

Standard Method Performance Requirements (SMPRs®) for Determination of Total Amino Acids in Foods, with an Emphasis on Novel Foods and Ingredients

Intended Use: Reference Method for Dispute Resolution

Purpose

What: AOAC Standard Method Performance Requirements (SMPRs®) are voluntary consensus standards developed in accordance with the AOAC policy, “AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents.” SMPRs describe a scientific community’s

recommended minimum method performance characteristics and analytical requirements for a specific method-related intended use.

Who: Drafted by AOAC working groups, SMPRs are adopted by AOAC by a consensus of stakeholders affiliated with its integrated science programs and projects which are composed of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.

Use: AOAC SMPRs are used in the AOAC core science programs as a resource for AOAC method experts, including expert review panels, in the evaluation of validation study data for methods submitted to the AOAC *Official Methods of Analysis*SM and AOAC *Performance Tested Methods*SM programs. AOAC SMPRs also may be used to provide acceptance criteria for the verification of methods and serve as a resource to guide method development and optimization.

1 Applicability

Quantitative analysis of individual proteogenic amino acids, hydroxyproline, hydroxylysine, and taurine (Table 1), with results

Table 1. Amino acids target list

Common name	IUPAC systematic name	CAS No. L-form ^a	CAS No. D-form ^a	CAS No. DL-form ^a
Alanine	2-Aminopropanoic acid	56-41-7	338-69-2	302-72-7
Arginine	2-Amino-5-(diaminomethylideneamino)pentanoic acid	74-79-3	157-06-2	7200-25-1
Asparagine ^{b,c}	2,4-Diamino-4-oxobutanoic acid	70-47-3	2058-58-4	3130-87-8
Aspartic acid ^c	2-Aminobutanedioic acid	56-84-8	1783-96-6	617-45-8
Cysteine ^d	2-Amino-3-sulfanylpropanoic acid	52-90-4	921-01-7	3374-22-9
Cystine ^d	2-Amino-3-[(2-amino-2-carboxyethyl)disulfanyl]propanoic acid	56-89-3	349-46-2	923-32-0
Glutamic acid ^e	2-Aminopentanedioic acid	56-86-0	6893-26-1	617-65-2
Glutamine ^{b,e}	2,5-Diamino-5-oxopentanoic acid	56-85-9	5959-95-5	6899-04-3
Glycine	2-Aminoacetic acid	56-40-6		
Histidine	2-Amino-3-(1H-imidazol-5-yl)propanoic acid	71-00-1	351-50-8	4998-57-6
Hydroxyproline ^f	4-Hydroxypyrrolidine-2-carboxylic acid	51-35-4	3398-22-9	NA
Hydroxylysine ^f	2,6-Diamino-5-hydroxyhexanoic acid	1190-94-9	NA	504-91-6
Isoleucine	2-Amino-3-methylpentanoic acid	73-32-5	319-78-8	3107-04-8
Leucine	2-Amino-4-methylpentanoic acid	61-90-5	328-38-1	328-39-2
Lysine	2,6-Diaminohexanoic acid	56-87-1	923-27-3	70-54-2
Methionine	2-Amino-4-methylsulfanylbutanoic acid	63-68-3	348-67-4	59-51-8
Phenylalanine	2-Amino-3-phenylpropanoic acid	63-91-2	673-06-3	150-30-1
Proline	Pyrrolidine-2-carboxylic acid	147-85-3	344-25-2	609-36-9
Serine	2-Amino-3-hydroxypropanoic acid	56-45-1	312-84-5	302-84-1
Taurine ^f	2-Aminoethanesulfonic acid	107-35-7		
Threonine	2-Amino-3-hydroxybutanoic acid	72-19-5	632-20-2	80-68-2
Tryptophan ^b	2-Amino-3-(1H-indol-3-yl)propanoic acid	73-22-3	153-94-6	54-12-6
Tyrosine	2-Amino-3-(4-hydroxyphenyl)propanoic acid	60-18-4	556-02-5	556-03-6
Valine	2-Amino-3-methylbutanoic acid	72-18-4	640-68-6	516-06-3

^a Preference will be given to methods able to distinguish D- and L- forms, but this distinction is not mandatory.

^b Not detectable when acid hydrolysis is used.

^c Reported together as aspartic acid when acid hydrolysis is used.

^d Generally not reported separately.

^e Reported together as glutamic acid when acid hydrolysis is used.

^f Only relevant in selected matrices (e.g., collagen-containing samples for hydroxyproline and hydroxylysine and cat food for taurine).

Table 2. Target matrices^{a,b}

Mandatory matrices	Primary representative matrices	Secondary representative matrices
Plant protein ingredients ^c	Pulses, seeds	Green leaves, cereals, whole plant
Protein concentrates, isolates	From pulses and seeds	From cereals, precision fermentation, insects
Plant-based products	Products resembling patties, sausages, nuggets, or similar forms	Products resembling cheese, egg Other products (e.g., falafel, tofu)
Protein bars	From novel ingredients and protein sources	
Protein drink mixes	From novel ingredients and protein sources	
Liquid products	Plant-based liquid products (e.g., from almond, soy, oat)	
Supplementary matrices	Primary representative matrices	Secondary representative matrices
Fermentation biomass	Yeast, fungi	Bacteria
Precision fermentation products	Liquid products	Structured products (resembling yogurt, cheese, egg...)
Algae	Microalgae, macroalgae	Algal finished products (salads, pastes, spreads)
Insects	Insect powders (including feed)	Protein bars, protein drink mixes
Collagen	Hydrolyzed collagen	Intact collagen
Cell-based products	Cell-based meat, fish, and seafood	
Pet food ^d	Dry pet food	Wet pet food
High-fat solid matrices	Peanut butter	From seeds or nuts

^a Validation must be conducted on at least one primary representative matrix for each mandatory matrix category. Method authors are encouraged to complement their validation with additional representative matrices (primary and secondary representative matrices) as well as supplementary matrices. Preference will be given to methods covering as many matrix categories as possible.

^b Methods that include analysis of both total and free amino acids should also be validated on mandatory matrices listed in AOAC SMPR 2026.006 for Determination of Free Amino Acids in Foods, with an Emphasis on Novel Foods and Ingredients.

^c Low refinement levels, usually flour.

^d Dry or wet pet foods that differ from traditional recipes to include a significant portion of novel ingredients, such as lentils, vegetables, and insects.

expressed as total concentration of each amino acid (sum of free and bound forms), in a variety of foods, with an emphasis on novel foods and ingredients (Table 2). Preference will be given to methods able to distinguish D- and L- forms but this distinction is not mandatory. The analytical method(s) must account for potential interferences such as those listed in Table 3.

For amino acids sensitive to modification during handling and/or processing (primarily methionine, lysine, and cysteine/cystine), which can result in modified forms different from the parent amino acids (e.g., methionine sulfoxide), preference will be given to methods best able to discriminate the parent forms of the target amino acids against their modified forms.

2 Analytical Technique

Any analytical technique that meets the method performance requirements is acceptable. It is expected that multiple methods will be required to completely fulfill the requirements.

3 Definitions

Free amino acids (FAA).—Amino acid molecules that are not incorporated into proteins or peptides.

Bound amino acids.—Amino acid molecules that are incorporated into proteins or peptides.

Limit of quantitation (LOQ).—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Calibration blank.—A calibration standard that does not contain the analyte(s) of interest at a detectable level. This can be the buffer in which the calibration standards are made.

Novel foods and ingredients.—For the purpose of this SMPR, novel foods and ingredients refer to foods from new sources, in new forms, or from new production technologies for the replacement of food and ingredients historically consumed in the region of interest. Examples of novel foods and ingredients are included in Table 2.

Table 3. Examples of possible interfering compounds

Interfering compound	Matrix category/ area of concern	CAS No.
Chitin (β-N-acetylglucosamine)	Insects, algae, fermentation biomass	1398-61-4 (14131-68-1)
Pyroglutamic acid	Process-induced modification	149-87-1
Methionine sulfoxide	Process-induced modification	62697-73-8
Furosine	Process-induced modification	19746-33-9
β-Alanine	Cell-based and fermentation products	107-95-9
3-Methylhistidine	Cell-based and fermentation products	368-16-1
γ-Aminobutyric acid	Fermentation products	56-12-2

Table 4. Method performance requirements

Parameter	Minimum acceptable criteria	
Limit of quantitation (LOQ) ^a	≤0.05	
Analytical range ^a	0.05–1	>1–50
Recovery, %	88–112	90–110
Repeatability, %	≤7	≤5
Reproducibility, %	≤11	≤8

^a Concentrations are expressed as g/100 g on as-received basis.

Procedural blank.—Sample that does not contain the matrix that is brought through the entire measurement procedure and analyzed in the same manner as a test sample.

Recovery.—Percentage of the analyte that is recovered when the test sample is analyzed using the entire method.

Repeatability.—Closeness of agreement between independent test results obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. Expressed as the standard deviation of repeatability (SD_r) or relative standard deviation of repeatability (RSD_r).

Reproducibility.—Closeness of agreement between independent test results obtained with the same method on identical test items in different laboratories with different operators using different equipment. Expressed as the standard deviation of reproducibility (SD_R) or relative standard deviation of reproducibility (RSD_R).

4 Method Performance Requirements

Method performance requirements are listed in Table 4.

5 System Suitability Tests and/or Analytical Quality Control

Calibration curve.—Every sequence must include a set of calibration standards covering the analytical range injected at the beginning and the end of the analytical series. The coefficient of determination (r²) of the standard curve must be a minimum of 0.995. The measured concentration of each standard level must be within ±10% of the theoretical value.

Blanks.—Suitable methods will include calibration blanks and procedural blanks. No carry-over should be observed after the highest calibration point. If this is the case, calibration blanks should be added accordingly to ensure carry-over minimization.

Check standards and positive control samples.—Suitable methods will include check standards at the midrange point of the analytical range. Check standard injections must be within ±10% of the theoretical value and can be used to detect deviation throughout the analytical series (bracketing). Positive control samples, such as quality control (QC) or reference materials, should also be included.

Resolution.—When measured in the same channel, it is recommended that analytes should have a peak resolution not lower than 1.5.

6 Reference Material(s)

Examples of reference materials are listed in Table 5.

7 Validation Guidance

Data must demonstrate the ability to identify and quantitate the amino acids listed in Table 1. Process-induced modifications and matrix-specific interferences should be evaluated for interference

Table 5. Reference materials

Matrix category	Reference material
Plant protein ingredients	NIST 1568c Rice Flour NIST 3233 Fortified Breakfast Cereal NIST 2386 Avocado Powder MUVA-VS-3001 Chickpea Base
Plant-based products	NIST 2383a Baby Food Composite NRC-CAME-1 Canola Meal Muva VK-3101 "Plant-based cheese alternative"
Protein-rich products	NIST 3252 Protein Drink Mix
Algae	NIST 3232 Kelp Powder
Insects	NRC-VORM-1 Mealworm Powder NRC BFLY-1 Black Soldier Fly Meal NRC KRIK-1 Cricket Flour
Cell-based products	NIST 1546a Meat Homogenate
Pet food	NIST 3290 Dry Cat Food
High-fat matrices	NIST 2387 Peanut Butter

with the quantitation of amino acids. Examples of possible interfering compounds are included in Table 3. Any interferences with the identification and quantitation of target amino acids should be reported in the method.

Validation must be conducted on at least one primary representative matrix for each mandatory matrix category (Table 2). Method authors are encouraged to complement their validation with additional representative matrices (primary and secondary representative matrices) as well as supplementary matrices. Preference will be given to methods covering as many matrix categories as possible. Method authors are encouraged to use reference materials as much as possible to assess method trueness.

Methods that include analysis of both total and free amino acids shall also be validated on the mandatory matrices listed in AOAC SMPR 2026.006 for Determination of Free Amino Acids in Foods, with an Emphasis on Novel Foods and Ingredients.

Single-laboratory validation studies must follow the general guidelines described in OMA Appendix L [AOAC Recommended Guidelines for Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation, *Official Methods of Analysis of AOAC INTERNATIONAL* (22nd Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (<https://doi.org/10.1093/9780197610145.005.012>)] for ruggedness, materials, linearity, LOD/LOQ, precision (assessed over at least six days in duplicate to calculate repeatability and intermediate reproducibility), trueness (using either reference materials or spike recovery experiments), and uncertainty (when both trueness and precision can be evaluated).

For matrices that naturally contain total amino acids above the LOQ, this concentration becomes the practical LOQ for each analyte/matrix pair. LOQ can be estimated based on extrapolation of signal to-noise ratio (S/N) obtained for a concentration level naturally present in the evaluated matrix to a concentration level that would correspond to S/N = 10.

For matrices with low levels of total amino acids (≤30% of the target LOQ), validation should be conducted at the target LOQ and 5x LOQ levels. LOQ is determined as the lowest spiking level that meets recovery and repeatability requirements.

Interlaboratory validation studies must follow OMA Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, *Official Methods of Analysis of AOAC INTERNATIONAL* (22nd Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (<https://doi.org/10.1093/9780197610145.005.004>)

Appendix F: Guidelines for *Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (22nd Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (<https://doi.org/10.1093/9780197610145.005.006>)

Appendix K: Guidelines for Dietary Supplements and Botanicals, *Official Methods of Analysis of AOAC INTERNATIONAL* (22nd Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (<https://doi.org/10.1093/9780197610145.005.011>)

8 Maximum Time-to-Result

None.

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