical technique is accentable				
${23}$							
24		4.	Definitions:				
25							
26			Protein				
27			Naturally occurring and synthetic polypeptides having molecular weights greater than about				
28			10000 daltons (the limit is not precise) (IUPAC Definition)				
29							
30			Probability of Identification (POI)				
31			The proportion of positive analytical outcomes for an identification method for a given				
32			matrix at a given analyte level or concentration. LPOI is the Laboratory Probability of				
33			Identification.				
34							
35		5.	Method Performance Requirements:				
36			See table 1.				
37							
38		6.	System suitability tests and/or analytical quality control:				
39			Suitable methods will include blank check samples, and check standards at the lowest point				
40			and midrange point of the analytical range.				
41		-					
4Z		7.	Potential Reference Material(s):				
43			Poter to Annox E: Davalonment and Use of In House Paterance Materials in Annondix E:				
44 45			Guidelines for Standard Method Performance Requirements 19 <sup>th</sup> Edition of the AOAC				
<del>т</del> 5 46			INTERNATIONAL Official Methods of Analysis (2012) Available at				
47			http://www.eoma.aoac.org/app_f.pdf				
48							
49							

#### 50 8. Validation Guidance:

- 51
- 52 Data demonstrating method performance for the plant-derived proteins listed 53 in table 3 in the presence of the potential non-protein ingredients including 54 adulterants listed in table 4 is recommended.
- 55 56 Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a 57 Method of Analysis; 19<sup>th</sup> Edition of the AOAC INTERNATIONAL Official Methods of Analysis 58 (2012). Available at: http://www.eoma.aoac.org/app\_d.pdf
- 59
- Appendix F: Guidelines for Standard Method Performance Requirements; 19<sup>th</sup> Edition of the 60
- AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: 61
- 62 http://www.eoma.aoac.org/app\_f.pdf
- 63
- Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of 64 65 Analysis (2016) 20th Ed., AOAC INTERNATIONAL.
- 66

#### 67 9. Maximum Time-To-Result: None

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## **Table 1: Method Performance Table**

	Study	Parameter	Parameter Requirements	Target Test Concentration	Minimum Acceptable Results
Single Laboratory Validation		POI @ low concentration	Minimum of 33 replicates representing all target analytes in Table 2.	0.1 %	90% POI <sup>†</sup> of the pooled data for all target compounds and matrices.
	Matrix Study	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 <sup>(1)</sup>
		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			Use Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods.	0.1 %	≥ 0.85 <sup>+</sup>
	Matrix Study <sup>(2)</sup>	LPOI		10 %.	≥ 0.95 <sup>†</sup>
		LPOI (0)		0 %	≤ 0.05 <sup>+</sup>

Notes:
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	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.
70	
71	
72	
72	Table 2 : Recommended non-animal Plant-Derived Proteins from these sources
73	Table 2 . <u>Recommended non-animal</u> Hant-Derived Proteins nom these sources
74	
75	
/6	Algae
77	<u>Canola (Rapeseed)</u>
78	Flax
79	Hemp
80	Pea
81	Potato
82	Pumpkin
83	Quinoa
84	Rice
85	Soy
86	Wheat
87	
88	Table 3: Non-Protein Ingredients Including Adulterants
89	
90	Melamine
91	Urea
92	Free amino acids
93	Creatine
94	Caffeine
95	Taurine
96	Surfactants
97	Hydrolized leather
98	Hydrolized neutrici
90	Pentides (less than 10,000 daltons)
100	
100	
101	