



Edition: BP 2025 (Ph. Eur. 11.6 update)

Hydrocortisone and Neomycin Cream

[General Notices](#)

Action and use

Corticosteroid + Aminoglycoside antibacterial.

DEFINITION

Hydrocortisone and Neomycin Cream contains Hydrocortisone and Neomycin Sulfate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for [thin-layer chromatography, Appendix III A](#), using the following solutions.

- (1) Add 10 mL of [hexane](#) saturated with [acetonitrile](#) to a quantity of the preparation being examined containing 5 mg of Hydrocortisone and shake for 2 to 3 minutes. Add 10 mL of [acetonitrile](#) saturated with [hexane](#), shake for 10 minutes and allow the layers to separate. Centrifuge, filter the acetonitrile layer if necessary, evaporate 5 mL to dryness and dissolve the residue in 5 mL of a mixture of equal volumes of [chloroform](#) and [ethanol \(96%\)](#).
- (2) 0.05% w/v of [hydrocortisone BPCRS](#) in a mixture of equal volumes of [chloroform](#) and [ethanol \(96%\)](#).

CHROMATOGRAPHIC CONDITIONS

- (a) Use as the coating [silica gel GF₂₅₄](#).
- (b) Use the mobile phase as described below.
- (c) Apply 10 µL of each solution.
- (d) Develop the plate to 15 cm.
- (e) After removal of the plate, dry in air and spray with [alkaline tetrazolium blue solution](#).

MOBILE PHASE

8 volumes of [methanol](#) and 90 volumes of [dichloromethane](#).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay for hydrocortisone the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (2).

C. Carry out the method for [thin-layer chromatography, Appendix III A](#), using the following solutions.

- (1) Disperse a quantity containing 7000 IU of Neomycin Sulfate with 10 mL of [chloroform](#), add 5 mL of [water](#), shake, centrifuge and use the clear, upper layer.

(2) 0.2% w/v of [neomycin sulfate EPCRS](#) in [water](#).

CHROMATOGRAPHIC CONDITIONS

- Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).
- Use the mobile phase as described below.
- Apply 5 µL of each solution.
- Develop the plate to 15 cm.
- After removal of the plate, dry in air, spray with a 1% w/v solution of [ninhydrin](#) in [butan-1-ol](#) and heat at 105° for 2 minutes.

MOBILE PHASE

20 volumes of [chloroform](#), 40 volumes of 13.5M [ammonia](#) and 60 volumes of [methanol](#).

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

TESTS

Neamine

Carry out the method for [thin-layer chromatography](#), [Appendix III A](#), using the following solutions.

- Dissolve a quantity containing 7000 IU of Neomycin Sulfate in 10 mL of [chloroform](#), shake gently with 5 mL of [water](#), centrifuge and use the aqueous layer.
- 0.004% w/v of [neamine EPCRS](#) in [water](#).

CHROMATOGRAPHIC CONDITIONS

- Use as the coating [silica gel H](#).
- Use the mobile phase as described below.
- Apply 2 µL of each solution.
- Develop the plate to 15 cm.
- After removal of the plate dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting [sodium hypochlorite solution](#) with [water](#) to contain 0.5% of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a very faint blue colour with a drop of a 0.5% w/v solution of [potassium iodide](#) in [starch mucilage](#); avoid prolonged exposure to the cold air. Spray the plate with a 0.5% w/v solution of [potassium iodide](#) in [starch mucilage](#).

MOBILE PHASE

Freshly prepared 3.85% w/v solution of [ammonium acetate](#).

CONFIRMATION

Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C

Carry out the method for [liquid chromatography](#), [Appendix III D](#), using the following solutions.

- Shake a quantity containing 3500 IU of Neomycin Sulfate with 10 mL of [chloroform](#), add 5 mL of 0.02M [sodium tetraborate](#), mix, allow to separate and centrifuge the upper layer. Proceed as for solution (2) but using 0.5 mL of the clear supernatant liquid in place of 0.5 mL of the neomycin sulfate solution.
- Add 1.5 mL of a freshly prepared 2% w/v solution of [1-fluoro-2,4-dinitrobenzene](#) in [methanol](#) to 0.5 mL of a 0.10% w/v solution of [neomycin sulfate EPCRS](#) in 0.02M [sodium tetraborate](#), heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 mL with the mobile phase, allow to stand and use the clear lower layer.

CHROMATOGRAPHIC CONDITIONS

- Use a stainless steel column (20 cm × 4.6 mm) packed with [silica gel for chromatography](#) (5 µm) (Nucleosil 100-5 is suitable).
- Use isocratic elution and the mobile phase described below.
- Use a flow rate of 1.6 mL per minute.
- Use an ambient column temperature.
- Use a detection wavelength of 350 nm.
- Inject 10 µL of each solution.
- Pass the mobile phase through the column for several hours before starting the analysis. Record the chromatogram for 1.4 times the retention time of the peak due to neomycin B.

MOBILE PHASE

0.5 mL of [glacial acetic acid](#), 1.0 mL of [water](#) and 97 mL of [tetrahydrofuran](#), with sufficient of a 2.0% v/v solution of [absolute ethanol](#) in [ethanol-free chloroform](#) to produce 250 mL.

SYSTEM SUITABILITY

The chromatogram obtained with solution (2) shows a principal peak due to neomycin B and a major [secondary peak](#) due to neomycin C with a retention time relative to neomycin B of about 0.6.

The [column efficiency](#), determined using the peak due to neomycin B in the chromatogram obtained with solution (2), should be at least 13,000 [theoretical plates](#) per metre.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak corresponding to neomycin C is 3 to 15% of the sum of the areas of the peaks corresponding to neomycin B and neomycin C.

ASSAY

For [hydrocortisone](#)

Carry out the method for [liquid chromatography, Appendix III D](#), using the following solutions in [chloroform](#).

- Shake together a quantity of the preparation being examined containing 25 mg of Hydrocortisone, several small glass beads and 25 mL of a 0.036% w/v solution of [fluoymesterone BPCRS](#) in [chloroform](#) for 15 minutes, add sufficient [chloroform](#) to produce 50 mL, mix and centrifuge. Remove any excipient material present at the interface and use the clear supernatant liquid.
- 0.010% w/v of [hydrocortisone BPCRS](#) and 0.018% w/v of [fluoymesterone BPCRS](#) (internal standard).

CHROMATOGRAPHIC CONDITIONS

- Use a stainless steel column (30 cm × 3.9 mm) packed with [silica gel for chromatography](#) (10 µm) (µPorasil is suitable).
- Use isocratic elution and the mobile phase described below.
- Use a flow rate of 1 mL per minute.
- Use an ambient column temperature.
- Use a detection wavelength of 254 nm.
- Inject 20 µL of each solution.

MOBILE PHASE

30 volumes of [glacial acetic acid](#), 35 volumes of [methanol](#), 70 volumes of [tetrahydrofuran](#), 425 volumes of [butyl chloride](#) and 425 volumes of [butyl chloride](#) saturated with [water](#).

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{30}O_5$ in the cream using the declared content of $C_{21}H_{30}O_5$ in [hydrocortisone BPCRS](#).

For neomycin sulfate

Stir a quantity containing 4200 IU with 15 mL of [chloroform](#) until the emulsion is completely broken. Transfer to a separating funnel with 25 mL of [phosphate buffer pH 8.0](#) and 5 mL of [chloroform](#), shake vigorously, allow to separate and reserve the aqueous phase. Extract the chloroform layer with two 25-mL quantities of [phosphate buffer pH 8.0](#) and discard the chloroform layer. Pass [nitrogen](#) through the combined aqueous solutions to remove dissolved chloroform and dilute to 100 mL with sterile [phosphate buffer pH 8.0](#). Dilute 10 mL of the resulting solution to 50 mL with the same solvent and carry out the [microbiological assay of antibiotics, Appendix XIV A](#). The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated amount.

LABELLING

The strength with respect to Neomycin Sulfate is stated as the number of IU (Units) per g.