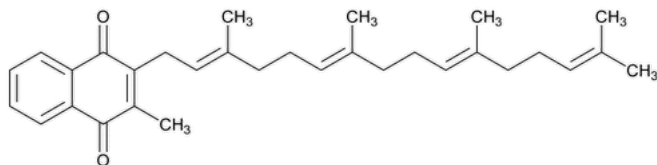


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## Menaquinone-4



$C_{31}H_{40}O_2$  444.65

2-Methyl-3-[(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]naphthalene-1,4-dione;

Menatetrenone CAS RN<sup>®</sup>: 863-61-6.

### DEFINITION

Menaquinone-4 contains NLT 98.0% and NMT 102.0% of menaquinone-4 ( $C_{31}H_{40}O_2$ ), calculated on the anhydrous basis.

### IDENTIFICATION

**Change to read:**

- **A.** ▲ [SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Infrared Spectroscopy: 197A](#) ▲ (CN 1-MAY-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol

[NOTE—Protect the *Standard solution* and *Sample solution* from light and inject immediately after preparation.]

**Standard solution:** 0.05 mg/mL of [USP Menaquinone-4 RS](#) in [isopropyl alcohol](#)

**System suitability solution:** 0.05 mg/mL of [USP Phytonadione RS](#) in *Standard solution*

**Sample stock solution:** Transfer 50 mg of Menaquinone-4 to a 50-mL volumetric flask. Dissolve in [isopropyl alcohol](#) and dilute with the same solvent to volume.

**Sample solution:** 0.05 mg/mL of Menaquinone-4 in [isopropyl alcohol](#) prepared from the *Sample stock solution*

#### Chromatographic system

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

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing [L1](#)

**Column temperature:** 40°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 μL

**Run time:** NLT 3 times the retention time of menaquinone-4

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for menaquinone-4 and phytonadione are 1.0 and 1.7, respectively.]

#### Suitability requirements

**Resolution:** NLT 4 between menaquinone-4 and phytonadione

**Relative standard deviation:** NMT 2.0% for menaquinone-4

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of menaquinone-4 ( $C_{31}H_{40}O_2$ ) in the portion of Menaquinone-4 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of menaquinone-4 from the *Sample solution*

$r_S$  = peak response of menaquinone-4 from the *Standard solution*

$C_s$  = concentration of [USP Menaquinone-4 RS](#) in the *Standard solution* (mg/mL)

$C_u$  = concentration of Menaquinone-4 in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

#### IMPURITIES

- [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

#### • ISOMERIC PURITY

**Standard solution:** 0.2 mg/mL of [USP Menaquinone-4 RS](#) in [hexane](#)

**Sample solution:** 10 mg/mL of Menaquinone-4 in [hexane](#)

#### Chromatographic system

(See [Chromatography \(621\), General Procedures, Thin-Layer Chromatography](#).)

**Mode:** TLC

**Adsorbent:** 0.2- to 0.3-mm layer of [chromatographic silica gel mixture](#)

**Application volume:** 10 µL

**Developing solvent system:** [Hexane](#) and [butyl ether](#) (17:3)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative  $R_F$  values of *cis*-menaquinone-4 and *trans*-menaquinone-4 are about 1.1 and 1.0, respectively.]

Proceed as directed in the chapter and develop the chromatogram until the solvent front has moved about 75% of the length of the plate.

Dry the plate in air and examine under short-wavelength UV light.

**Acceptance criteria:** NMT 2.0%. Any spot corresponding to the relative  $R_F$  value of 1.1 in the *Sample solution* is neither larger nor more intense than the principal spot from the *Standard solution*.

#### • ORGANIC IMPURITIES

**Mobile phase, System suitability solution, Sample stock solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Standard solution:** 1 µg/mL of [USP Menaquinone-4 RS](#) in [isopropyl alcohol](#)

**Sample solution:** Use the *Sample stock solution* from the Assay.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Menaquinone-4 taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of any impurity from the *Sample solution*

$r_s$  = peak response of menaquinone-4 from the *Standard solution*

$C_s$  = concentration of [USP Menaquinone-4 RS](#) in the *Standard solution* (mg/mL)

$C_u$  = concentration of Menaquinone-4 in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Any individual impurity:** NMT 0.1%

**Total impurities:** NMT 1.0%

#### SPECIFIC TESTS

- [WATER DETERMINATION \(921\), Method I, Method Ia](#): NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in a tight container in a cool place. Protect from light.

- [USP REFERENCE STANDARDS \(11\)](#).

[USP Menaquinone-4 RS](#)

[USP Phytanadione RS](#)

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

| Topic/Question | Contact                                                                     | Expert Committee                           |
|----------------|-----------------------------------------------------------------------------|--------------------------------------------|
| MENAQUINONE-4  | <a href="#">Fatkhulla K Tadjimukhamedov</a><br>Associate Scientific Liaison | NBDS2020 Non-botanical Dietary Supplements |