

Ergocalciferol Oral Solution

Ergocalciferol Oral Solution is a solution of Ergocalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains not less than 100.0 percent and not more than 120.0 percent of the labeled amount of C₂₈H₄₄O.

Packaging and storage— Preserve in tight, light-resistant containers.

Labeling— Label the Oral Solution to indicate the concentration of ergocalciferol in mg. The activity may be expressed also in terms of USP Units, on the basis that 40 USP Vitamin D Units = 1 µg.

Assay—

Mobile phase— Use chloroform containing alcohol as preservative.

Standard solution A— Dissolve an accurately weighed quantity of [USP Ergocalciferol RS](#) in chloroform, and dilute quantitatively with chloroform to obtain a solution having a known concentration of about 500 µg per mL. [**note**—Prepare this Standard solution A fresh daily.]

Standard solution B— Transfer 5.0 mL of Standard solution A to a 50-mL volumetric flask, dilute with chloroform to volume, and mix to obtain a solution having a known concentration of 50 µg per mL. [**note**—Reserve a portion of this solution for the test for Pre-ergocalciferol response factor.]

Standard preparation— By quantitative dilution, with chloroform, of 5 mL of Standard solution B, prepare a solution having a known concentration of about 5 µg per mL. [**note**—Store this Standard preparation at a temperature not above 0 °C.]

Assay preparation— Transfer an accurately measured volume of Oral Solution, equivalent to about 250 µg of ergocalciferol, to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

Chromatographic system—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L3. The flow rate is about 1 mL per minute. The relative retention times of pre-ergocalciferol and ergocalciferol are about 0.8 and 1.0, respectively.

Calibration— Inject a suitable volume (10 μL to 20 μL) of the Standard preparation into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the Ergocalciferol response factor, F_D , by the formula: C_S / R_S

in which C_S is the concentration, in μg per mL, of [USP Ergocalciferol RS](#) in the Standard preparation, and R_S is the peak response of ergocalciferol.

Pre-ergocalciferol response factor— Transfer 5.0 mL of Standard solution B into a round-bottomed flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 hour in a water bath under a nitrogen atmosphere to obtain a solution containing ergocalciferol and pre-ergocalciferol. Cool, transfer, with the aid of several portions of chloroform, to a 50-mL volumetric flask, dilute with chloroform to volume, and mix to obtain the Working mixture. Inject a suitable volume (about 10 μL to 20 μL) of the Working mixture into the chromatograph, record the chromatogram, and measure the peak responses for ergocalciferol and pre-ergocalciferol.

Calculate the concentration, $C^I S$, in μg per mL, of ergocalciferol in the (heated) Working mixture taken by the formula:

$$F_D R_E$$

in which R_E is the peak response for ergocalciferol. Calculate the concentration, $C^I \text{Pre}$, in μg per mL, of pre-ergocalciferol in the (heated) Working mixture taken by the formula:

$$C_S - C^I S.$$

Calculate the response factor, F_{Pre} , for pre-ergocalciferol taken by the formula:

$$C^I \text{Pre} / R_P$$

in which R_P is the peak response of pre-ergocalciferol.

System suitability— The resolution, R , between the pre-ergocalciferol peak and the ergocalciferol peak determined during the test for Pre-ergocalciferol response factor is not less than 1.0, and the relative standard deviation for the peak response of replicate injections of the Standard preparation is not more than 2.0%.

Procedure— Separately inject equal volumes (10 μL to 20 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the ergocalciferol peaks. Calculate the quantity, in μg , of $\text{C}_{28}\text{H}_{44}\text{O}$ in the Assay preparation taken by the formula:

$$(F_D S_E) + (F_{\text{Pre}} S_{\text{Pre}})$$

in which S_E and S_{Pre} are the peak responses of ergocalciferol and pre-ergocalciferol, respectively.