**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of phytonadione ($C_{31}H_{46}O_2$) 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 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 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%

**OTHER COMPONENTS**
- ***Z*-ISOMER CONTENT**
[NOTE—Protect solutions containing phytonadione from exposure to light.]
**Mobile phase, Internal standard solution, Sample solution, Chromatographic system,** 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**
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Phytonadione RS

## Phytonadione Injectable Emulsion

» Phytonadione Injectable Emulsion is a sterile, aqueous dispersion of Phytonadione. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{31}H_{46}O_2$. It contains suitable solubilizing and/or dispersing agents.

**Packaging and storage—**Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** ⟨11⟩**—**
USP Endotoxin RS
USP Phytonadione RS

**Identification—**The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

**Bacterial endotoxins** ⟨85⟩**—**It contains not more than 14.0 USP Endotoxin Units per mg of phytonadione.

**pH** ⟨791⟩: between 3.5 and 7.0.

**Other requirements—**It meets the requirements under *Injections* ⟨1⟩.

**Assay—**[NOTE—Use low-actinic glassware throughout this assay, and otherwise protect the solutions from exposure to light.]

*Mobile phase*—Prepare a suitable degassed mixture of dehydrated alcohol and water (95:5).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Phytonadione RS in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.1 mg per mL.

*Assay preparation 1* (containing 10 mg or more of phytonadione per mL)—Pipet a volume of Injectable Emulsion, equivalent to 10 mg of phytonadione, into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation 2* (containing less than 10 mg of phytonadione per mL)—Pipet a volume of Injectable Emulsion, equivalent to 1 mg of phytonadione, into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 0.7 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 1.5%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak response for the major peak. Calculate the quantity, in mg, of $C_{31}H_{46}O_2$ in each mL of the Injectable Emulsion taken by the formula:

$$D(C/V)(r_U / r_S)$$

in which *D* is 100 if the Injectable Emulsion contains 10 mg or more of phytonadione per mL, or 10 if the Injectable Emulsion contains less than 10 mg of phytonadione per mL; *C* is the concentration, in mg per mL, of USP Phytonadione RS in the *Standard preparation; V* is the volume, in mL, of Injectable Emulsion taken; and $r_U$ and $r_S$ are the peak responses of phytonadione obtained from the appropriate *Assay preparation* and the *Standard preparation,* respectively.

## Phytonadione Tablets

**DEFINITION**
Phytonadione Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phytonadione ($C_{31}H_{46}O_2$).

**IDENTIFICATION**
- **A. ULTRAVIOLET ABSORPTION**
**Standard solution:** 0.01 mg/mL of USP Phytonadione RS in dehydrated alcohol
**Sample solution:** A portion of finely powdered Tablets, equivalent to 0.01 mg/mL of phytonadione in dehydrated alcohol. Shake vigorously, and filter. Use the filtrate.
**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same