

Standard Method Performance Requirements for Determination of Selected Carotenoids in Infant and Adult/Pediatric Nutritional Formula

Intended Use: Reference Method for Dispute Resolution

1 Applicability

Determinations of all-*trans* α -carotene (CAS 7488-99-5), *cis* isomers of α -carotene, all-*trans* β -carotene (CAS 7235-40-7), *cis* isomers of β -carotene, all-*trans* lutein (CAS 127-40-2), *cis* isomers of lutein, and lycopene in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

2 Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

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.

α -Carotene.—All-*trans* α -carotene (IUPAC name: 1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-2-en-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene, CAS No.: 7488-99-5) and its *cis* isomers (Figure 1).

β -Carotene.—All-*trans* β -carotene (IUPAC name: 1,3,3-trimethyl-2-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-1-yl)

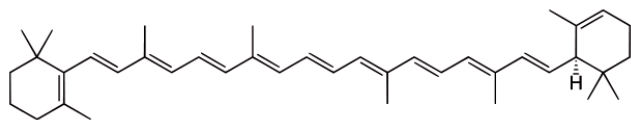


Figure 1. Molecular structure of all-*trans* α -carotene.

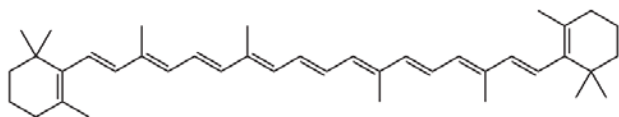


Figure 2. Molecular structure of all-*trans* β -carotene.

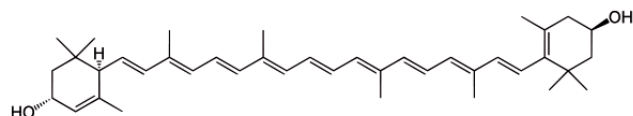


Figure 3. Molecular structure of all-*trans* lutein.

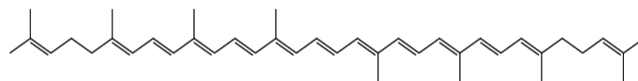


Figure 4. Molecular structure of lycopene.

octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene, CAS No.: 7235-40-7) and its *cis* isomers (Figure 2).

Carotenoids.—A class of organic pigments consisting of four 10-carbon terpene units, which in turn are formed from eight 5-carbon isoprene units. For the purposes of this standard, only the four carotenoids listed in the Applicability section are considered biologically important enough for consideration in this standard for in infant formula/adult nutritionals.

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.

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.

Lutein.—All-*trans* lutein (IUPAC name: (1R)-4-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-18-[(1R,4R)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethylcyclohex-3-en-1-ol, CAS No.: 127-40-2) and its *cis* isomers (Figure 3).

Lycopene.—IUPAC name: (6E,8E,10E,12E,14E,16E,18E,20E,22E,24E,26E)-2,6,10,14,19,23,27,31-octamethyldotriacont-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene, CAS No.: 502-65-8 (Figure 4).

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).

4 Method Performance Requirements

See Table 1.

Table 1. Method performance requirements^a

Parameter	Minimum acceptable criteria	
Analytical range	1–1300 ^b	
Limit of quantitation (LOQ)	≤1 ^b	
Recovery	90–110%	
Repeatability (RSD _r)	1–100	8%
	>100–1300	5%
Reproducibility (RSD _R)	1–100	15%
	>100–1300	10%
^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.		
^b µg/100 g reconstituted final product; range and LOQ are based on total of <i>cis+trans</i> isomers.		

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. Methods must be capable of resolving lutein from zeaxanthin.

6 Reference Material(s)

Neither NIST nor IRMM produce a certified reference material for carotenoids in infant formula. The carotenoid content of SRM 1849a has not been determined (as of November 2014).

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.