

Status: Currently Official on 15-Feb-2025
 Official Date: Official as of 01-May-2024
 Document Type: Dietary Supplement Monographs
 DocId: GUID-F0E343CC-B4C3-40F8-BF5E-3695A790B519_4_en-US
 DOI: https://doi.org/10.31003/USPNF_M8406_04_01
 DOI Ref: gb7xb

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Menaquinone-7 Preparation

Change to read:

DEFINITION

Menaquinone-7 Preparation is a combination of menaquinone-7 ($C_{46}H_{64}O_2$) or concentrated menaquinone-7 extract with one or more inert substances. It may be in a solid or liquid form. It contains NLT 90% and NMT 120% of the labeled amount ▲▲ (USP 1-May-2024) of menaquinone-7 calculated on the dried basis for the solid form and on the as-is basis for the liquid and microencapsulated powder forms.

IDENTIFICATION

Change to read:

• A. THIN-LAYER CHROMATOGRAPHY

Standard solution: Transfer 10 mg of [USP Menaquinone-7 RS](#) into a 10-mL volumetric flask, add 1 mL of [tetrahydrofuran](#), and dissolve in and dilute with the same solvent to volume.

Sample solution: Prepare the appropriate solution as follows.

For solid Preparation: Transfer an amount of Preparation, equivalent to about 1 mg of menaquinone-7, to a 2-mL centrifuge tube, and add 1 mL of [tetrahydrofuran](#). Shake, and allow the mixture to settle (if necessary, centrifuge). Use the supernatant for the test.

For liquid Preparation (oil suspension): Transfer an amount of Preparation, equivalent to about 4 mg of menaquinone-7, to a 10-mL centrifuge tube, and add 4 mL of [tetrahydrofuran](#). Shake, and allow the mixture to settle (if necessary, centrifuge). Use the supernatant for the test.

For microencapsulated powder Preparation: ▲ Prepare using one of the following suitable procedures.

Procedure 1: ▲ (USP 1-May-2024) Transfer an amount of Preparation, equivalent to about 1 mg of menaquinone-7, to a 50-mL centrifuge tube, and add 10 mL of [water](#). Heat to 50° over a water bath, and shake well. Add 10 mL of [ethyl acetate](#), shake well, and allow the mixture to settle. Use the supernatant for the test.

▲ Procedure 2: If the Preparation complies with this procedure, the labeling indicates that the Preparation complies with *Procedure 2*. Transfer an accurately weighed microencapsulated powder Preparation, equivalent to about 2 mg of menaquinone-7, to a 50-mL centrifuge tube. Add 6.0 mL of [water](#), shake the suspension well, and sonicate for 15 min at 40°–50° by shaking well every 5 min. Transfer this solution to a 100-mL Erlenmeyer flask, rinse the centrifuge tube with 25 mL of [dehydrated alcohol](#), and transfer it into the same flask. Place a magnetic stirring bar in the flask and mix at 120 rpm for 15 min at room temperature. Transfer this solution to a 50-mL volumetric flask, rinse the Erlenmeyer flask with [dehydrated alcohol](#), transfer it to the same volumetric flask, and dilute with [dehydrated alcohol](#) to volume. Shake the flask to disperse the sample evenly and pass the solution through a membrane filter of 0.45-µm pore size. ▲ (USP 1-May-2024)

Chromatographic system

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

Mode: HPTLC

Adsorbent: Nano silica gel F₂₅₄ for HPTLC¹

Application volume: 5 µL

Developing solvent system: [Hexane](#) and [ethyl acetate](#) (95:5)

Spray reagent: Transfer 1 g of [cerium sulfate](#) and 21 g of [ammonium molybdate](#) into a 500-mL volumetric flask, dissolve with 33 mL of 98% (w/w) [sulfuric acid](#), and dilute with deionized [water](#) to volume. Stir to complete dissolution. Cool, and store the solution at 0°. Allow the *Spray reagent* to come to room temperature before use.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths the length of the plate. Remove the plate from the chamber, and air-dry. Examine the plate under visible light and short-wavelength UV light, and record the spots. Spray the plate with the *Spray reagent*, and heat at 100°–110° for 5 min. Examine the plate under white light, and record the spots.

Acceptance criteria: Under visible light and short-wavelength UV light, the spots from the *Sample solution* correspond in color (light yellow), shape, and R_F value to those from the *Standard solution*. After applying the *Spray reagent*, the spots from the *Sample solution*, under white light, correspond in color (dark blue), shape, and R_F value to those from the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Menaquinone-7*.

COMPOSITION

Change to read:

• CONTENT OF MENAQUINONE-7

[NOTE—Use low-actinic glassware.]

Mobile phase: [Dehydrated alcohol](#) and [water](#) (97:3)

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

Standard solution: Transfer 20 mg of [USP Menaquinone-7 RS](#) into a 50-mL volumetric flask, add 1.0 mL of [tetrahydrofuran](#), and dilute with [dehydrated alcohol](#) to volume. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, and dilute with [dehydrated alcohol](#) to volume.

Sample solution: Prepare the appropriate solution as follows.

For solid Preparation: Transfer an accurately weighed solid Preparation, equivalent to about 0.8 mg of menaquinone-7, to a 25-mL volumetric flask, add 2 mL of [tetrahydrofuran](#), and dilute with [dehydrated alcohol](#) to volume. Shake the suspension for 2 min, and pass through a membrane filter of 0.45-μm pore size.

For liquid Preparation (oil suspension): Transfer an accurately weighed liquid Preparation, equivalent to about 1.0 mg of menaquinone-7, to a 25-mL volumetric flask, add 2 mL of [tetrahydrofuran](#), and dilute with [dehydrated alcohol](#) to volume. Pass this solution through a membrane filter of 0.45-μm pore size.

For microencapsulated powder Preparation: ▲ Prepare using one of the following suitable procedures.

Procedure 1: ▲ (USP 1-May-2024) Transfer an accurately weighed microencapsulated powder Preparation, equivalent to about 0.6 mg of menaquinone-7, to a 50-mL centrifuge tube, add 10 mL of [water](#), shake the suspension well, and sonicate for 15 min at 40°–50° by shaking well every 5 min. Cool the solution to room temperature over an ice bath and add 25 mL of [ethyl acetate](#), shake the suspension well, and sonicate for another 15 min by shaking well every 5 min. Allow the suspension to cool to room temperature and separate the phases by centrifuging or by allowing it to settle for 30 min. Pass the supernatant through a membrane filter of 0.45-μm pore size.

▲ **Procedure 2:** If the Preparation complies with this procedure, the labeling indicates that the Preparation complies with *Procedure 2*.

Transfer an accurately weighed microencapsulated powder Preparation, equivalent to about 0.8 mg of menaquinone-7, to a 50-mL centrifuge tube. Add 6.0 mL of [water](#), shake the suspension well, and sonicate for 15 min at 40°–50° by shaking well every 5 min. Transfer this solution to a 100-mL Erlenmeyer flask, rinse the centrifuge tube with 60 mL of [dehydrated alcohol](#), and transfer it into the same flask. Place a magnetic stirring bar in the flask and mix at 120 rpm for 15 min at room temperature. Transfer this solution to a 100-mL volumetric flask, rinse the Erlenmeyer flask with [dehydrated alcohol](#), transfer it to the same volumetric flask, and dilute with [dehydrated alcohol](#) to volume. Shake the flask to disperse the sample evenly and pass the solution through a membrane filter of 0.45-μm pore size. ▲ (USP 1-May-2024)

Chromatographic system

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

Mode: LC

Detector: UV 268 nm

Column: 4.6-mm × 10-cm; 2.6-μm packing [L1](#)

Column temperature: 25°

Flow rate: 0.7 mL/min

Injection volume: 10 μL

Run time: NLT 3 times the retention time of menaquinone-7

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0% from 6 replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of menaquinone-7 ($C_{46}H_{64}O_2$), in ▲ μg/g, ▲ (USP 1-May-2024) in the portion of Preparation taken:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times V$$

r_U = peak response of menaquinone-7 from the *Sample solution*

r_S = peak response of menaquinone-7 from the *Standard solution*

C_S = concentration of [USP Menaquinone-7 RS](#) in the *Standard solution* (μg/mL)

W = weight of Preparation used to prepare the *Sample solution* (g)

V = volume of the *Sample solution* (mL)