AOAC SMPR® 2014.013

Standard Method Performance Requirements (SMPRs®) for Determination of Amino Acids in Infant Formula and Adult/Pediatric Nutritional Formula

Intended Use: Reference Method for Dispute Resolution

1 Applicability

Determination of free and/or total proteinogenic L-α-amino acids and taurine (as shown in Table 1) in all forms of infant, adult, and/or pediatric formulas (powders, ready-to-feed liquids, and liquid concentrates). 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, preference will be given to methods best able to discriminate against these modified forms. Structures to be excluded include, but are not necessarily limited to, methionine sulfone, methionine sulfoxide, cysteic acid, and lysine derived Maillard products. Method authors should specifically discuss, with appropriate supporting data, the ability to determine only parent forms of the target amino acids.

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

Accuracy (corresponds to the VIM definition for "trueness").—The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

Adult/pediatric formula.—Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment [AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN); 2010], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant formula.—Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding (Codex Standard 72–1981), made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Table 1. Proteinogenic L-α-amino acids and taurine

Amino acid	IUPAC name	CAS Registry No.
L-alanine	(2*S*)-2-aminopropanoic acid	56-41-7
L-arginine	(2*S*)-2-amino-5-(diaminomethylideneamino)pentanoic acid	74-79-3
L-asparagine[a,b]	(2*S*)-2,4-diamino-4-oxobutanoic acid	70-47-3
L-aspartic acid[b]	(2*S*)-2-aminobutanedioic acid	56-84-8
L-cysteine[c]	(2*R*)-2-amino-3-sulfanylpropanoic acid	52-90-4
L-cystine[c]	(2*R*)-2-amino-3-[[(2*R*)-2-amino-2-carboxyethyl]disulfanyl]propanoic acid	56-89-3
L-glutamic acid[b]	(2*S*)-2-aminopentanedioic acid	617-65-2
L-glutamine[a,b]	(2*S*)-2,5-diamino-5-oxopentanoic acid	56-85-9
L-glycine	2-Aminoethanoic acid	56-40-6
L-histidine	(2*S*)-2-amino-3-(1*H*-imidazol-5-yl)propanoic acid	71-00-1
L-isoleucine	(2*S*,3*S*)-2-amino-3-methylpentanoic acid	73-32-5
L-leucine	(2*S*)-2-amino-4-methylpentanoic acid	61-90-5
L-lysine	(2*S*)-2,6-diaminohexanoic acid	56-87-1
L-methionine	(2*S*)-2-amino-4-methylsulfanylbutanoic acid	63-68-3
L-phenylalanine	(2*S*)-2-amino-3-phenylpropanoic acid	63-91-2
L-proline	(2*S*)-pyrrolidine-2-carboxylic acid	147-85-3
L-serine	(2*S*)-2-amino-3-hydroxypropanoic acid	56-45-1
L-threonine	(2S,3R)-2-amino-3-hydroxybutanoic acid	72-19-5
L-tryptophan	(2*S*)-2-amino-3-(1H-indol-3-yl)propanoic acid	73-22-3
L-tyrosine	(2*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid	60-18-4
L-valine	(2*S*)-2-amino-3-methylbutanoic acid	72-18-4
Taurine	2-Aminoethanesulfonic acid	107-35-7

[a] Determined only as free amino acids.

[b] Not reported separately when acid hydrolysis is used.

[c] Generally not reported separately.

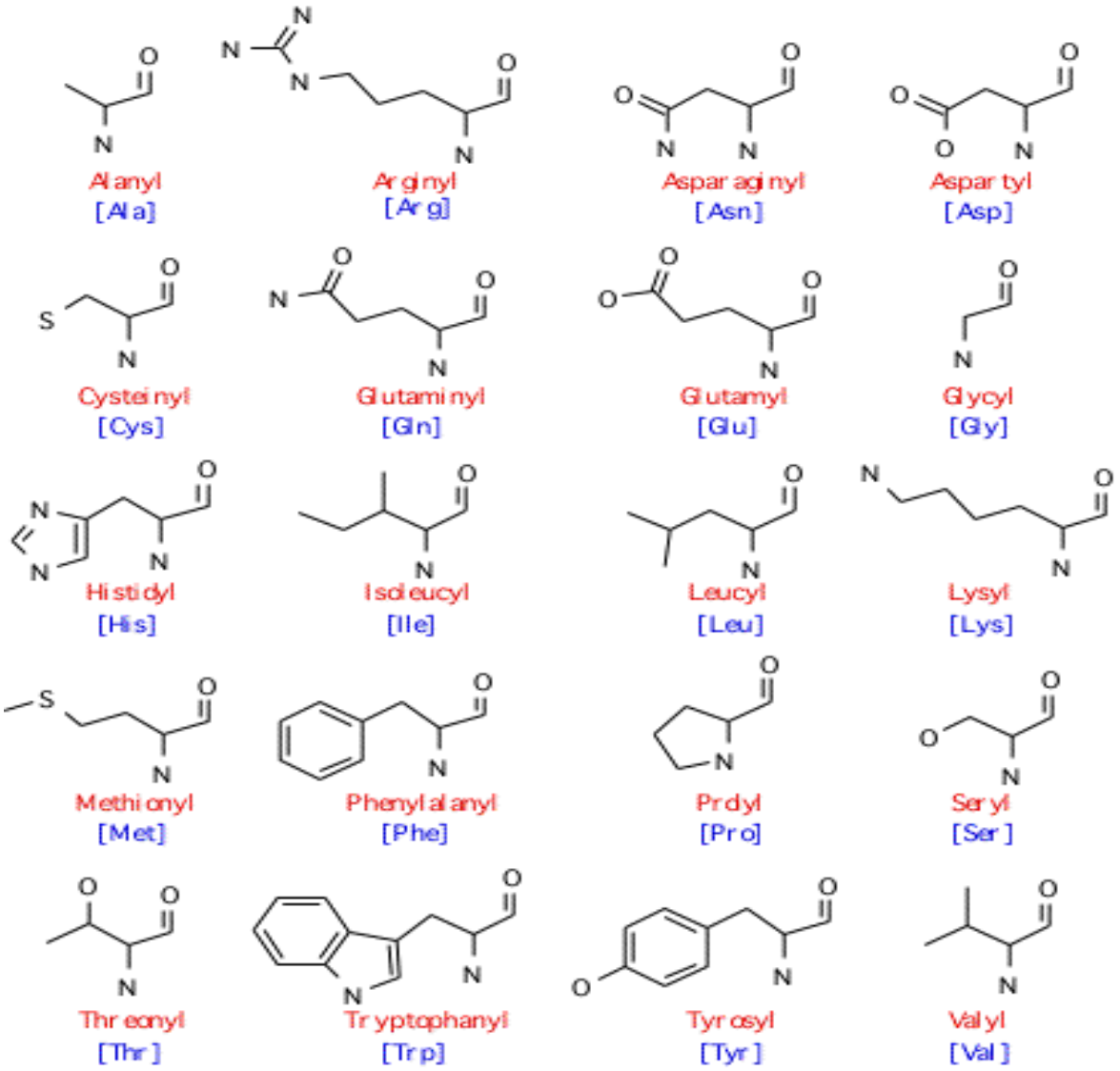

Figure 1. Molecular structure of amino acids.

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.

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

Proteinogenic L-α-amino acids.—Amino acids that are precursors to proteins. *See* Figure 1 for molecular structures.

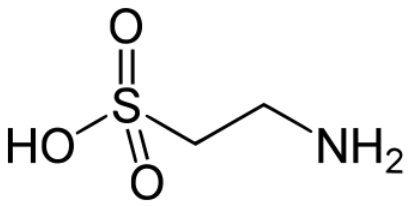

Figure 2. Molecular structure of taurine.

Recovery.—The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

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).

Taurine.—2-Aminoethanesulfonic acid. Taurine is sometimes called an amino acid, but it does not contain a carboxyl group necessary to be classified as an amino acid. It is in fact an acid containing an amino group. *See* Figure 2 for the molecular structure.

4 Method Performance Requirements

See Table 2.

Table 2. Method performance requirements[a]

Parameter	Minimum acceptable criteria	
Analytical range	0.5–2500[b]	
Limit of quantitation (LOQ)	≤0.5[b]	
Recovery	0.5–5.0[b]	±12%
	5.0–150	±10%
	150–2500	±7%
Repeatability (RSD_r)	0.5–5.0[b]	≤7%
	5.0–150	≤5%
	150–2500	≤3%
Reproducibility (RSD_R)	0.5–5.0[b]	≤11%
	5.0–150	≤8%
	150–2500	≤5%
[a] Concentrations apply to: (*a*) 'ready-to-feed" liquids "as is"; (*b*) reconstituted powders (25 g into 200 g water); and (*c*) liquid concentrates diluted 1:1 by weight using water.		
[b] mg/100 g reconstituted final product.		

5 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.

6 Reference Material(s)

NIST Standard Reference Material® 1849a Infant/Adult Nutritional Formula, or equivalent. SRM 1849a is a milk-based, hybrid infant/adult nutritional powder. One unit of SRM 1849a contains 10 packets each containing approximately 10 g of material. The SRM 1849a certificate values for amino acids and taurine are reference values and are for total content (Table 3). Other reference materials may be specified.

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*SM.

8 Maximum Time-to-Result

No maximum time.

Approved by Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN). Final Version Date: September 6, 2014. Effective Date: November 13, 2014. Revised: August 25, 2018.

Table 3. NIST 1849a reference values for amino acids and taurine

Amino acid	Value, mg/100 g[a]	Expanded uncertainty	Coverage factor
Alanine	0.455	0.021	2.31
Arginine	0.400	0.029	2.31
Aspartic acid[b]	1.070	0.057	2.31
Cystine[c]	0.1286	0.0071	2.00
Glutamic acid[d]	2.59	0.27	2.31
Glycine	0.241	0.019	2.31
Histidine	0.315	0.036	2.31
Isoleucine	0.660	0.071	2.31
Leucine	1.261	0.050	2.31
Lysine	1.010	0.071	2.31
Methionine	0.482	0.051	
Phenylalanine	0.580	0.021	2.31
Proline	1.195	0.086	2.31
Serine	0.720	0.030	2.31
Taurine	0.0366	0.0018	2.00
Threonine	0.640	0.022	2.31
Tryptophan	0.184	0.010	2.45
Tyrosine	0.510	0.043	2.31
Valine	0.7600	0.11	2.31
[a] As-is basis.			
[b] Sum of Asp+Asn.			
[c] Sum of CSSC and Cys.			
[d] Sum of Glu+Gln.			