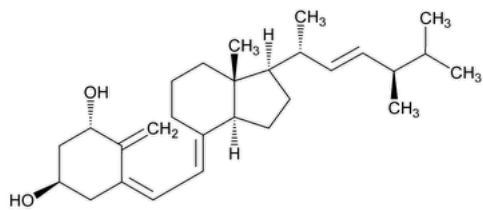


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# Doxercalciferol



$C_{28}H_{44}O_2$  412.65  
1 $\alpha$ -Hydroxyvitamin D<sub>2</sub>;  
1 $\alpha$ -Hydroxyergocalciferol;  
9,10-Secoergosta-5,7,10(19),22-tetraene-1,3-diol, (1 $\alpha$ ,3 $\beta$ ,5Z,7E,22E)-;  
(5Z,7E,22E)-9,10-Secoergosta-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ -diol CAS RN®: 54573-75-0.

**DEFINITION**

Doxercalciferol contains NLT 98.0% and NMT 102.0% of doxercalciferol ( $C_{28}H_{44}O_2$ ), calculated on the dried basis.

[CAUTION—Great care should be taken in handling Doxercalciferol, because it is a potentially cytotoxic agent.]

**IDENTIFICATION**

Change to read:

- **A.** ▲ [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), *Infrared Spectroscopy: 197K* ▲ (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**

[NOTE—Protect doxercalciferol solutions from light.]

**Solution A:** [Water](#)

**Solution B:** [Acetonitrile](#)

**Mobile phase:** See [Table 1](#). Return to original conditions and re-equilibrate the system for 5 min.

Table 1

| Time (min) | Solution A (%) | Solution B (%) |
|------------|----------------|----------------|
| 0          | 25             | 75             |
| 15         | 23             | 77             |
| 20         | 10             | 90             |
| 25         | 10             | 90             |

**Standard solution:** 1.0 mg/mL of [USP Doxercalciferol RS](#) in [acetonitrile](#). Use sonication to dissolve if necessary.

**System suitability solution:** Heat a portion of the *Standard solution* at 40° for 1 h to obtain at least 0.1%–0.2% of pre-doxercalciferol.

**Sample solution:** 1.0 mg/mL of Doxercalciferol in [acetonitrile](#). Use sonication to dissolve if necessary.

[NOTE—Doxercalciferol solutions are stable for at least 9 h when stored at room temperature, and up to 7 days when stored at 5°.]

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm × 15-cm; 3- $\mu$ m packing L1

**Column temperature:** 35°

**Flow rate:** 1.7 mL/min

**Injection volume:** 5 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for pre-doxercalciferol and doxercalciferol are 0.94 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between pre-doxercalciferol and doxercalciferol, *System suitability solution*

**Tailing factor:** 0.7–1.3, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

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

Calculate the percentage of doxercalciferol ( $C_{28}H_{44}O_2$ ) in the portion of Doxercalciferol taken:

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

$r_U$  = peak response of doxercalciferol from the *Sample solution*

$r_S$  = peak response of doxercalciferol from the *Standard solution*

$C_S$  = concentration of [USP Doxercalciferol RS](#) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Doxercalciferol in the *Sample solution* (mg/mL)

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

#### IMPURITIES

##### • ORGANIC IMPURITIES

[NOTE—Protect doxercalciferol solutions from light.]

**Solution A, Solution B, and Chromatographic system:** Proceed as directed in the Assay.

**Mobile phase:** See [Table 2](#). Return to original conditions and re-equilibrate the system for 5 min.

**Table 2**

| Time<br>(min) | Solution A<br>(%) | Solution B<br>(%) |
|---------------|-------------------|-------------------|
| 0             | 25                | 75                |
| 15            | 23                | 77                |
| 20            | 10                | 90                |
| 30            | 10                | 90                |
| 36            | 0                 | 100               |
| 50            | 0                 | 100               |

**Standard stock solution:** 4.0 mg/mL of [USP Doxercalciferol RS](#). Dissolve first in [ethyl acetate](#) using about 20% of the final volume with sonication in an ice-water bath, if necessary, and dilute with [acetonitrile](#) to volume.

**Standard solution:** 0.004 mg/mL of [USP Doxercalciferol RS](#) in [acetonitrile](#) from the *Standard stock solution*

**System suitability solution:** Heat a portion of the *Standard stock solution* at 40° for 1 h or let it stand at room temperature for about 4–6 h, to obtain at least 0.1%–0.2% of pre-doxercalciferol

**Sample solution:** 4.0 mg/mL of Doxercalciferol. Dissolve first in [ethyl acetate](#) using about 20% of the final volume with sonication in an ice-water bath, if necessary, and dilute with [acetonitrile](#) to volume. [NOTE—The *Sample solution* should be prepared fresh before injection and injected within 5 min of completing its preparation.]

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between pre-doxercalciferol and doxercalciferol

#### Analysis

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

Calculate the percentage of each individual impurity in the portion of Doxercalciferol taken:

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