DRAFT AOAC Free Alpha Amino Acids SMPR, v6.1, 30 November 2016.

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 Methods* or AOAC *Official Methods of Analysis*, 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) 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.[1]

   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.

[1] Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)

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 reproducibilitystandard deviation (SD_R); or % reproducibilityrelative 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 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*, 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 must demonstrate ability to identify and quantitate the free amino acids in Table 1 in the presence of the non-target compounds 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; 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; 19thEdition 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

102 **Table 1: Free alpha amino acids and related compounds**

Common name	IUPAC Systematic Name	CAS No.*
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
cystine	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
*CAS numbers specify the racemic forms, except for glycine and taurine which are achiral.		

103
104

Table 2 : Dietary Ingredients and Supplements

Powder
Tablets
Liquids
Capsules

Table 3: Method performance requirements ~~(Free Amino)~~ (part 1)

Parameters	**Acceptable Criteria**
Analytical Range (%)	0.04 - 100
LOQ (%)	≤0.04
Recommended LOD (%)	≤0.01
For individual free amino acid components measured.	

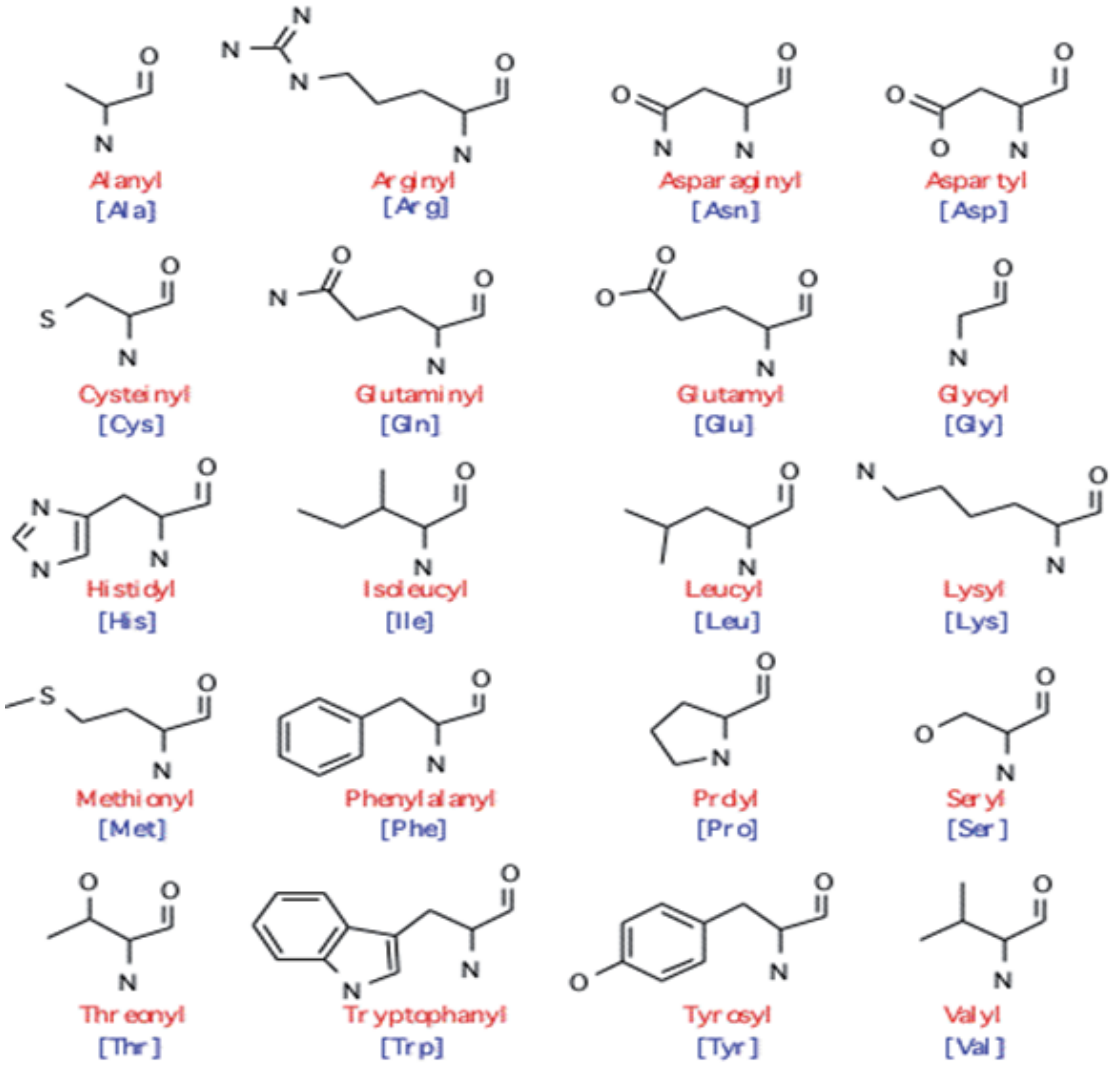
Table 4: Method performance requirements (part 2)

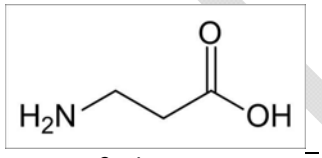
Ranges (%)	0.04 -10	> 10
Recovery (%)	90 - 107	98 – 102
% RSD_r	≤ 5	≤ 3
% RSD_R	≤ 8	≤4
For individual free amino acid components measure.d		

Table 5 : Non-target Compounds

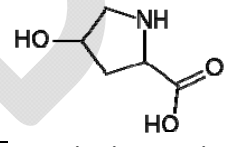
Norvaline
Sarcosine
Carnitine
Citrulline
Ornithine
Selenomethionine
GABA
Selenocystine
5HTP

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





β-alanine



hydoxyproline