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Gentamicin and Hydrocortisone Acetate Ear Drops

General Notices

Action and use

Aminoglycoside antibacterial (Gentamicin Sulfate); corticosteroid (Hydrocortisone Acetate).

DEFINITION

Gentamicin and Hydrocortisone Acetate Ear Drops contain Gentamicin Sulfate and Hydrocortisone Acetate suspended in a suitable vehicle.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Dilute a volume of the ear drops, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.3% w/v of gentamicin, filter and use the filtrate.
(2) 0.5% w/v of *gentamicin sulfate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).
(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, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

MOBILE PHASE

The lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *dichloromethane* and *methanol* and allowing to separate.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatogram obtained with solution (2).

B. Comply with the test for Composition of gentamicin sulfate.
C. Filter 20 mL of the well-mixed ear drops (Whatman No. 1 filter paper is suitable), wash the residue with the minimum amount of *water*, dry at 105° and dissolve 50 mg of the residue obtained in 50 mL of *ethanol (96%)*. Add 2 mL of *sulfuric acid* to 2 mL of the resulting solution; an intense yellow colour is produced which exhibits a green fluorescence which is particularly intense when viewed under *ultraviolet light (365 nm)*. Add the solution to 10 mL of *water* and mix; the fluorescence under *ultraviolet light (365 nm)* does not disappear.

D. In the Assay for hydrocortisone acetate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

Composition of gentamicin sulfate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 mL of *methanol* to 10 mL of a solution prepared by diluting a suitable volume of the ear drops with *water* to contain the equivalent of 0.045% w/v of gentamicin. Swirl and add 4 mL of *phthalaldehyde reagent*, mix and add sufficient *methanol* to produce 25 mL, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.
(2) Prepare in the same manner as solution (1) but using 10 mL of a 0.065% w/v solution of *gentamicin sulfate BPCRS* in place of 10 mL of the preparation being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS and Kromasil C18 are suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 330 nm.
(f) Inject 5 µL of each solution.

MOBILE PHASE

0.025M *sodium heptanesulfonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (1) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the ear drops by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

ASSAY

For gentamicin

Dilute a volume of the ear drops containing the equivalent of 15 mg of gentamicin to 50 mL with sterile *phosphate buffer pH 8.0* and dilute 10 mL of the resulting solution to 50 mL with the same solvent. 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.

Calculate the content of gentamicin in the ear drops, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the ear drops containing 0.1 g of Hydrocortisone Acetate with 25 mL of *methanol*, add sufficient *methanol* to produce 100 mL and dilute 10 mL of this solution to 50 mL with the mobile phase.
(2) Dilute 10 mL of a 0.1% w/v solution of *hydrocortisone acetate BPCRS* in *methanol* to 50 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 256 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

400 volumes of *water* and 600 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}O_6$ in the ear drops using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

STORAGE

Gentamicin and Hydrocortisone Acetate Ear Drops should not be allowed to freeze.

LABELLING

The quantity of Gentamicin Sulfate is stated in terms of the equivalent amount of gentamicin.