

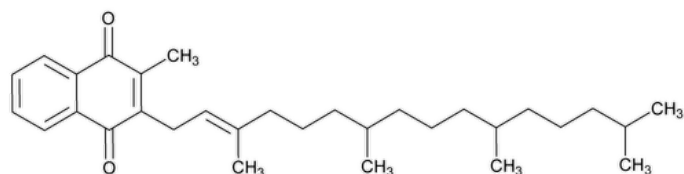
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Phytonadione

Change to read:

▲



▲2S (USP41)

$C_{31}H_{46}O_2$ 450.70

▲2-Methyl-3-(3,7,11,15-tetramethylhexadec-2-en-1-yl)naphthalene-1,4-dione; ▲2S (USP41)

Phylloquinone CAS RN®: ▲81818-54-4. ▲2S (USP41)

DEFINITION

Phytonadione is a mixture of *E*- and *Z*-isomers containing NLT 97.0% and NMT 103.0% of phytonadione isomers ($C_{31}H_{46}O_2$). It contains NMT 21.0% of the *Z*-isomer.

IDENTIFICATION

Change to read:

- A. ▲ [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy: 197F](#) ▲ (CN 1-MAY-2020)

Change to read:

- B. ▲ [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Ultraviolet-Visible Spectroscopy: 197U](#) ▲ (CN 1-MAY-2020)

Analytical wavelength: 248 nm

Sample solution: 10 µg/mL in [n-hexane](#)

Acceptance criteria: Meets the requirements. Absorptivities do not differ by more than 3.0%.

ASSAY

PROCEDURE

[NOTE—Protect solutions containing phytonadione from exposure to light.]

Mobile phase: [n-Hexane](#) and *n*-amyl alcohol (2000:1.5)

Internal standard solution: 2.5 mg/mL of [cholesteryl benzoate](#) in *Mobile phase*

Standard solution: Prepare a 96-µg/mL solution of [USP Phytonadione RS](#) in *Mobile phase*. Pipet 10 mL of this solution and 7 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Prepare as directed for the *Standard solution*, using Phytonadione instead of [USP Phytonadione RS](#).

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L3

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for the internal standard, (*Z*)-phytonadione, and (*E*)-phytonadione are 0.7, 0.9, and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 1.5 between (Z)-phytonadione and (E)-phytonadione**Relative standard deviation:** NMT 2.0% for the ratios of the sum of peak areas of (Z)-phytonadione and (E)-phytonadione to the peak area of the internal standard**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of phytonadione isomers ($C_{31}H_{46}O_2$) in the portion of Phytonadione taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 R_U = internal standard ratio [sum of the peak areas of (Z)-phytonadione and (E)-phytonadione/peak area of the internal standard] from the *Sample solution*
 R_S = internal standard ratio [sum of the peak areas of (Z)-phytonadione and (E)-phytonadione/peak area of the internal standard] from the *Standard solution*
 C_S = concentration of [USP Phytonadione RS](#) in the *Standard solution* (µg/mL)

 C_U = concentration of Phytonadione in the *Sample solution* (µg/mL)
Acceptance criteria: 97.0%–103.0% of phytonadione isomers**OTHER COMPONENTS****• Z-ISOMER CONTENT**

[NOTE—Protect solutions containing phytonadione from exposure to light.]

Mobile phase, Internal standard solution, Standard solution, Sample solution, Chromatographic system, System suitability, and**Analysis:** Proceed as directed in the Assay, except calculate the percentage of Z-isomer in the portion of Phytonadione taken:

$$\text{Result} = [r_Z/(r_Z + r_E)] \times 100$$

 r_Z = peak area of the (Z)-phytonadione isomer from the *Sample solution*
 r_E = peak area of the (E)-phytonadione isomer from the *Sample solution*
Acceptance criteria: NMT 21.0%**IMPURITIES****• LIMIT OF MENADIONE****Sample:** 20 mg of Phytonadione**Analysis:** Mix the *Sample* with 0.5 mL of a mixture of [6 N ammonium hydroxide](#) and [alcohol](#) (1:1). Add 1 drop of [ethyl cyanoacetate](#), and shake gently.**Acceptance criteria:** No purple or blue color is produced.**SPECIFIC TESTS****• REFRACTIVE INDEX (831):** 1.523–1.526**• REACTION:** A 50-mg/mL solution of Phytonadione in [dehydrated alcohol](#) is neutral to litmus.**ADDITIONAL REQUIREMENTS****Change to read:****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store in a cool place.▲2S (USP41)**• USP REFERENCE STANDARDS (11).**
[USP Phytonadione RS](#)**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
PHYTONADIONE	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements  Documents

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