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Method Name: **Determination of Vitamins K_1 and K_2 in Dietary Supplements and Dietary Ingredients**

Approved by: Stakeholder Panel on Dietary Supplements (SPDS)

Intended Use:

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 3.

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).

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.

Vitamin K_1 .— Phyilloquinone. IUPAC name: 2-methyl-3-[(2*E*)-3,7,11,15-tetramethyl hexadec-2-en-1-yl]naphthoquinone. CAS number: 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 number :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 number :2124-57-4

4. Method Performance Requirements:

Table 1: Analytical Range & LOQ Based on Matrix

	Vitamin K_1 & K_2*	
Parameter	Dietary Supplements	Dietary Ingredients
Analytical range	1– 3000 ppm	1,000 – 1M ppm
Limit of Quantitation	0.5 ppm	200 ppm

* Measured as individual forms of Vitamin K1 and K2 and their isomers

Table 2: Method Performance Requirements as a Function of Range

Parameter	**Range***		
	1 – 100 ppm	>100 – 3,000	>3,000 ppm
Recovery (%)	80 – 110	90-107	97 – 103
% RSD_r	< 11	< 6	< 5
% RSD_R	< 15	< 8	< 6

* Measured as individual forms of Vitamin K1 and K2 and their 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. A control sample must be included.

6. Reference Material(s):

NIST SRM 3280
NIST SRM 1849a
NIST SRM 3232
MK4 from Sigma Aldrich V031 Cerilliant
MK7: 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*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: 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 3 shall be evaluated. One analyte 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; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Also at: . AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447 and available at: http://www.eoma.aoac.org/app_k.pdf

8. Maximum Time-To-Determination: No maximum time.

Figure 1: Molecular structures of vitamin K_1 and K_2

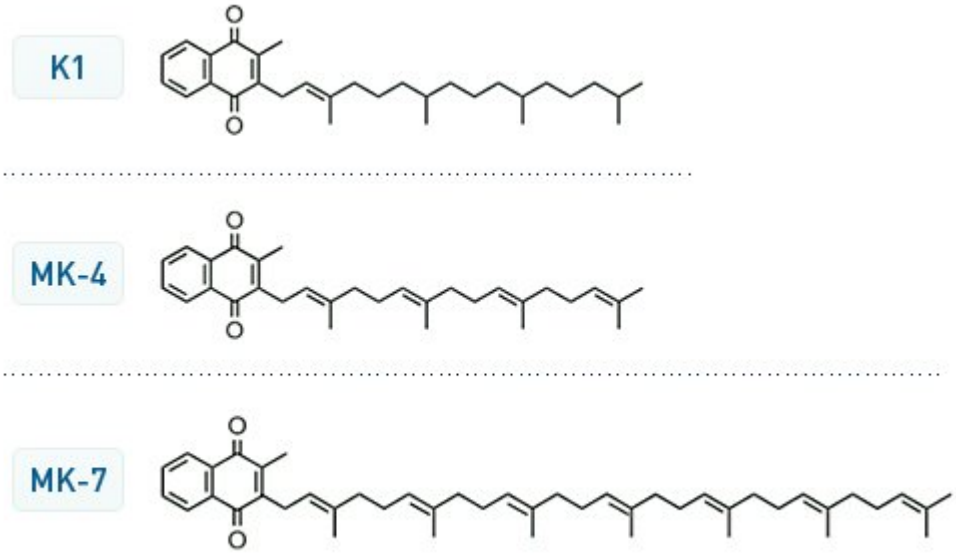

Table 3: Matrices

Dietary Ingredients:

powders
oils
extracts
encapsulated

Dietary Supplements :

powders
tablets
gummies
oils
liquids
capsules
softgel capsules
tinctures
gelcaps
chewables