TESTS

Solution S. Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 or Y_7 (*2.2.2, Method II*).

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a). Dissolve 50 mg of the substance to be examined in a mixture of 10 volumes of *water R* and 15 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with a mixture of 10 volumes of *water R* and 15 volumes of *methanol R*.

Reference solution (a). Dissolve 10 mg of *flucytosine CRS* in a mixture of 10 volumes of *water R* and 15 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b). Dilute 1 ml of test solution (b) to 100 ml with a mixture of 10 volumes of *water R* and 15 volumes of *methanol R*.

Reference solution (c). Dissolve 5 mg of *fluorouracil CRS* in 5 ml of reference solution (a).

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm in an unsaturated tank using a mixture of 1 volume of *anhydrous formic acid R*, 15 volumes of *water R*, 25 volumes of *methanol R* and 60 volumes of *ethyl acetate R*. Allow the solvents to evaporate. At the bottom of a chromatography tank place an evaporating dish containing a mixture of 2 volumes of a 15 g/l solution of *potassium permanganate R*, 1 volume of *hydrochloric acid R1* and 1 volume of *water R*, close the tank and allow to stand for 15 min. Place the dried plate in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 5 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of the coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution R*. Spray with *potassium iodide and starch solution R*. Examine the plate in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Fluoride. Not more than 200 ppm. Carry out a potentiometric determination of fluoride ion, using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode.

Prepare and store all solutions in plastic containers.

Buffer solution. Dissolve 58 g of *sodium chloride R* in 500 ml of *water R*. Add 57 ml of *glacial acetic acid R* and 200 ml of a 100 g/l solution of *cyclohexylenedinitrilotetra-acetic acid R* in *1 M sodium hydroxide*. Adjust the pH to 5.0 to 5.5 with a 200 g/l solution of *sodium hydroxide R* and dilute to 1000.0 ml with *water R*.

Test solution. Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions. Dissolve 4.42 g of *sodium fluoride R*, previously dried at 120 °C for 2 h, in 300 ml of *water R* and dilute to 1000.0 ml with the same solvent (solution (a): 1.9 g/l of fluoride). Prepare three reference solutions by dilution of solution (a) 1 in 100, 1 in 1000 and 1 in 10 000. To 20.0 ml of each reference solution, add 10.0 ml of the buffer solution and stir with a magnetic stirrer. Introduce the electrodes into the solution and allow to stand for 5 min with constant stirring. Determine the potential difference between the electrodes. Plot on semi-logarithmic graph paper the potential difference obtained for each solution as a function of concentration of fluoride. Using exactly the same conditions, determine the potential difference obtained with the test solution and calculate the content of fluoride.

Heavy metals (*2.4.8*). 1.0 g complies with limit test C for heavy metals (20 ppm). *Use aplatinum crucible.* Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.100 g in 40 ml of *anhydrous acetic acid R* and add 100 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 12.91 mg of $C_4H_4FN_3O$.

STORAGE

Store protected from light.

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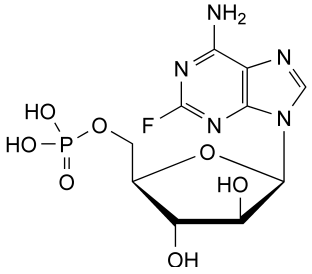
FLUDARABINE PHOSPHATE

Fludarabini phosphas



$C_{10}H_{13}FN_5O_7P$ M_r 365.2
[75607-67-9]

DEFINITION

2-Fluoro-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-6-amine.

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder, hygroscopic.

Solubility: slightly soluble in water, freely soluble in dimethylformamide, very slightly soluble in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *fludarabine phosphate CRS*.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 50 mg in 5.0 ml of *dimethylformamide R* with the aid of ultrasound.

Specific optical rotation (*2.2.7*): + 10.0 to + 14.0 (anhydrous substance).

Dissolve 0.100 g in *water R* and dilute to 20.0 ml with the same solvent with the aid of ultrasound.

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution. With the aid of ultrasound, dissolve 20 mg of the substance to be examined in 50 ml of *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a). With the aid of ultrasound, dissolve 20 mg of *fludarabine phosphate CRS* in 50 ml of *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b). With the aid of ultrasound, dissolve 20 mg of the substance to be examined in 20 ml of *0.1 M hydrochloric acid.* Heat in a water-bath at 80 °C for 15 min, cool to room temperature, mix and dilute to 100.0 ml with *water R*.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 20.0 ml with *water R*.

Blank solution: *0.02 M hydrochloric acid.*

A. Early eluting impurities.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 60 volumes of *methanol R* and 940 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 260 nm and 292 nm.

Injection: 10 µl; inject the solutions and record the chromatograms at 260 nm.

Run time: 4.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Identification of impurities: identify the impurity peaks in the chromatogram obtained with reference solution (a) and in the chromatogram obtained with the test solution by comparison with Figure 1781.-1. Additionally, inject the test solution and reference solution (a) and record the chromatograms at 292 nm to identify impurities A and B, the response of which is much higher than that at 260 nm.

Relative retention with reference to fludarabine phosphate (retention time = about 9 min): impurity A = about 0.26; impurity B = about 0.34; impurity C = about 0.42.

System suitability: reference solution (b) at 292 nm:

- *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits: at 260 nm:

- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 4.0; impurity B = 2.5; impurity C = 1.9;
- *impurity A*: maximum 0.8 per cent;
- *impurity B*: maximum 0.2 per cent;
- *impurity C*: maximum 0.4 per cent;
- *any other impurity preceding fludarabine phosphate*: maximum 0.1 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent), and any peak eluting after fludarabine phosphate.

B. Late eluting impurities.

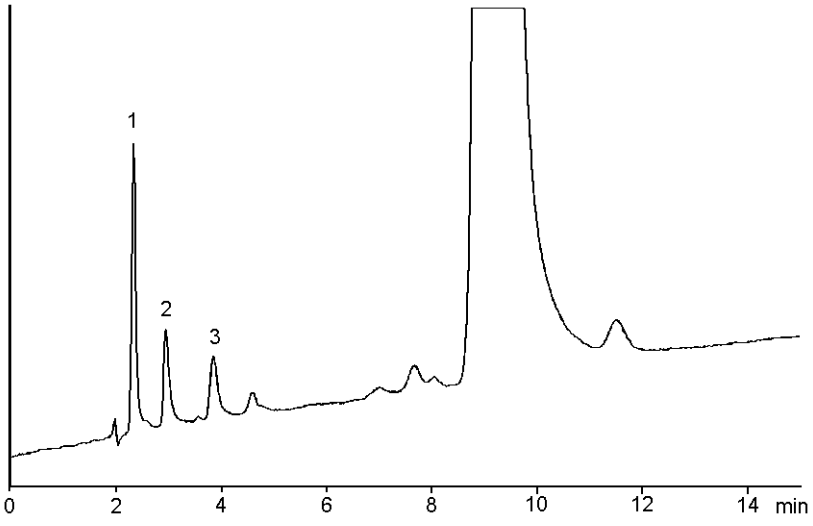
Conditions as described under Test A with the following modifications.

Mobile phase: mix 200 volumes of *methanol R* and 800 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R*.

Detection: spectrophotometer at 260 nm.

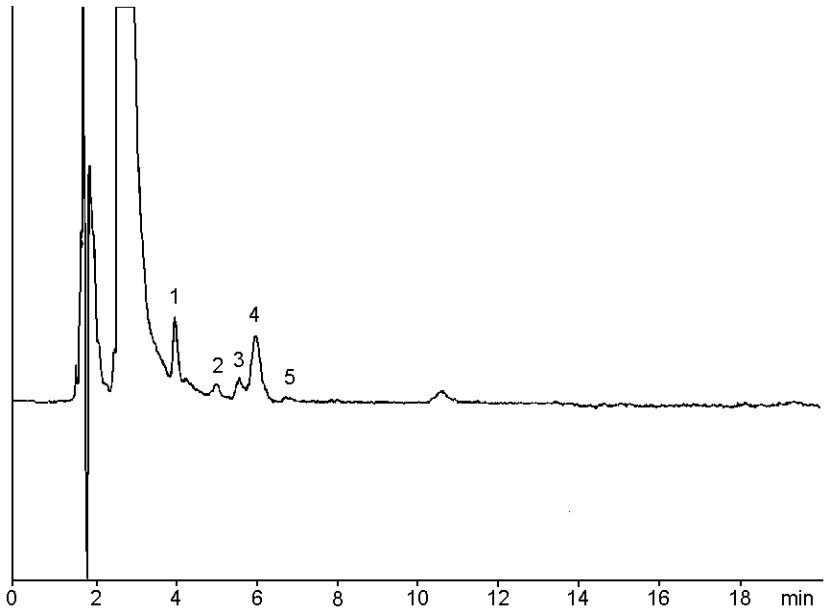
Injection: 10 µl.

Run time: 8 times the retention time of the principal peak in the chromatogram obtained with the test solution.



1. impurity A
2. impurity B
3. impurity C

Figure 1781.-1. – *Chromatogram for test A for related substances of fludarabine phosphate: reference solution (a) at 260 nm*



1. impurity D
2. impurity E
3. impurity G
4. impurity H
5. impurity F

Figure 1781.-2. – *Chromatogram for test B for related substances of fludarabine phosphate: reference solution (a) at 260 nm.*

Identification of impurities: identify the impurity peaks in the chromatogram obtained with reference solution (a) and in the chromatogram obtained with the test solution by comparison with Figure 1781.-2.

Relative retention with reference to fludarabine phosphate (retention time = about 2.5 min): impurity D = about 1.5; impurity E = about 1.9; impurity G = about 2.2; impurity H = about 2.4; impurity F = about 2.5.

System suitability: reference solution (a):

– *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity H.

Limits:

– *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.5; impurity E = 0.6; impurity F = 1.8;
– *impurity D*: maximum 0.1 per cent;
– *impurity E*: maximum 0.2 per cent;
– *impurity F*: maximum 0.2 per cent;
– *any other impurity eluting after fludarabine phosphate*: maximum 0.1 per cent;
– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent), and any peak eluting before fludarabine phosphate.

Total of impurities eluting before fludarabine phosphate in test A, apart from impurities A, B and C, and of impurities eluting after fludarabine phosphate in test B, apart from impurities D, E and F: maximum 0.5 per cent.

Total of all impurities eluting before fludarabine phosphate in test A and after fludarabine phosphate in test B: maximum 2.0 per cent.

Ethanol (*2.4.24, System A*): maximum 1.0 per cent.

Heavy metals (*2.4.8*): maximum 20 ppm.

Dissolve 1.0 g by heating in 10 ml of *water R*. Allow to cool. Add *ammonia R* until the litmus paper reaction is slightly alkaline. Adjust to pH 3.0-4.0 with *dilute acetic acid R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*): maximum 3.0 per cent, determined on 0.200 g (ground to a very fine powder). Stir the substance in 15 ml of *anhydrous methanol R* for about 15 s before titrating.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Test solution. With the aid of ultrasound, dissolve 24.0 mg of the substance to be examined in 50 ml of *water R* and dilute to 100.0 ml with the same solvent. Dilute 25.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. With the aid of ultrasound, dissolve 24.0 mg of *fludarabine phosphate CRS* in 50 ml of *water R* and dilute to 100.0 ml with the same solvent. Dilute 25.0 ml of the solution to 100.0 ml with the mobile phase.

Detection: spectrophotometer at 260 nm.

Injection: 10 µl.

Calculate the percentage content of $C_{10}H_{13}FN_5O_7P$ using the chromatograms obtained with the test solution and the reference solution, and the declared content of *fludarabine phosphate CRS*.

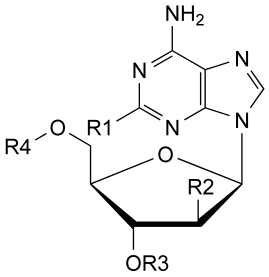
STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

Specified impurities: *A, B, C, D, E, F, G.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *H, I, J.*



A. R1 = R2 = OH, R3 = H, R4 = PO_3H_2: 6-amino-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-2-ol,

C. R1 = F, R2 = OH, R3 = R4 = PO_3H_2: 9-(3,5-di-*O*-phosphono-β-D-arabinofuranosyl)-2-fluoro-9*H*-purin-6-amine,

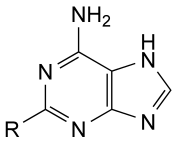
E. R1 = F, R2 = OH, R3 = R4 = H: 9-β-D-arabinofuranosyl-2-fluoro-9*H*-purin-6-amine,

F. R1 = O-C_2H_5, R2 = OH, R3 = H, R4 = PO_3H_2: 2-ethoxy-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-6-amine,

G. R1 = F, R2 = Cl, R3 = H, R4 = PO_3H_2: 9-(2-chloro-2-deoxy-5-*O*-phosphono-β-D-arabinofuranosyl)-2-fluoro-9*H*-purin-6-amine,

I. R1 = NH_2, R2 = OH, R3 = H, R4 = PO_3H_2: 9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purine-2,6-diamine,

J. R1 = OCH_3, R2 = OH, R3 = H, R4 = PO_3H_2: 2-methoxy-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-6-amine,



B. R = OH: 6-amino-7*H*-purin-2-ol,

D. R = F: 2-fluoro-7*H*-purin-6-amine,

H. 9-(2,5-anhydro-β-D-arabinofuranosyl)-2-fluoro-9*H*-purin-6-amine.

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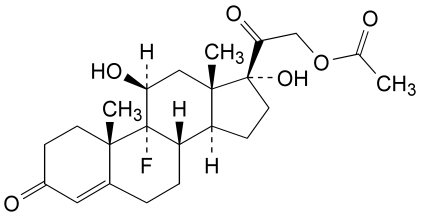
corrected 6.0

FLUDROCORTISONE ACETATE

Fludrocortisoni acetas



$C_{23}H_{31}FO_6$ M_r 422.5
[514-36-3]

DEFINITION

9-Fluoro-11β,17-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate.

Content: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, sparingly soluble in anhydrous ethanol.

IDENTIFICATION

First identification: A, B.

Second identification: C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *fludrocortisone acetate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture: *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution. Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a). Dissolve 10 mg of *fludrocortisone acetate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *cortisone acetate CRS* in 5 ml of reference solution (a).

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R.*

Application: 5 µl.

Development: over a path of 15 cm.

Drying: in air.

Detection A: examine in ultraviolet light at 254 nm.

Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).