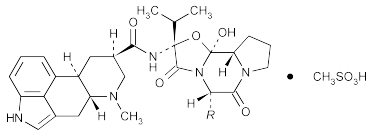
• **B. COLOR REACTION**
Sample solution: Triturate a quantity of powdered Tablets, equivalent to 5 mg of ergocalciferol, with 10 mL of chloroform, and filter.
Analysis: To 5 mL of the *Sample solution* add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake vigorously.
Acceptance criteria: A bright red color is produced and rapidly changes through violet and blue to green.

ASSAY
• **PROCEDURE:** Proceed with Tablets as directed in *Vitamin D Assay* ⟨581⟩, *Chemical Method*.
Acceptance criteria: 100.0%–120.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨**701**⟩
Time: 30 min
• **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** Label the Tablets to indicate the content 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.
• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Ergocalciferol RS

Ergoloid Mesylates



R	
Dihydroergocornine	—$CH(CH_3)_2$
Dihydroergocristine	—$CH_2C_6H_5$
Dihydro-alpha-ergocryptine	—$CH_2CH(CH_3)_2$
Dihydro-beta-ergocryptine	—$CH(CH_3)CH_2CH_3$

$C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$ (dihydroergocornine mesylate) 659.79
$C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$ (dihydroergocristine mesylate) 707.84
$C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$ (dihydro-α-ergocryptine mesylate) 673.82
$C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$ (dihydro-β-ergocryptine mesylate) 673.82
Ergotaman-3′,6′,18-trione, 9,10-dihydro-12′-hydroxy-2′,5′-bis(1-methylethyl)-, (5′α,10α)-, monomethanesulfonate (salt) mixture with 9,10α-dihydro-12′-hydroxy-2′-(1-methylethyl)-5′α-(phenylmethyl)ergotaman-3′,6′,18-trione monomethanesulfonate (salt), 9,10α-dihydro-12′-hydroxy-2′-(1-methylethyl)-5′α-(2-methylpropyl)ergotaman-3′,6′,18-trione monomethanesulfonate (salt), and 9,10α-dihydro-12′-hydroxy-2′-(1-methylethyl)-5′α-(1-methylpropyl)ergotaman-3′,6′,18-trione monomethanesulfonate (salt).
Dihydroergotoxine monomethanesulfonate (salt).
An equiproportional mixture of dihydroergocornine mesylate, dihydroergocristine mesylate, and ratio of dihydro-α-ergocryptine mesylate to dihydro-β-ergocryptine mesylate is (1.5-2.5:1) [8067-24-1].

» Ergoloid Mesylates is a mixture of the methanesulfonate salts of the three hydrogenated alkaloids, dihydroergocristine ($C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$), dihydroergocornine ($C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$), and dihydroergocryptine ($C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$), in an approximate weight ratio of 1:1:1. Ergoloid Mesylates contains not less than 97.0 percent and not more than 103.0 per cent of the alkaloid methanesulfonate mixture, calculated on the anhydrous basis, and not less than 30.3 percent and not more than 36.3 per cent of the methanesulfonate salt of each of the individual alkaloids. Dihydroergocryptine mesylate exists as a mixture of *alpha-* and *beta-* isomers. The ratio of *alpha-* to *beta-* isomers is not less than 1.5:1.0 and not more than 2.5:1.0.

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

USP Reference standards ⟨11⟩**—**
USP Ergoloid Mesylates RS

Identification—

A: The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar, undried preparation of USP Ergoloid Mesylates RS.

B: In a suitable chromatographic chamber, arranged for thin-layer chromatography, place a volume of a solvent system consisting of a mixture of acetone, *n*-butyl alcohol, ammonium hydroxide, and water (65:20:10:5) sufficient to develop the chromatogram. Prepare a test solution of Ergoloid Mesylates in a mixture of chloroform and methanol (9:1) containing 40 mg per mL. Apply 10 μL of this solution and 10 μL of a reference solution of methanesulfonic acid containing 0.4 mL in 100 mL of a mixture of chloroform and methanol (9:1) to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram until the solvent front has moved 10 cm. Remove the plate from the developing chamber, mark the solvent front, and dry in a current of cold air. Spray the plate with a 1 in 1000 solution of bromocresol purple in alcohol that previously has been adjusted to the purple color with 6 N ammonium hydroxide, then place in a stream of warm air until the spots appear: the R_F value of the methanesulfonic acid spot obtained from the test solution corresponds to that obtained from the reference solution.

Specific rotation ⟨781S⟩: between +11.0° and +15.0°.
Test solution: 10 mg per mL, in dilute alcohol (1 in 2).

pH ⟨791⟩: between 4.2 and 5.2 in a solution (1 in 200).

Water, *Method I* ⟨921⟩: not more than 5.0%.

Limit of ergotamine—Prepare three solutions in a mixture of chloroform and methanol (9:1) containing 5 mg of Ergoloid Mesylates per mL, 5 mg of USP Ergoloid Mesylates RS per mL, and 5 mg of Ergotamine Tartrate per mL. Apply 5-μL volumes of the solutions at points about 2 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Add the solvent system consisting of a mixture of chloroform and methanol (9:1) and a small beaker of ammonium hydroxide to a suitable chamber, seal, and allow to equilibrate for 30 minutes. Develop the chromatogram in the equilibrated chamber until the solvent front has moved about 15 cm from the points of application. Remove the plate, air-dry, and locate the spots, first by viewing under long-wavelength UV light, and then by spraying with a reagent prepared by dissolving 800 mg of *p*-(dimethylamino)-benzaldehyde in a mixture of 80 mL of alcohol and 11 mL of sulfuric acid: the chromatogram from Ergoloid Mesylates shows primary spots that correspond in size and color to the spots obtained from the USP Ergoloid Mesylates RS solution, and shows no spot corresponding to the principal spot in the chromatogram of Ergotamine Tartrate.

Limit of nonhydrogenated alkaloids—Prepare a solution in alcohol containing 0.4 mg of Ergoloid Mesylates per mL, and prepare a 1 in 10 dilution of the first solution. Determine the absorbances in 1-cm cells of the first solution at 317.5 nm and the dilution at 280 nm, using alcohol as the blank: the absorb-

ance of the first solution is not more than 0.15 times that of the dilution.

Assay—

Mobile phase—Prepare a degassed solution containing a mixture of water, acetonitrile, and triethylamine (80:20:2.5). Adjust the ratio as necessary.

Standard preparation—Transfer about 10 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 10-mL volumetric flask. Dissolve in a mixture of acetonitrile and water (1:1), dilute with the same solvent to volume, and mix. Prepare this solution fresh.

Assay preparation—Using about 10 mg of Ergoloid Mesylates, accurately weighed, proceed as directed for *Standard preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R,* between dihydro-α-ergocryptine mesylate and dihydroergocristine mesylate is not less than 1.35, the resolution, *R,* between dihydroergocristine and dihydro-β-ergocryptine is not less than 1.0; the column efficiency determined for the dihydro-β-ergocryptine mesylate peak is not less than 950 theoretical plates; the tailing factor for dihydro-β-ergocryptine mesylate is not more than 2.5; and the relative standard deviation of the sum of the four peaks for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The order of elution is dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine, and dihydro-β-ergocryptine. Calculate the total quantity, in mg, of these alkaloids in the portion of Ergoloid Mesylates taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation;* and r_U and r_S are the sums of the responses of the four major peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the percentage of each alkaloid taken by the formula:

$$100r_i(MW)_i / \Sigma[r_i(MW)_i]$$

in which r_i is the peak response of an individual alkaloid; $(MW)_i$ is the molecular weight of that alkaloid; and $\Sigma[r_i(MW)_i]$ is the summation of the products of peak responses and molecular weights calculated for the four alkaloids.

Ergoloid Mesylates Capsules

» Ergoloid Mesylates Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergoloid mesylates, consisting of not less than 30.3 per cent and not more than 36.3 per cent of the methane-sulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha-* to *beta-*dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

Packaging and storage—Preserve in tight, light-resistant containers between 15° and 25°. Do not freeze.

USP Reference standards ⟨11⟩**—**
USP Ergoloid Mesylates RS

Identification—

A: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, both relative to the internal standard as obtained in the *Assay*.

B: Using a sharp blade, carefully open 1 Capsule and transfer the entire contents into a 250-mL flask. Inspect the encapsulated liquid to ensure that crystallization or agglomeration of the drug substance has not taken place. Add 5 mL of water to the flask, and swirl to dissolve. Add 10 mL of *p*-dimethylaminobenzaldehyde TS: a blue color develops within 2 minutes and persists for not less than 10 minutes.

Microbial enumeration tests ⟨61⟩ **and Tests for specified microorganisms** ⟨62⟩**—**The total bacterial count does not exceed 1000 per g, and the total combined molds and yeasts count does not exceed 200 per g. Capsules meet also the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The test specimen shows an absence of the members of the *Enterobacteriaceae* family and *Pseudomonadaceae* family at levels greater than 100 per g of each.

Dissolution ⟨711⟩**—**

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

Procedure—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and note if there is membrane formation. Record the time that each Capsule ruptures.

Tolerances—All of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 Capsules fail to rupture in 15 minutes but rupture in not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements, chloroform being used as the solvent in the procedure for *Weight Variation*.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and triethylamine (32:18:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve an accurately weighed quantity of *m*-chloroacetanilide in acetonitrile to obtain a solution having a known concentration of about 0.12 mg per mL.

Tartaric acid solution—Dissolve an accurately weighed quantity of tartaric acid in water to obtain a solution having a known concentration of about 5.6 mg per mL.

Standard preparation—Transfer about 25.0 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix. Transfer 40.0 mL of this solution to a 200-mL volumetric flask, add 40.0 mL of *Tartaric acid solution*, and mix.

Assay preparation—Pipet 40.0 mL of *Tartaric acid solution* into a 200-mL volumetric flask, and heat in a water bath maintained at 50°. Add 10 Capsules (or the equivalent of 10 mg of ergoloid mesylates), and shake the flask by mechanical means for 10 minutes or until the gelatin has dissolved. Pipet 40.0 mL of *Internal standard solution* into the flask, and shake for an additional 10 minutes. Remove the flask from the bath and cool to room temperature. Transfer about 20 mL of the solution to a 30-mL centrifuge tube, and centrifuge at 10,000 rpm for 60 minutes. Filter a portion of the supernatant through a filter having a porosity of 0.45 µm, discarding the first 5 mL of the filtrate. Use the remainder of the filtrate as the *Assay preparation*.