Medroxyprogesterone injection (Medroxyprogesteroni injectio)

Category. Contraceptive.

Storage. Medroxyprogesterone injection should be protected from light. On standing solid matter may separate; it should be resuspended before use.

Additional information. Strength in the current WHO Model List of Essential Medicines: 150 mg/mL in 1 mL vial.

Requirements

Complies with the monograph for *Parenteral preparations*.

Definition. Medroxyprogesterone injection is a sterile aqueous suspension of Medroxyprogesterone acetate. It contains not less than 90.0% and not more than 110.0% of the amount of Medroxyprogesterone acetate ($C_{24}H_{34}O_4$) stated on the label.

Identity tests

-Either test A alone or tests B and C or tests C and D may be applied.

A. Centrifuge a volume of the injection to be examined containing 50 mg of Medroxyprogesterone acetate. Decant the supernatant liquid and discard. Dry the residue at 105 °C for 3 hours and carry out the examination as described under <u>1.7 Spectrophotometry in the infrared region</u>. The infrared absorption spectrum is concordant with the spectrum obtained from medroxyprogesterone acetate RS or with the *reference spectrum* of medroxyprogesterone acetate.

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1 Carry out the test as described under <u>1.14.1 Chromatography</u>, Thin-layer chromatography using silica R5 as the coating substance and a mixture of 10 volumes of dichloromethane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µL of each of the following two solutions in dichloromethane R. For solution (A) measure a volume of injection to be examined containing about 40 mg of Medroxyprogesterone acetate, add 15 mL of dichloromethane R, shake vigorously for 20 minutes, allow to stand for 30 minutes, add 2.5 g sodium sulphate anhydrous R, shake for 5 minutes and allow to stand for another 10 minutes. For solution (B) use 2.5 mg of medroxyprogesterone acetate RS per mL. After removing the plate from the chromatographic chamber heat it at 120 °C for 30 minutes, spray with 4-toluenesulfonic acid/ethanol TS and heat further at 120 °C for 10 minutes. Allow the plate to cool and examine the chromatogram in ultraviolet light (365 nm).

The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

B.2 Carry out the test as described under <u>1.14.1 Chromatography</u>, <u>Thin-layer chromatography</u> using the conditions described under test B.1, but spray the plate with a mixture of equal volumes of sulfuric acid R and ethanol (~750 g/L) TS and heat further at 120 °C for 10 minutes. Allow the plate to cool and examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

C. Centrifuge a volume of injection to be examined containing 30 mg of Medroxyprogesterone acetate. Decant the supernatant liquid, dissolve the residue in 5 mL of sulfuric acid R and introduce 5 mL of ethanol (~750 g/L) TS to form an upper layer; a bluish violet ring is formed at the interface of the two layers.

D. See the test described below under "Assay". The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

pH. pH of the injection, 3.0–7.0.

Impurity F (4,5-Dihydromedroxyprogesterone acetate). Carry out the test as described under <u>1.14.1 Chromatography</u>, <u>Thin-layer</u> <u>chromatography</u> using silica gel R5 as the coating substance and a mixture of 10 volumes of tetrahydrofuran R, 45 volumes of *tert*-butyl methyl ether R and 45 volumes of hexane R as the mobile phase.

Apply separately to the plate 10 µL of each of the following three solutions in dichloromethane R. For solution (A) transfer a volume of the injection to be examined containing about 300 mg of Medroxyprogesterone acetate into a flask, add 15 mL of dichloromethane R, stopper the flask and shake vigorously for 20 minutes, allow to stand for 30 minutes, add 10 g sodium sulphate anhydrous R, shake again for 5 minutes and allow to stand for another 10 minutes. For solution (B) dilute 0.5 volume of

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solution (A) to 100 volumes. For solution (C) use a solution containing 20 mg of medroxyprogesterone acetate RS and 0.1 mg of medroxyprogesterone acetate impurity F RS per mL.

Develop the plate for a distance of about 10 cm. Allow it to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase. After removing the plate from the chromatographic chamber heat it at 100 °C to 105 °C for 30 minutes and spray with 4-toluenesulfonic acid/ethanol TS. Heat again at 120 °C for 10 minutes, allow to cool and examine the chromatogram in ultraviolet light (365 nm).

In the chromatogram obtained with solution (C) impurity F has a R_f value of about 0.66 and medroxyprogesterone acetate a R_f value of about 0.56. The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots. In the chromatogram obtained with solution (A) any spot due to impurity F is not more intense than the principal spot in the chromatogram obtained with solution (B) (0.5%).

Related substances

Prepare fresh solutions and perform the tests without delay.

Carry out the test as described under <u>1.14.1 Chromatography</u>, <u>High-performance liquid chromatography</u> using the conditions given under "Assay".

Prepare the following solutions with the mobile phase. For solution (1) dilute a suitable volume of the injection to be examined to obtain a concentration of 0.4 mg of Medroxyprogesterone acetate per mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) dissolve 4 mg of medroxyprogesterone acetate for system suitability RS (containing medroxyprogesterone acetate and the impurities A, B, C, D, E, G and I) and dilute to 2.0 mL. For solution (4) use a solution containing 3.65 µg of methyl hydroxybenzoate R and 0.4 µg of propyl hydroxybenzoate R per mL.

Inject separately 20 µL of solution (1), (2), (3) and (4). Record the chromatogram for about twice the retention time of medroxyprogesterone acetate in solution (2).

Use the chromatogram supplied with medroxyprogesterone acetate for system suitability RS and the chromatograms obtained with solution (3) and (4) to identify the peaks due to the impurities A, B, C, D, E, G and I and possible preservatives (depending on the formulation of the injection there may be the preservatives methyl hydroxybenzoate and propyl hydroxybenzoate present). The impurities are eluted at the following relative retention with reference to the peak of medroxyprogesterone acetate (retention time about 27 minutes): impurity A about 0.3; impurity I about 0.5; impurity H about 0.65; impurity B about 0.7; impurity C about 0.8; impurity G about 0.85; impurity D about 0.9; impurity E about 0.95.

The test is not valid unless in the chromatogram obtained with solution (3) the peak-to-valley ratio (Hp/Hv) is at least 2.5, where Hp is the height above the baseline of the peak due to impurity E and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to medroxyprogesterone acetate.

In the chromatogram obtained with solution (1):

-the area of any impurity peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

-the sum of all impurity peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%). Disregard any peak due to hydroxybenzoate derivatives in the chromatogram obtained with solution (4).Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (2) (0.05%).

Assay

Carry out the test as described under <u>1.14.1 Chromatography</u>, High-performance liquid chromatography using a stainless steel column (25 cm \times 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 μ m).

As the mobile phase use a solution prepared as follows: mix 100 volumes of tetrahydrofuran R, 350 volumes of acetonitrile R and 500 volumes of water R.

Prepare the following solutions in the mobile phase. For solution (1) dilute a suitable volume of the injection to be examined to obtain a concentration of 40 µg of Medroxyprogesterone acetate per mL. For solution (2) dissolve 10 mg of medroxyprogesterone acetate RS and dilute to 50.0 mL. Dilute 5.0 mL of this solution to 25.0 mL.

Operate with a flow rate of 0.9 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 241 nm.

Inject separately 20 μ L of solution (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of medroxyprogesterone acetate ($C_{24}H_{34}O_4$), using the declared content of $C_{24}H_{34}O_4$ in medroxyprogesterone acetate RS.