

## Standard Method Performance Requirements (SMPRs) for Determination of $\alpha$ -Carotene in Infant and Adult/Pediatric Nutritional Formula

Intended Use: Reference Method for Dispute Resolution

### 1 Applicability

Determination of total (include *cis* and *trans* isomers if they are separated)  $\alpha$ -carotene (CAS 7488-99-5) 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.

*$\alpha$ -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). See Figure 1.

*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.

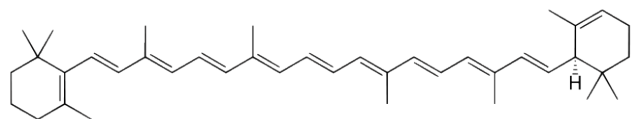


Figure 1. Molecular structure of all-*trans*  $\alpha$ -carotene.

Table 1. Method performance requirements<sup>a</sup>

Analytical range	1–50 <sup>b</sup>
Limit of quantitation (LOQ)	$\leq 1^b$
Recovery	90–110%
Repeatability (RSD <sub>r</sub> )	$\leq 8\%$
Reproducibility (RSD <sub>R</sub> )	$\leq 15\%$

<sup>a</sup> 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.

<sup>b</sup>  $\mu\text{g}/100\text{ g}$  reconstituted final product.

*Limit of detection (LOD).*—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).*—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result

*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<sub>r</sub>); or % repeatability relative standard deviation (%RSD<sub>r</sub>).

*Reproducibility.*—Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD<sub>R</sub>); or % reproducibility relative standard deviation (%RSD<sub>R</sub>).

### 4 Method Performance Requirements

See Table 1.

### 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  $\alpha$ -carotene from lycopene and  $\beta$ -carotene.

### 6 Reference Material(s)

Neither NIST nor JRC produce a certified reference material for  $\alpha$ -carotene in infant formula.

### 7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*<sup>SM</sup>.

### 8 Maximum Time-to-Result

No maximum time.

Approved by the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN). Final Version Date: March 15, 2017.