Quality standards

Edition: BP 2025 (Ph. Eur. 11.6 update)

Adenine

General Notices

(Ph. Eur. monograph 0800)



C₅H₅N₅ 135.1 73-24-5

Action and use

Constituent of anticoagulant and preservative solutions for blood.

Ph Eur

DEFINITION

Adenine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7*H*-purin-6-amine, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, very slightly soluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A.

Second identification: B, C.

- A. Examine by infrared absorption spectrophotometry (<u>2.2.24</u>), comparing with the spectrum obtained with <u>adenine CRS</u>. Examine the substances prepared as discs.
- B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).
- C. To 1 g add 3.5 mL of <u>propionic anhydride R</u> and boil for 15 min with stirring. Cool. To the resulting crystalline mass add 15 mL of <u>light petroleum R</u> and heat to boiling with vigorous stirring. Cool and filter. Wash the precipitate with two quantities, each of 5 mL, of <u>light petroleum R</u>. Dissolve the precipitate in 10 mL of <u>water R</u> and boil for 1 min. Filter the mixture at 30 °C to 40 °C. Allow to cool. Filter, and dry the precipitate at 100 °C to 105 °C for 1 h. The melting point (2.2.14) of the precipitate is 237 °C to 241 °C.

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TESTS

Solution S

Suspend 2.5 g in 50 mL of <u>distilled water R</u> and boil for 3 min. Cool and dilute to 50 mL with <u>distilled water R</u>. Filter. Use the filtrate as solution S.

Appearance of solution

Dissolve 0.5 g in <u>dilute hydrochloric acid R</u> and dilute to 50 mL with the same acid. The solution is clear ($\underline{2.2.1}$) and colourless ($\underline{2.2.2}$, <u>Method II</u>).

Acidity or alkalinity

To 10 mL of solution S add 0.1 mL of <u>bromothymol blue solution R1</u> and 0.2 mL of <u>0.01 M sodium hydroxide</u>. The solution is blue. Add 0.4 mL of <u>0.01 M hydrochloric acid</u>. The solution is yellow.

Related substances

Examine by thin-layer chromatography ($\underline{2.2.27}$), using $\underline{silica\ gel\ GF}_{254}$ R as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in <u>dilute acetic acid R</u>, with heating if necessary, and dilute to 10 mL with the same acid.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with <u>dilute acetic acid R</u>.

Reference solution (a) Dissolve 10 mg of <u>adenine CRS</u> in <u>dilute acetic acid R</u>, 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 <u>adenine CRS</u> and 10 mg of <u>adenosine R</u> in <u>dilute acetic acid R</u>, 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 <u>concentrated</u> <u>ammonia R</u>, 40 volumes of <u>ethyl acetate R</u> and 40 volumes of <u>propanol R</u>. 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 <u>concentrated ammonia R</u> and 3 mL of <u>silver nitrate solution R2</u>. Filter. Wash the precipitate with a little <u>water R</u> and dilute the filtrate to 15 mL with <u>water R</u>. The solution complies with the limit test for chlorides (100 ppm). When carrying out the test, add 2 mL of <u>dilute nitric acid R</u> instead of 1 mL of <u>dilute nitric acid R</u>.

Sulfates (2.4.13)

Dilute 10 mL of solution S to 15 mL with <u>distilled water R</u>. The solution complies with the limit test for sulfates (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 <u>red litmus paper R</u> 5 mm square and wetted with a few drops of <u>water R</u>. Finely powder the substance to be examined, place 0.5 g in the lower watch-glass and suspend in 0.5 mL of <u>water R</u>. To the suspension add 0.30 g of <u>heavy magnesium oxide R</u>. 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 <u>ammonium standard solution (100 ppm NH₄) R</u>, 0.5 mL of <u>water R</u> and 0.30 g of <u>heavy magnesium oxide R</u> (10 ppm).

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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.

Sulfated 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 <u>acetic anhydride R</u> and 30 mL of <u>anhydrous acetic acid R</u>. Titrate with <u>0.1 M</u> <u>perchloric acid</u>, determining the end-point potentiometrically (<u>2.2.20</u>).

1 mL of $\underline{0.1~M~perchloric~acid}$ is equivalent to 13.51 mg of $C_5H_5N_5$.

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