

Standard Method Performance Requirements (SMPRs) for Identification and Quantitation of Free Alpha Amino Acids in Dietary Ingredients and 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 MethodsSM* or *AOAC Official Methods of AnalysisSM*, and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Methods must identify and quantify free alpha amino acids and related compounds (*see* Table 1 and Figure 1) in dietary ingredients and finished dietary supplement products as listed in Table 2. May not address purity of ingredients. One or more methods may be needed to meet the entire range.

3 Analytical Technique

Any analytical technique is acceptable.

4 Definitions

Dietary ingredients.—A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients {Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]}.

Dietary supplements.—A product intended for ingestion that contains a “dietary ingredient” intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

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 Tables 3 and 4.

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, Official Methods of Analysis of AOAC INTERNATIONAL* (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

Table 1. Free alpha amino acids and related compounds

Common name	IUPAC systematic name	CAS No. ^a
β-Alanine	3-aminopropanoic acid	107-95-9
Alanine	2-aminopropanoic acid	302-72-7
Arginine	2-Amino-5-(diaminomethylideneamino) pentanoic acid	2500-25-7
Asparagine	2,4-Diamino-4-oxobutanoic acid	3130-87-8
Aspartic acid	2-Aminobutanedioic acid	617-45-8
Cysteine	2-Amino-3-sulfanylpropanoic acid	3374-22-9
Cysteine	2-Amino-3-[[[(2R)-2-amino-2-carboxyethyl] disulfanyl]propanoic acid	923-32-0
Glutamic acid	2-Aminopentanedioic acid	617-65-2
Glutamine	2,5-Diamino-5-oxopentanoic acid	585-21-7
Glycine	2-Aminoethanoic acid	56-40-6
Histidine	2-Amino-3-(1H-imidazol-5-yl)propanoic acid	4998-57-6
Hydroxyproline	4-Hydroxypyrrolidine-2-carboxylic acid	51-35-4
Isoleucine	2-Amino-3-methylpentanoic acid	443-79-8
Leucine	2-Amino-4-methylpentanoic acid	328-39-2
Lysine	2,6-Diaminohexanoic acid	70-54-2
Methionine	2-Amino-4-methylsulfanylbutanoic acid	59-51-8
Phenylalanine	2-Amino-3-phenylpropanoic acid	63-91-2
Proline	Pyrrolidine-2-carboxylic acid	609-36-9
Serine	2-Amino-3-hydroxypropanoic acid	302-84-1
Taurine	2-Aminoethanesulfonic acid	107-35-7
Threonine	2-Amino-3-hydroxybutanoic acid	80-68-2
Tryptophan	2-Amino-3-(1H-indol-3-yl)propanoic acid	54-12-6
Tyrosine	2-Amino-3-(4-hydroxyphenyl)propanoic acid	556-03-6
Valine	2-Amino-3-methylbutanoic acid	516-06-3

^a CAS numbers specify the racemic forms, except for glycine and taurine which are achiral.

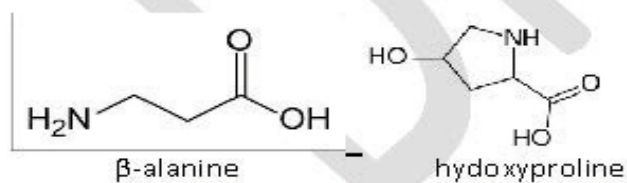
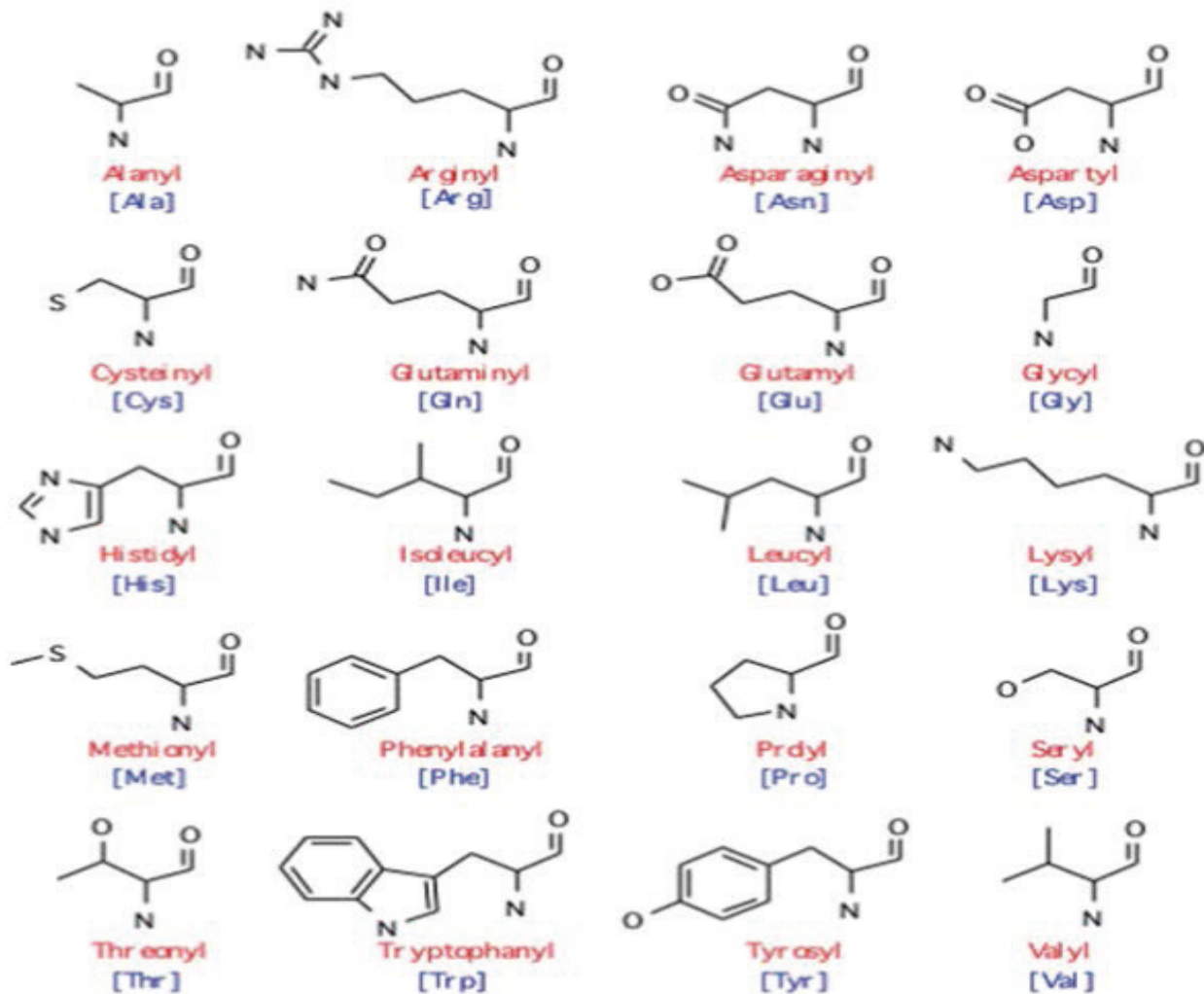


Figure 1. Molecular structures of free amino acids and related compounds identified in Table 1.

Table 2. Dietary ingredients and supplements

Powder
Tablets
Liquids
Capsules

Table 3. Method performance requirements (part 1)^a

Parameter	Acceptable criteria
Analytical range, %	0.04–100
LOQ, %	≤0.04
Recommended LOD, %	≤0.01

^a For individual free amino acid components measured.

8 Validation Guidance

Data must demonstrate ability to identify and quantitate the free amino acids in Table 1 in the presence of the nontarget compounds of interest in Table 5. Interferences with the identification and quantitation of target compounds should be reported in the method.

Method developers should be able to demonstrate that candidate methods can in fact identify and quantitate minor target compounds in the presence of greater concentrations of other amino acids and their related compounds.

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, Official Methods of Analysis of AOAC INTERNATIONAL* (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_d.pdf)

Appendix F: *Guidelines for Standard Method Performance Requirements, Official Methods of Analysis of AOAC*

Table 4. Method performance requirements (part 2)^a

Range, %	0.04–10	>10
Recovery, %	90–107	98–102
RSD _r , %	≤5	≤3
RSD _R , %	≤8	≤4

^a For individual free amino acid components measured

Table 5. Nontarget compounds

Norvaline
Sarcosine
Carnitine
Citrulline
Ornithine
Selenomethionine
GABA
Selenocystine
5HTP

INTERNATIONAL (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

Appendix K: *Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis of AOAC INTERNATIONAL* (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf)

9 Maximum Time-to-Result

None

Approved by the AOAC Stakeholder Panel on Dietary Supplements (SPDS) on March 17, 2017. Final Version Date: March 17, 2017.