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3 **Method Name: Determination of  $\beta$ -Carotene in Infant and Adult/  
4 Pediatric Nutritional Formula**

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6 **Approved by:** Stakeholder Panel for Infant Formula and Adult Nutritionals

7 **Final version date:**

8 **Effective date:**

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10 **Intended Use:** Reference method for dispute resolution.

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12 **1. Applicability:**

13 Determinations of all-*trans*  $\beta$ -carotene (CAS 7235-40-7) and *cis* isomers of  $\beta$ -  
14 carotene in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed  
15 liquids, and liquid concentrates).

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17 **2. Analytical Technique:**

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

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21 **3. Definitions:**

22 Accuracy (Corresponds to the VIM definition for “trueness”).

23 The closeness of agreement between the average of an infinite number of replicate  
24 measured quantity values and a reference quantity value.

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26 **Adult/Pediatric Formula**

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

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34  **$\beta$ -Carotene**

35 All-*trans* beta-carotene (IUPAC name: 1,3,3-trimethyl-2-  
36 [(1E,3E,5E,7E,9E,11E,13E,15E,17E)-3,7,12,16-tetramethyl-18-(2,6,6-  
37 trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene,  
38 CAS number: 7235-40-7) and its *cis* isomers. Figure 1.

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40 **Infant formula**

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

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47 **Limit of Detection (LOD)**

48 The minimum concentration or mass of analyte that can be detected in a given matrix  
49 with no greater than 5% false positive risk and 5% false negative risk.

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51 **Limit of Quantitation (LOQ)**

52 The minimum concentration or mass of analyte in a given matrix that can be reported  
53 as a quantitative result

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**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<sub>r</sub>).

**Reproducibility**

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation ( $SD_R$ ); or % reproducibility relative standard deviation (% RSD<sub>R</sub>).

**4. Method Performance Requirements:  
See Table 1.**

**Table 1. Method Performance requirements<sup>a</sup>**

Analytical range	1–1300 <sup>b</sup>
Limit of Quantitation (LOQ)	≤ 1 <sup>b</sup>
Recovery	90-110%
Repeatability (RSD <sub>r</sub> )	
1-100 <sup>b</sup>	8%
>100-1300 <sup>b</sup>	5%
Reproducibility (RSD <sub>R</sub> )	
1-100 <sup>b</sup>	15%
>100-1300 <sup>b</sup>	10%
<sup>a</sup> Concentrations apply to: a) 'ready-to-feed' liquids "as is"; b) reconstituted powders (25 g into 200 g of water); and c) liquid concentrates diluted 1:1 by weight.	
<sup>b</sup> μg /100 g reconstituted final product; range and LOQ are based on total of <i>cis+trans</i> isomers.	

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**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 β-carotene from α-carotene and lycopene.

**6. Reference Material(s):**

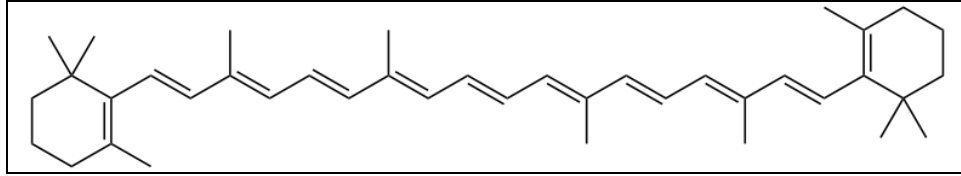
SRM 1869. Please contact Dr. Melissa Phillips, Research Chemist, NIST for materials at [melissa.phillips@nist.gov](mailto:melissa.phillips@nist.gov) or (301) 975-4134.

**7. Validation Guidance:**

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

**8. Maximum Time-To-Result:** No maximum time.

89 Figures:  
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Figure 1: Molecular structure of all-*trans*  $\beta$ -Carotene

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