DRAFT AOAC SMPR 2016.XXX; Version 4; November 19, 2015

SMPR Name: 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* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA.]

2. Applicability:

Separate quantitative determination of β-cryptoxanthin, lutein, and zeaxanthin 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 registry number: 472-70-8. See figure 1 for chemical structure.

Lutein

IUPAC name: β,ε-carotene-3,3'-diol. CAS registry number 1 27-40-2. 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 registry number: 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.[1]

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.

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 table 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*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

USP Lutein
USP Zeaxanthin

[1] Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)

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

8. Validation Guidance:

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available on line at: http://www.eoma.aoac.org/app_k.pdf

All matrices 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

Table 1: Analytical Range and LOQ Requirements

Analytical Range	0.0005% to 100%
	5 to 1,000,000 ppm
Limit of Quantitation (LOQ)	≤ 0.0002%
	≤ 2 ppm

Table 2: Recovery, Repeatability, and Reproducibility Parameters

Range	**5 to 20 ppm**	**>20 to 1000ppm**	**>0.1% to 1%**	**>1%**
% Recovery	80 to 110	95 to 105	97 to 102	98 – 102
% RSDr	≤ 8	≤ 5	≤ 4	≤ 2
% RSD_R	≤ 12	≤ 8	≤ 6	≤ 3

% recovery, % RSDr, and % RSD_R shall be determined individually for each claimed matrice.

Table 3: Matrices

Tablets
Capsules
Liquids
Powders
Extracts
Plant products
Gummies

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

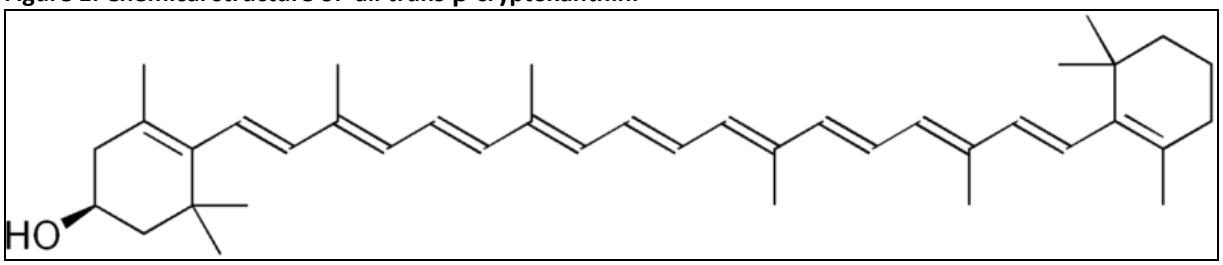

Figure 2: Chemical structure of all-*trans* lutein.

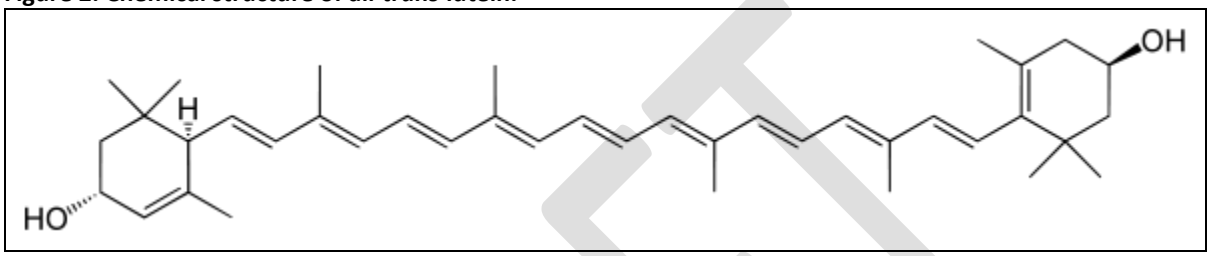

Figure 3: Chemical structure of all-*trans* zeaxanthin.