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Method Name: **Determination of Lutein in Infant and Adult/ Pediatric Nutritional Formula**

Approved by: Stakeholder Panel for Infant Formula and Adult Nutritionals
Final version date:
Effective date:

Intended Use: Reference method for dispute resolution.

1. **Applicability:**

Determinations of all-*trans* lutein (CAS 127-40-2) and *cis* isomers of lutein 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.

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 number: 127-40-2) and its *cis* isomers. Figure 1.

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 relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

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

Table 1. Method Performance requirements[a]

Analytical range	1–800[b]
Limit of Quantitation (LOQ)	≤ 1[b]
Recovery	90-110%
Repeatability (RSD_r)	
1-100[b]	8%
>100-800[b]	5%
Reproducibility (RSD_R)	
1-100[b]	15%
>100-800[b]	10%
[a]Concentrations apply to: a) 'ready-to-feed" liquids "as is"; b) re-constituted powders (25 g into 200 g of 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 *cis*+*trans* 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)**:
SRM 1869. Please contact Dr. Melissa Phillips, Research Chemist, NIST for materials at melissa.phillips@nist.gov or (301) 975-4134.

7. **Validation Guidance**:
Recommended level of validation: *Official Methods of Analysis*^{SM}.

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

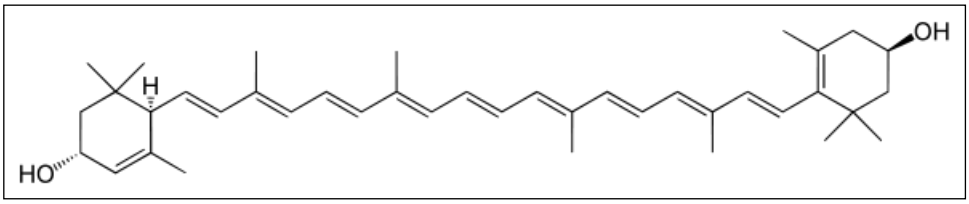

Figure 1: Molecular structure of all-*trans* lutein.