AOAC SMPR 2016.004

Standard Method Performance Requirements (SMPRs®) for Quantitative Measurement of β-Cryptoxanthin, Lutein, and Zeaxanthin in Ingredients and Dietary Supplements

Intended Use: Reference method for cGMP compliance

1 Purpose

AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for method being considered for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠, and can be used as acceptance criteria for verification at user laboratories [refer to Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA].

2 Applicability

Separate quantitative determination the *cis* and *trans* isomers of β-cryptoxanthin, lutein, and zeaxanthin and their fatty acid esters in ingredients and dietary supplements.

3 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

4 Definitions

Analytes:

β-Cryptoxanthin.—IUPAC name: (*R*)-3,5,5-trimethyl-4-[3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-1-enyl)-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-cyclohex-3-enol. CAS No. 472-70-8. *See* Figure 1 for chemical structure.

Lutein.—IUPAC name: β,ε-carotene-3,3'-diol. CAS No. 127-40-2. *See* Figure 2 for chemical structure.

Zeaxanthin.—IUPAC name: 4-[18-(4-hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethyl-cyclohex-3-en-1-ol. CAS No. 144-68-3. *See* Figure 3 for chemical structure.

Dietary ingredients.—A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients {Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]}.

Dietary supplements.—A product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

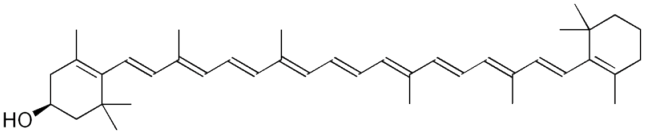

Figure 1. Chemical structure of all-*trans* β-cryptoxanthin.

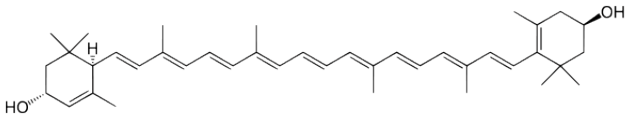

Figure 2. Chemical structure of all-*trans* lutein.

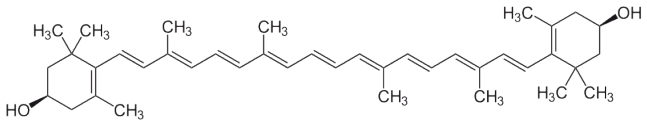

Figure 3. Chemical structure of all-*trans* zeaxanthin.

Limit of quantitation (LOQ).—The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Quantitative method.—Method of analysis which response is the amount of the analyte measured either directly (enumeration in a mass or a volume), or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

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).

Recovery.—The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

5 Method Performance Requirements

See Tables 1 and 2.

6 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.

7 Reference Material(s)

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, (http://www.eoma.aoac.org/app_f.pdf)

USP Lutein
USP Zeaxanthin
NIST 3280 Lutein (Multivitamin)
NIST list of lutein, zeaxanthin, and β-cryptoxanthin in foods

Table 1. Analytical range and LOQ requirements	
Analytical range	0.0005 to 100%
	5 to 1000000 ppm
Limit of quantitation (LOQ)	≤0.0002%
	≤2 ppm

Table 2. Recovery, repeatability, and reproducibility parameters				
Range[a]	5 to 20 ppm	>20 to 1000 ppm	>0.1 to 1%	>1%
Recovery, %	80 to 110	95 to 105	97 to 102	98–102
RSD_r, %	≤8	≤5	≤4	≤2
RSD_R, %	≤12	≤8	≤6	≤5

[a] Percent recovery, $\%RSD_r$, and $\%RSD_R$ shall be determined individually for each claimed matrix and analyte in total.

Table 3. Matrixes
Tablets
Capsules
Liquids
Powders
Extracts
Plant products
Gummies

8 Validation Guidance

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, *Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_d.pdf)

Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

Appendix K: *Guidelines for Dietary Supplements and Botanicals*, *Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf)

All matrixes in Table 3 shall be evaluated, or the scope (applicability) of AOAC-adopted method must expressly state the applicable dietary supplement forms.

9 Maximum Time-to-Result

None.

Approved by AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: March 17, 2016. Effective Date: March 17, 2016.