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Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Ointment

DEFINITION

Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Ointment contains NLT 90.0% and NMT 130.0% of the labeled amounts of nystatin, neomycin, and thiostrepton, and NLT 90.0% and NMT 110.0% of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution: 100 µg/mL of USP Triamcinolone Acetonide RS in chloroform

Sample solution: Transfer 2 g of Ointment into a conical flask, add 5.0 mL of chloroform, and shake for 10 min. Add 15 mL of alcohol, and shake for an additional 10 min. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg/mL of triamcinolone acetonide.

Chromatographic system

(See *Chromatography ⟨621⟩, General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Chloroform, benzene, and methanol (5:2:1)

Spray reagent: A mixture of a 1-in-5 solution of sodium hydroxide and a 1-in-500 solution of blue tetrazolium in methanol (1:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* on a line parallel to and about 1.5 cm from the bottom edge of the TLC plate. Proceed as directed in the chapter and develop in the *Developing solvent system* until the solvent front has moved about 12 cm above the application line. Remove the plate, allow the solvent to evaporate, and spray with *Spray reagent*.

Acceptance criteria: The intensity of the blue color and the R_F of the spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

Change to read:

• **Nystatin**

[Note—Protect solutions that contain nystatin from ambient light. Store at 8° until they can be injected.]

Solution A: Dissolve 10.8 ± 1.0 g of ammonium acetate in 2500 mL of water. Adjust with acetic acid to a pH of 6.50 ± 0.05.

Solution B: 1.0 mg/mL of butylated hydroxytoluene in methanol

Mobile phase: Acetonitrile, methanol, and *Solution A* (2:1:5). [Note—Pass through a 0.45-µm nylon filter.]

System suitability solution: Transfer about 50 mg of USP Nystatin RS into a 50-mL low-actinic volumetric flask. Add 0.5 mL of 0.01 N sodium hydroxide, and allow to sit for 1 min. Add 5 mL of *Solution A*. Add about 25 mL of methanol, and sonicate to dissolve. Dilute with methanol to volume, and store in low-actinic glassware.

Standard solutions: 5400 USP Nystatin Units/mL of USP Nystatin RS in *Solution B*, in duplicate. Store in low-actinic glassware.

Sample solutions: Thoroughly mix Ointment prior to sampling. In duplicate, transfer about 1.0 g of Ointment having a known density, accurately weighed, into a low-actinic sample bottle. Add 20.0 mL of *Solution B*, and insert a polytef-coated magnetic stir bar with dimensions of about 12.7 mm × 7.9 mm. Clamp the bottles onto a suitable mixer mill,[1] and mix for a minimum of 5 min at about 30 Hz. Centrifuge at about 1350 × *g* for 5 min, or until the supernatant is clear. Transfer the supernatant to low-actinic glassware.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 304 nm

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Column: 3.9-mm × 15-cm; 4-µm packing L1. [NOTE—After each analysis, rinse the column with a mixture of acetonitrile and water (85:15) until the baseline is stable, and store in this solution. At the beginning of the next run, rinse with *Mobile phase* until the baseline is stable.]

Column temperature: 40°

Flow rate: 2.0 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solutions*

[NOTE—The relative retention times, using the *System suitability solution*, for nystatin A1 and nystatin A2 are about 1.0 and 1.4, respectively. The relative retention times are provided as information that could aid in peak assignment.]

Suitability requirements

Column efficiency: NLT 1200 theoretical plates for the nystatin A1 peak

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0% for replicate injections

Analysis

Samples: *Standard solutions* and *Sample solutions*

Separately inject equal volumes of the duplicate *Standard solutions* and duplicate *Sample solutions*, record the chromatograms, and measure the peak areas for nystatin A1 and nystatin A2.

▲Calculate the percentage of the labeled amount of nystatin in the portion of Ointment taken:

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

r_U = average peak areas of the sum of nystatin A1 and nystatin A2 from the *Sample solutions*

r_S = average peak areas of the sum of nystatin A1 and nystatin A2 from the *Standard solutions*

C_S = concentration of USP Nystatin RS in the *Standard solutions* (USP Nystatin Units/mL)

C_U = nominal concentration of nystatin in the *Sample solutions* (USP Nystatin Units/mL)▲ (USP 1-Dec-2024)

Acceptance criteria: 90.0%–130.0%

• **NEOMYCIN**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Standard: USP Neomycin Sulfate RS

Sample stock solution: Transfer an accurately weighed portion of Ointment, equivalent to about 2.5 mg of neomycin, into a 250-mL conical flask. Add 50 mL of hexanes and shake to disperse the Ointment. Transfer the mixture to a 250-mL separator. Wash the flask with 50 mL of 0.01 N hydrochloric acid, with shaking, and transfer the washing to a separator. Stopper the separator, shake, and allow the layers to separate. Draw off the lower aqueous layer, collecting it in a 250-mL volumetric flask. Repeat the extraction of the hexanes layer remaining in the separator with two or more 50-mL portions of 0.01 N hydrochloric acid, combining the aqueous extracts in the 250-mL volumetric flask. Dilute the contents of the volumetric flask with 0.01 N hydrochloric acid to volume, and mix.

Sample solution: Dilute the *Sample stock solution* quantitatively and stepwise with *Buffer B.3* to obtain a nominal concentration of neomycin equal to the median concentration of the *Standard*.

Analysis: Proceed as directed for the turbidimetric assay for neomycin in the chapter.

Acceptance criteria: 90.0%–130.0% of the labeled amount of neomycin

• **THIOSTREPTON**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Standard: USP Thiostrepton RS

Sample stock solution: Transfer a suitable, accurately weighed portion of Ointment into a blender jar. Add a sufficient, accurately measured volume of dimethyl sulfoxide, blend on high speed to obtain a convenient concentration, and filter.

Sample solution: Dilute an accurately measured volume of the *Sample stock solution* quantitatively with dimethyl sulfoxide to obtain a nominal concentration of thiostrepton equal to the median concentration of the *Standard*.

Analysis: Proceed as directed for thiostrepton in the chapter.

Acceptance criteria: 90.0%–130.0% of the labeled amount of thiostrepton

Change to read:

• **TRIAMCINOLONE ACETONIDE**

Mobile phase: Acetonitrile and water (approximately 30:70)

Internal standard solution: 50 µg/mL of fluoxymesterone in isopropyl alcohol

Standard stock solution: 75 µg/mL of USP Triamcinolone Acetonide RS in the *Internal standard solution*

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