

Standard Method Performance Requirements (SMPRs®) for Determination of Free 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 free amino acids (Table 1) 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

Table 1. Free 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	2,4-Diamino-4-oxobutanoic acid	70-47-3	2058-58-4	3130-87-8
Aspartic acid	2-Aminobutanedioic acid	56-84-8	1783-96-6	617-45-8
Cysteine ^b	2-Amino-3-sulfanylpropanoic acid	52-90-4	921-01-7	3374-22-9
Cystine ^b	2-Amino-3-[(2-amino-2-carboxyethyl)disulfanyl]propanoic acid	56-89-3	349-46-2	923-32-0
Glutamic acid	2-Aminopentanedioic acid	56-86-0	6893-26-1	617-65-2
Glutamine	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
Hydroxylysine ^c	2,6-Diamino-5-hydroxyhexanoic acid	1190-94-9	NA	6000-08-4
Hydroxyproline ^c	4-Hydroxypyrrolidine-2-carboxylic acid	51-35-4	3398-22-9	NA
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 ^c	2-Aminoethanesulfonic acid	107-35-7		
Threonine	2-Amino-3-hydroxybutanoic acid	72-19-5	632-20-2	80-68-2
Tryptophan	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 Generally not reported separately.

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

Note: Additional amino acids, amino acid derivatives, or other compounds relevant to selected matrices or intended method application should be included in the validation dataset. As follows is a nonexhaustive list of supplementary analytes provided as guidance only:

- Tea and botanical products: theanine
- Fermentation-derived, cell-based, insect, algae, or microbial products: γ-aminobutyric acid (GABA), ornithine, citrulline, creatine, β-alanine, sarcosine, α-aminobutyric acid (AABA), β-aminobutyric acid (BABA), kynurenine, N-acetyl-L-ornithine, 3-methyl-L-histidine
- Process-induced modifications: methionine sulfoxide, cysteic acid

Table 2. Target matrices^{a,b}

Mandatory matrices	Primary representative matrices	Secondary representative matrices
Milk and dairy products	Bovine milk	Other dairy products (e.g., fermented milk products, colostrum, milk from other species)
Protein hydrolysates	Plant-based	Other sources (e.g., dairy, algae, insects, collagen)
Ingredients	Premixes	Single AA ingredients Protein ingredients (flour, concentrates, isolates) from plants, algae, insects...
FAA fortified dietary supplements	Powder drink mixes	Liquid supplements
Liquid products	Plant-based liquid products (e.g., from almond, soy, oat)	Fruit juice (e.g., orange)
Supplementary matrices	Representative matrices	
Protein bars	From novel ingredients and protein sources	
Plant-based products	Products resembling patties, sausages, and nuggets Products resembling cheese or egg Other products (e.g., falafel, tofu)	
Matrices for authentication or adulteration detection	Tea, cheese, honey, mushrooms	
Pet food	Dry/wet dog or cat food	
Animal feed and ingredients	Animal feed and pet food ingredients, spent grains	
Emerging technology products	Broth from fermentation, biomass, cell-based products	

^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 2026.005 for Determination of Total Amino Acids in Foods, with an Emphasis on Novel Foods and Ingredients.

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 may be required to completely fulfill the requirements.

3 Definitions

Free amino acids (FAA).—Amino acid molecules that are not 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.

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 (r^2) of the standard curve must be a minimum of 0.995. The measured concentration of each standard level must be within $\pm 10\%$ of the theoretical value.

Blanks.—Suitable methods will include calibration blanks and procedural blanks. No carry-over should be observed after the

Table 3. Examples of possible interfering compounds^a

Possible interfering compound	Matrix category/area of concern	CAS No.
Chitin (β- <i>N</i> -acetylglucosamine)	Insects, algae, fermentation biomass	1398-61-4 (14131-68-1)
Ammonia	Metabolic waste	7664-41-7
Urea	Metabolic waste, milk and dairy products	57-13-6
Sweeteners, such as aspartame	Powder drink mixes, liquid supplements	
Biogenic amines, such as ethanolamine, ethylamine, methylamine, tyramine, cadaverine, putrescine, spermine, spermidine, histamine	Fermented products, fish, meat, cheese	

^a Method authors should demonstrate that these compounds do not interfere with identification and quantitation of target amino acids within the specified matrix category or area of concern.

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 $\pm 10\%$ 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)

Method authors are encouraged to use certified reference materials when available to assess method trueness. In the absence of certified reference materials, trueness must be assessed by spike recovery experiments (see below).

7 Validation Guidance

Data must demonstrate the ability to identify and quantitate the FAA listed in Table 1 on at least one primary representative matrix for each mandatory matrix category listed in 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.

If supplementary analytes are relevant to the selected matrices or to the intended method application, method developers should include them in their validation dataset. A nonexhaustive list of supplementary analytes is provided as a note in Table 1.

Examples of possible interfering compounds are provided in Table 3. These compounds should be evaluated for potential interferences with identification and quantitation of target amino acids in the specified matrix category or area of concern.

Method developers should report any additional compounds evaluated during method development, including compounds assessed as additional target analytes and/or compounds evaluated for potential analytical interferences.

Table 4. Method performance requirements

Parameter	Minimum acceptable criteria		
Analytical range ^a	0.01–100		
Limit of quantification (LOQ) ^a	≤ 0.01		
Concentration range ^a	≤ 0.01 –0.1	>0.1–1	>1–100
Recovery, %	80–120	85–115	90–110
Repeatability, %	≤ 10	≤ 7	≤ 5
Reproducibility, %	≤ 20	≤ 15	≤ 8

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

Methods that include analysis of both total and FAA shall also be validated on the mandatory matrices listed in AOAC SMPR 2026.005 for Determination of Total Amino Acids in Foods, with an Emphasis on Novel Foods and Ingredients.

Single-laboratory validation studies must follow 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 certified reference materials or spike recovery experiments), and uncertainty (when both trueness and precision can be evaluated).

For matrices that naturally contain FAA 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 FAA ($\leq 30\%$ 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.

For samples that contain high levels of certain amino acids relative to others (e.g., premixes, fortified products), minor amino acid components should be detectable at $\leq 1\%$ the concentration of the most abundant amino acid.

Interlaboratory validation studies must follow 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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