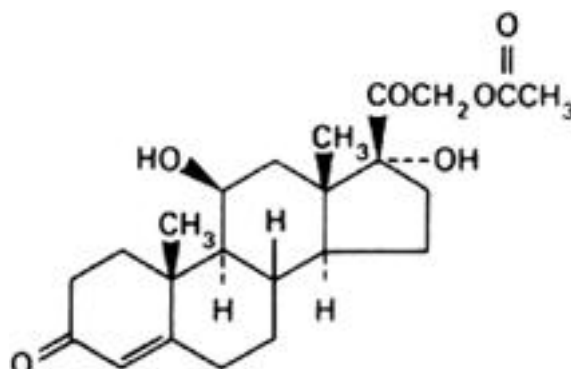


**Hydrocortisone acetate (Hydrocortisoni acetat)****Molecular formula.**  $C_{23}H_{32}O_6$ **Relative molecular mass.** 404.5**Graphic formula.****Chemical name.** 21-(Acetyloxy)-11 $\beta$ ,17-dihydroxypregn-4-ene-3,20-dione; 11 $\beta$ ,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate; CAS Reg. No. 50-03-3.**Other name.** Cortisol acetate.**Description.** A white or almost white, crystalline powder; odourless.**Solubility.** Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.**Category.** Adrenocortical steroid.**Storage.** Hydrocortisone acetate should be kept in a well-closed container, protected from light.**Additional information.** Hydrocortisone acetate melts at about 220°C with decomposition.**Requirements****Definition.** Hydrocortisone acetate contains not less than 97.0% and not more than 102.0% of  $C_{23}H_{32}O_6$ , calculated with reference to the dried substance.**Identity tests**

- Either test A or test B may be applied.

A. Carry out the examination as described under [1.7 Spectrophotometry in the infrared region](#). The infrared absorption spectrum is concordant with the spectrum obtained from hydrocortisone acetate RS or with the *reference spectrum* of hydrocortisone acetate.

B. Carry out the test as described under [1.14.1 Chromatography, Thin-layer chromatography](#), using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours and carry out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 75 volumes of toluene R and 25 volumes of chloroform R. Apply separately to the plate 2  $\mu$ l of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per mL and (B) 2.5 mg of hydrocortisone acetate RS per mL. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120°C for 15 minutes, spray with sulfuric acid/ethanol TS, and then heat at 120°C for 10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

**Specific optical rotation.** Use a 10 mg/mL solution in dioxan R;  $[\alpha]_D^{20} = +157^\circ$  to  $+168^\circ$ .

**Loss on drying.** Dry to constant weight at 105°C; it loses not more than 10 mg/g.**Related substances.** Carry out the test as described under [1.14.1 Chromatography, Thin-layer chromatography](#) using silica gel R2 as the coating substance and a mixture of 95 volumes of dichloroethane R, 5 volumes of methanol R and 0.2 volumes of

water as the mobile phase. Apply separately to the plate 1  $\mu$ l of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per mL and (B) 0.30 mg of the test substance per mL. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated; then heat at 105°C for 10 minutes, allow to cool, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

**Assay.** Dissolve about 20 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 mL; dilute 5.0 mL of this solution to 100 mL with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 242 nm. Calculate the amount of  $C_{23}H_{32}O_6$  in the substance being tested by comparison with hydrocortisone acetate RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be  $0.40 \pm 0.02$  (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).