

Status: Currently Official on 15-Feb-2025  
Official Date: Official as of 01-Aug-2015  
Document Type: Dietary Supplement Monographs  
DocId: GUID-CB0EF344-523B-4066-B1E9-3D14CE697FEA\_2\_en-US  
DOI: [https://doi.org/10.31003/USPNF\\_M8046\\_02\\_01](https://doi.org/10.31003/USPNF_M8046_02_01)  
DOI Ref: ze5rx

© 2025 USPC  
Do not distribute

# Menaquinone-7 Capsules

**DEFINITION**  
Menaquinone-7 Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of menaquinone-7 ( $C_{46}H_{64}O_2$ ).

**IDENTIFICATION**  
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Method 1* or *Method 2* of the test for *Content of Menaquinone-7*.

**STRENGTH**  
• **CONTENT OF MENAQUINONE-7, Method 1**  
[NOTE—Menaquinone-7 is extremely light sensitive. Protect samples from light.]  
**Diluent:** 0.1 mg/mL of butylated hydroxytoluene in hexane  
**Mobile phase:** Methanol and ethyl acetate. See [Table 1](#) for gradient.

Table 1

| Time<br>(min) | Flow Rate<br>(mL/min) | Methanol<br>(%) | Ethyl<br>Acetate<br>(%) |
|---------------|-----------------------|-----------------|-------------------------|
| 0             | 1.00                  | 94              | 6                       |
| 5.5           | 1.00                  | 94              | 6                       |
| 6.5           | 1.25                  | 80              | 20                      |
| 13            | 1.25                  | 80              | 20                      |
| 14            | 1.25                  | 94              | 6                       |
| 15            | 1.00                  | 94              | 6                       |
| 25            | 1.00                  | 94              | 6                       |

**Standard stock solution:** Transfer 20 mg of [USP Menaquinone-7 RS](#) into a 50-mL volumetric flask, dilute with hexane to volume, and mix well.  
[NOTE—The solution can be kept in the freezer for 1 month.]

**Standard solution:** Transfer 1.0 mL of the *Standard stock solution* into a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix well.

**Sample solution A** (for hard shell capsules): Empty and mix thoroughly the contents of NLT 30 Capsules. Transfer a portion of the powder, nominally equivalent to about 1.0 mg of menaquinone-7, to a 25-mL volumetric flask. Add 10 mL of *Diluent*, and shake for 2 min. Dilute with *Diluent* to volume, mix, and pass through a membrane filter of 0.45-µm pore size.

**Sample solution B** (for soft shell capsules): Weigh NLT 30 Capsules. With a sharp blade carefully open the Capsules, without loss of the shell material, and transfer as much as possible of the combined Capsule contents to a suitable container. Remove any adhering substances from the emptied Capsules and shell remains by washing with several small portions of hexane. Discard the washings, and allow the empty Capsules and shell remains to dry in a current of dry air until the odor of hexane is no longer perceptible. Weigh the empty Capsules and shell remains, and calculate the average net weight per Capsule by difference. Transfer accurately weighed combined Capsule contents equivalent to about 1.0 mg of menaquinone-7 to a 25-mL volumetric flask, add 10 mL of *Diluent*, and mix on a vortex mixer for 15 s. Dilute with *Diluent* to volume, mix, and pass through a membrane filter of 0.45-µm pore size.

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 268 nm

**Column:** 4.6-mm × 25-cm; 4-μm packing L87. [NOTE—A suitable column is Synergy Max-RP from [www.phenomenex.com](http://www.phenomenex.com).]

**Column temperature:** 35°

**Flow rate:** See [Table 1](#).

**Injection volume:** 15 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 3.0% for six sequential injections

**Retention time:** NMT 0.5 min difference between the first and last injection for a total of six injections

#### Analysis

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

Calculate the percentage of the labeled amount of menaquinone-7 ( $C_{46}H_{64}O_2$ ) in the portion of Capsules taken:

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

$r_U$  = 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)

$C_U$  = nominal concentration of menaquinone-7 in the *Sample solution* (μg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### • CONTENT OF MENAQUINONE-7, Method 2

[NOTE—Menaquinone-7 is extremely light sensitive. Protect samples from light, and inject immediately after preparation.]

**Mobile phase:** Ethanol and water (97:3)

**Standard stock solution:** Transfer 25 mg of [USP Menaquinone-7 RS](#) into a 50-mL volumetric flask, and add 1 mL of tetrahydrofuran. Dilute with dehydrated alcohol to volume, and mix well. [NOTE—The solution can be kept in the freezer for 1 month.]

**Standard solution:** 10 μg/mL of menaquinone-7 in dehydrated alcohol from the *Standard stock solution*

**Sample solution:** Treat Capsules as directed for *Sample solution A* or *Sample solution B* in *Method 1*. Transfer accurately weighed Capsule contents equivalent to about 0.25 mg of menaquinone-7 to a 25-mL volumetric flask, and add 2 mL of tetrahydrofuran. Dilute with dehydrated alcohol to volume, shake for 2 min, and pass through a membrane filter of 0.45-μm pore size.

#### 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

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 3.0% for six replicate injections

#### Analysis

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

Calculate the percentage of the labeled amount of menaquinone-7 ( $C_{46}H_{64}O_2$ ) in the portion of Capsules taken:

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

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

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