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Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Ointment

DEFINITION

Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Ointment contains NLT 90.0% and NMT 140.0% of the labeled amounts of nystatin, neomycin, and gramicidin, 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

• **Nystatin**

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

Standard: USP Nystatin RS

Sample stock solution: Transfer a suitable, accurately weighed portion of Ointment into a blender jar. Add a sufficient, accurately measured volume of dimethylformamide and blend at high speed for 3–5 min to obtain a convenient concentration. Dilute quantitatively an accurately measured volume of the solution so obtained with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units/mL.

Sample solution: Dilute an accurately measured volume of the *Sample stock solution* quantitatively with *Buffer B.6* to obtain a nominal concentration of nystatin equal to the median concentration of the *Standard*.

Analysis: Proceed as directed for nystatin in the chapter.

Acceptance criteria: 90.0%–140.0% of the labeled amount of nystatin

• **Neomycin**

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

Standard: USP Neomycin Sulfate RS

Sample stock solution: Transfer an accurately weighed portion of Ointment, equivalent to about 3.5 mg of neomycin, to a separator, add about 50 mL of ether, and shake well. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to an appropriate volume to obtain a stock solution of convenient concentration.

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 neomycin in the chapter.

Acceptance criteria: 90.0%–140.0% of the labeled amount of neomycin

• **Gramicidin**

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

Standard: USP Gramicidin RS

Sample stock solution: Dissolve an accurately weighed portion of Ointment in 50 mL of hexanes, transfer into a separator, and extract with four 20-mL portions of 80% alcohol. Combine the extracts in a suitable volumetric flask, dilute with alcohol to volume, and mix.

Sample solution: Dilute an accurately measured volume of the *Sample stock solution* quantitatively and stepwise with alcohol to obtain a nominal concentration of gramicidin equal to the median concentration of the *Standard*.

Analysis: Proceed as directed for gramicidin in the chapter.

Acceptance criteria: 90.0%–140.0% of the labeled amount of gramicidin

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

• **Water Determination ⟨921⟩, *Method I***

[Note—Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.]

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards ⟨11⟩**

USP Gramicidin RS
USP Neomycin Sulfate RS
USP Nystatin RS
USP Triamcinolone Acetonide RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
NYSTATIN, NEOMYCIN SULFATE, GRAMICIDIN, 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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