AOAC SMPR® 2017.013

Standard Method Performance Requirements (SMPRs) for Determination of Vitamins K_1 and K_2 in Dietary Supplements and Dietary Ingredients

Intended Use: Reference Method for cGMP Compliance

1 Applicability

Individually separate and quantify *cis* and *trans* forms of vitamin K_1 (phylloquinone); all-*trans* forms of both MK-4 and MK-7 (vitamin K_2); and determine area % for total *cis* forms of vitamin K_2 in dietary ingredients and dietary supplements as listed in Table 1.

2 Analytical Technique

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

3 Definitions

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. {United States 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.

Limit of quantitation (LOQ).—The 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 ($\%RSD_r$).

Table 1. Matrices

Dietary ingredients
Powders
Oils
Extracts
Encapsulated
Dietary supplements
Powders
Tablets
Gummies
Oils
Liquids
Capsules
Softgel capsules
Tinctures
Gelcaps
Chewables

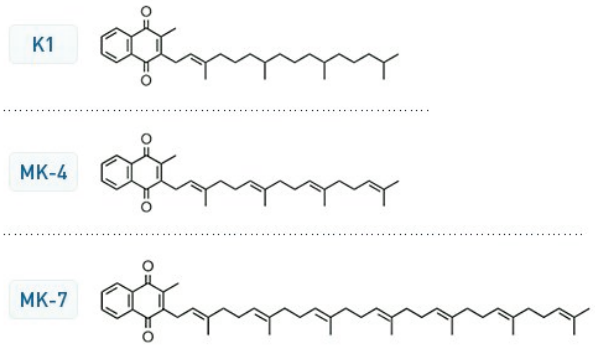

Figure 1. Molecular structures of vitamins K_1 and K_2.

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 ($\%RSD_R$).

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

Vitamin K_1.—Phylloquinone. IUPAC name: 2-methyl-3-[(2*E*)-3,7,11,15-tetramethyl hexadec-2-en-1-yl]naphthoquinone (CAS No. 084-80-0). *See* Figure 1.

Vitamin K_2.—Menaquinone with several subtypes designated as MK-n. "MK" identifies the basic quinone ring structure and "n" designating the number of attached isoprenoid units. *See* Figure 1.

MK-4.—IUPAC name: 2-methyl-3-[(2E,6E,10E)-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-yl]-1,4-naphthalenedione (CAS No. 863-61-6).

MK-7.—IUPAC name: 2-[(2E,6E,10E,14E,18E,22E)-3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaenyl]-3-methylnaphthalene-1,4-dione (CAS No. 2124-57-4).

4 Method Performance Requirements

See Tables 2 and 3.

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. A control sample must be included.

6 Reference Material(s)

NIST SRM 3280
NIST SRM 1849a
NIST SRM 3232
MK-4: Sigma-Aldrich V031 Cerilliant
MK-7: USP 1381119
K1: USP 1538006
K1: NIST SRM 3280 Multivitamin Tablet

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 of AOAC INTERNATIONAL* (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

7 Validation Guidance

All target analytes (vitamin K_1, MK-4, and MK-7) and all *claimed* matrixes listed in Table 1 shall be evaluated. One analyte

Table 2. Analytical range and LOQ based on matrix

	Vitamins K_1 and K_2[a]	
Parameter	Dietary supplements	Dietary ingredients
Analytical range, ppm	1–3000	1000–1M
LOQ, ppm	0.5	200

[a] Measured as individual forms of vitamins K_1 and K_2 and their isomers.

per *claimed* matrix is acceptable provided all three analytes are represented in the complete evaluation.

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

Appendix K: *Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis of AOAC INTERNATIONAL* (20th Ed.), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf). Also at *J. AOAC Int*. **95**, 268(2012) DOI: 10.5740/jaoacint.11-447

Table 3. Method performance requirements as a function of range

	Range, ppm[a]		
Parameter	1–100	>100–3000	>3000
Recovery, %	80–110	90–107	97–103
RSD_r, %	≤11	≤6	≤5
RSD_R, %	≤15	≤8	≤6

[a] Measured as individual forms of vitamins K_1 and K_2 and their isomers.

8 Maximum Time-to-Determination

No maximum time.

Approved by the AOAC Stakeholder Panel on Dietary Supplements (SPDS) on March 17, 2017. Final Version Date: March 17, 2017.