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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 polytetrafluoroethylene-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

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*

Standard solution: Mix an accurately measured volume of the *Standard stock solution* with an equal volume of *Mobile phase* to obtain a solution containing about 37.5 µg/mL of [USP Triamcinolone Acetonide RS](#).

Sample solution: Transfer an accurately weighed quantity of Ointment, equivalent to about 1.5 mg of triamcinolone acetonide, to a screw-capped tube. Add 20.0 mL of the *Internal standard solution*, and cap securely. Heat at 60° for 5 min, and then swirl vigorously for NLT 30 s. Repeat the heating and swirling sequence 3 times. Cool in a methanol-ice bath for 15–20 min, and then centrifuge at –5° for 15 min. Dilute an accurately measured volume of the supernatant with an equal volume of *Mobile phase*. Cool in a methanol-ice bath for 10–15 min, with occasional agitation. Pass first through a pledget of glass wool or a prefilter disk, and then pass through a membrane of 0.45-µm pore size to obtain a clear solution.

Chromatographic system

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

[NOTE—Adjust the operating parameters with *Mobile phase*, such that the separation of triamcinolone acetonide and the internal standard is optimized, with a retention time of about 14.5 min for triamcinolone acetonide.]

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing [L1](#)

Column temperature: Room temperature

Injection volume: 15–25 µL of equal volumes of the *Standard solution* and *Sample solution*

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the triamcinolone acetonide and fluoxymesterone peaks

Relative standard deviation: NMT 3.0% for 5 replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak heights of the internal standard and triamcinolone acetonide at the same retention times obtained from the *Standard solution* and *Sample solution*.

▲ Calculate the percentage of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of triamcinolone acetonide to the internal standard from the *Sample solution*

R_S = peak height ratio of triamcinolone acetonide to the internal standard from the *Standard solution*

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

C_U = nominal concentration of triamcinolone acetonide in the *Sample solution* (mg/mL)

▲ (USP 1-Dec-2024)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **MINIMUM FILL (755):** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS (11).**
[USP Nystatin RS](#)
[USP Neomycin Sulfate RS](#)
[USP Thiostrepton RS](#)
[USP Triamcinolone Acetonide RS](#)

¹ A suitable mixer mill can be obtained from Retsch Inc., product no. MM 301.

Topic/Question	Contact	Expert Committee
NYSTATIN, NEOMYCIN SULFATE, THIOSTREPTON, AND TRIAMCINOLONE ACETONIDE OINTMENT	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: [Chromatographic Database](#)

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