AOAC SMPR® 2017.005

Standard Method Performance Requirements (SMPRs) for Determination of α-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) α-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.

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). *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.

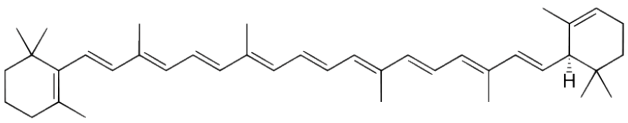

Figure 1. Molecular structure of all-*trans* α-carotene.

Table 1. Method performance requirements[a]

Analytical range	1–50[b]
Limit of quantitation (LOQ)	≤1[b]
Recovery	90–110%
Repeatability (RSD_r)	≤8%
Reproducibility (RSD_R)	≤15%

[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.

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_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—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.

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 α-carotene from lycopene and β-carotene.

6 Reference Material(s)

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

7 Validation Guidance

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

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.

AOAC SMPR® 2017.006

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

Intended Use: Reference Method for Dispute Resolution

1 Applicability

Determinations of all-*trans* β-carotene (CAS 7235-40-7) and *cis* isomers of β-carotene 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.

β-Carotene.—All-*trans* beta-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-trimethylcyclohexen-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene, CAS No.: 7235-40-7) and its *cis* isomers. *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.

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.

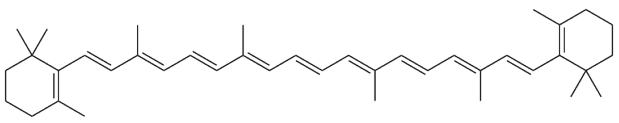

Figure 1. Molecular structure of all-*trans* β-carotene.

Table 1. Method performance requirements[a]

Analytical range	1–1300[b]
Limit of quantitation (LOQ)	≤1[b]
Recovery	90–110%
Repeatability (RSD_r)	
1–100[b]	≤8%
>100–1300[b]	≤5%
Reproducibility (RSD_R)	
1–100[b]	≤15%
>100–1300[b]	≤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 *cis+trans* isomers.

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_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.

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. Contact Dr. Melissa Phillips, Research Chemist, NIST, for materials at email: melissa.phillips@nist.gov, Tel: (301) 975-4134.

7 Validation Guidance

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

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.

AOAC SMPR® 2017.007

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

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. *See* Figure 1.

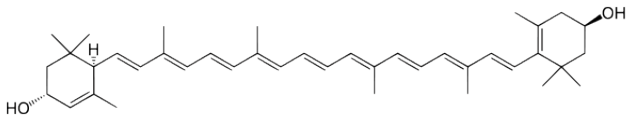

Figure 1. Molecular structure of all-*trans* lutein.

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) 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 *cis+trans* isomers.

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.

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. Contact Dr. Melissa Phillips, Research Chemist, NIST, for materials at email: melissa.phillips@nist.gov, Tel: (301) 975-4134.

7 Validation Guidance

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

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.

AOAC SMPR® 2017.008

Standard Method Performance Requirements (SMPRs) for Determination of Lycopene 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) lycopene (CAS 502-65-8) 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.

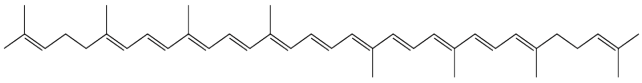

Figure 1. Molecular structure of lycopene.

Table 1. Method performance requirements[a]

Analytical range	1–50[b]
Limit of quantitation (LOQ)	≤1[b]
Recovery	90–110%
Repeatability (RSD_r)	≤8%
Reproducibility (RSD_R)	≤15%

[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.

Lycopene.—IUPAC name: (6E,8E,10E,12E,14E,16E,18E,20E,22E,24E,26E)-2,6,10,14,19,23,27,31-octamethyldotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene, CAS No. 502-65-8. *See* 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 standard deviation (SD_R); or % reproducibility relative standard deviation ($\%RSD_R$).

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 lycopene from α-carotene and β-carotene.

6 Reference Material(s)

SRM 1869. Contact Dr. Melissa Phillips, Research Chemist, NIST, for materials at email: melissa.phillips@nist.gov, Tel: (301) 975-4134.

7 Validation Guidance

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

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.