Reference solution (a). Dissolve 10 mg of *adenine CRS* in *dilute acetic acid R*, with heating if necessary, and dilute to 10 ml with the same acid.

Reference solution (b). Dilute 1 ml of test solution (b) to 20 ml with *dilute acetic acid R*.

Reference solution (c). Dissolve 10 mg of *adenine CRS* and 10 mg of *adenosine R* in *dilute acetic acid R*, with heating if necessary, and dilute to 10 ml with the same acid.

Apply to the plate 5 µl of each solution. Develop over a path of 12 cm using a mixture of 20 volumes of *concentrated ammonia R*, 40 volumes of *ethyl acetate R* and 40 volumes of *propanol R*. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. 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) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*). To 10 ml of solution S add 1 ml of *concentrated ammonia R* and 3 ml of *silver nitrate solution R2*. Filter. Wash the precipitate with a little *water R* and dilute the filtrate to 15 ml with *water R*. The solution complies with the limit test for chlorides (100 ppm). When carrying out the test, add 2 ml of *dilute nitric acid R* instead of 1 ml of *dilute nitric acid R*.

Sulphates (*2.4.13*). Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium. Prepare a cell consisting of two watch-glasses 60 mm in diameter placed edge to edge. To the inner wall of the upper watch-glass stick a piece of *red litmus paper R* 5 mm square and wetted with a few drops of *water R*. Finely powder the substance to be examined, place 0.5 g in the lower watch-glass and suspend in 0.5 ml of *water R*. To the suspension add 0.30 g of *heavy magnesium oxide R*. Briefly triturate with a glass rod. Immediately close the cell by putting the two watch-glasses together. Heat at 40 °C for 15 min. The litmus paper is not more intensely blue coloured than a standard prepared at the same time and in the same manner using 0.05 ml of *ammonium standard solution (100 ppm NH_4) R*, 0.5 ml of *water R* and 0.30 g of *heavy magnesium oxide R* (10 ppm).

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

Loss on drying (*2.2.32*). Not more than 0.5 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.

ASSAY

Dissolve 0.100 g in a mixture of 20 ml of *acetic anhydride R* and 30 ml of *anhydrous acetic acid 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 13.51 mg of $C_5H_5N_5$.

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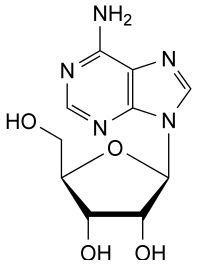
ADENOSINE

Adenosinum



$C_{10}H_{13}N_5O_4$ M_r 267.2
[58-61-7]

DEFINITION

9-β-D-Ribofuranosyl-9*H*-purin-6-amine.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, soluble in hot water, practically insoluble in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute mineral acids.

mp: about 234 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *adenosine CRS*.

TESTS

Solution S. Suspend 5.0 g in 100 ml of *distilled water R* and heat to boiling. Allow to cool, filter with the aid of vacuum and dilute to 100 ml with *distilled water R*.

Appearance of solution. Solution S is colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S, add 0.1 ml of *bromocresol purple solution R* and 0.1 ml of *0.01 M hydrochloric acid*. The solution is yellow. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is violet-blue.

Specific optical rotation (*2.2.7*): – 45 to – 49 (dried substance).

Dissolve 1.25 g in *1 M hydrochloric acid* and dilute to 50.0 ml with the same acid. Examine within 10 min of preparing the solution.

Related substances

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

Test solution. Dissolve 0.20 g of the substance to be examined in *dilute acetic acid R* with slight heating and dilute to 5 ml with the same acid.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with *water R*.

Reference solution (b). Dissolve 10 mg of *adenosine CRS* and 10 mg of *adenine CRS* in *dilute acetic acid R*, with heating if necessary, and dilute to 10 ml with the same acid.

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

Mobile phase: *water R, concentrated ammonia R, propanol R* (10:30:60 *V/V/V*).

Application: 5 µl.

Development: over a path of 12 cm.

Drying: in a current of warm air.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

Detection A: examine in ultraviolet light at 254 nm.

Results A: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent).

Detection B: spray with a 5 g/l solution of *potassium permanganate R* in *1 M sodium hydroxide*. Allow the plate to dry in a current of warm air and examine in daylight.

Results B: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent).

B. Liquid chromatography (*2.2.29*).

Solvent mixture. Dissolve 6.8 g of *potassium hydrogen sulphate R* and 3.4 g of *tetrabutylammonium hydrogen sulphate R* in *water R*, adjust to pH 6.5 with a 60 g/l solution of *potassium hydroxide R* and dilute to 1000 ml with the same solvent. Use freshly prepared solvent mixture.

Test solution. Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *adenine R* (impurity A) and 5 mg of *inosine R* (impurity G) in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 4 ml of this solution to 100 ml with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *water R*, solvent mixture (40:60 *V/V*).

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl.

Run time: 1.5 times the retention time of adenosine.

Relative retention with reference to adenosine (retention time = about 13 min): impurity A = about 0.3; impurity G = about 0.4.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to impurities A and G.

Limits:

– *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity G = 1.4;

– *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

– *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*): maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*): maximum 200 ppm, determined on solution S.

Ammonium (*2.4.1, Method B*): maximum 10 ppm, determined on 0.5 g.

Prepare the standard using 5 ml of *ammonium standard solution (1 ppm NH_4) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g, warming slightly if necessary, in a mixture of 20 ml of *acetic anhydride R* and 30 ml of *anhydrous acetic acid 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 26.72 mg of $C_{10}H_{13}N_5O_4$.

IMPURITIES

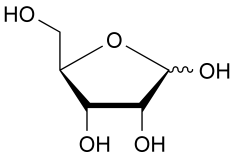
Specified impurities: *A, B, C, D, E, G.*

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*): *F, H.*

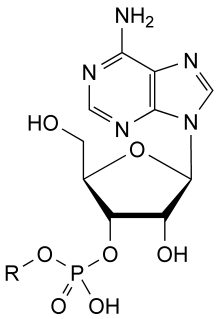
By thin-layer chromatography: B, C, D, E.

By liquid chromatography: A, F, G, H.

A. adenine,



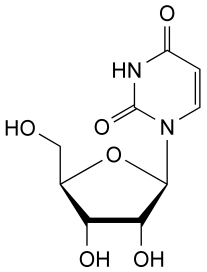
B. D-ribose,



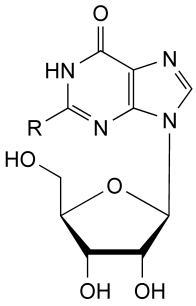
C. R = H: adenosine 3′-(dihydrogen phosphate),

D. R = PO_3H_2: adenosine 3′-(trihydrogen diphosphate),

E. R = PO_2H-O-PO_3H_2: adenosine 3′-(tetrahydrogen triphosphate),



F. 1-β-D-ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione (uridine),



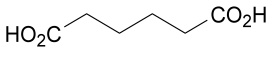
G. R = H: 9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (inosine),

H. R = NH_2: 2-amino-9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (guanosine).

01/2008:1586
corrected 6.0

ADIPIC ACID

Acidum adipicum



$C_6H_{10}O_4$ M_r 146.1
[124-04-9]

DEFINITION

Hexanedioic acid.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water, soluble in boiling water, freely soluble in alcohol and in methanol, soluble in acetone.

IDENTIFICATION

A. Melting point (*2.2.14*): 151 °C to 154 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *adipic acid CRS*.

TESTS

Solution S. Dissolve 5.0 g with heating in *distilled water R* and dilute to 50 ml with the same solvent. Allow to cool and to crystallise. Filter through a sintered-glass filter (40) (*2.1.2*). Wash the filter with *distilled water R*. Collect the filtrate and the washings until a volume of 50 ml is obtained.

Appearance of solution. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.20 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 20 mg of *glutaric acid R* in 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase, dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.125 m, Ø = 4.0 mm,

– *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m^2/g and a pore size of 10 nm,

– *temperature*: 30 °C.

Mobile phase: mix 3 volumes of *acetonitrile R* and 97 volumes of a 24.5 g/l solution of *dilute phosphoric acid R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 209 nm.

Injection: 20 µl.

Run time: 3 times the retention time of adipic acid.

System suitability: reference solution (a):

– *resolution*: minimum 9.0 between the peaks due to glutaric acid and adipic acid.

Limits:

– *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*): maximum 200 ppm.

2.5 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides.